

Comparison of two alpha-cyano pyrethroids when impregnated into bednets against a pyrethroid resistant and susceptible strain of *Anopheles stephensi* (Diptera: Culicidae) and their F₁ progeny

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Abstract

The two alpha-cyano pyrethroid insecticides lambda-cyhalothrin and alpha-cypermethrin were tested as bednet treatments at a target dose of 20 mg m⁻². To establish their efficacy, female pyrethroid resistant and susceptible *Anopheles stephensi* Liston, and the F₁-hybrids were allowed to fly freely in a room with a human subject under an impregnated net. Both treatments provided good personal protection by significantly reducing the number of blood fed mosquitoes compared to an untreated control net. Mortality after 24 h was significantly higher for the alpha-cypermethrin treated net when compared to lambda-cyhalothrin. For each insecticide there were no significant differences in the proportion of susceptible homozygotes and F₁-hybrids found dead after a 24 h holding period, which suggests that there would be no selection for pyrethroid resistant heterozygotes by either of the insecticides.

Introduction

Impregnation of bednets with pyrethroid insecticides reduces the incidence of malaria morbidity and mortality (Lengeler, 1998). However, bednets impregnated with a pyrethroid may select for genes that render mosquitoes resistant to these insecticides. It was pointed out by Taylor & Georgiou (1979) and Curtis *et al.* (1978) that the effectiveness of selection for a rare insecticide resistance gene depends on the effective dominance of the gene at the field dose.

To test for possible selection of pyrethroid impregnated bednets for resistance in host seeking anopheline mosquitoes under realistic conditions, free-flying mosquitoes may be released into a mosquito-proof room containing a human subject under a treated net (Curtis *et al.*, 1993). By using a pyrethroid resistant and a susceptible strain of *Anopheles stephensi* Liston (Diptera: Culicidae) and their F₁-progeny, the efficacy of each treatment against all three can be

measured and the likelihood of selection for resistance established under much more realistic conditions than in a bioassay with a fixed exposure time.

Such experiments have shown that 145 mg permethrin m⁻² kills both susceptible homozygotes and pyrethroid resistant heterozygotes of *A. stephensi*, which indicates that this dose is not likely to select for pyrethroid resistance in the early stages, when resistance genes are expected (according to the Hardy-Weinberg ratio) to occur almost entirely in the heterozygous form (Curtis *et al.*, 1993). Furthermore, it was shown that the dose of 145 mg permethrin m⁻² is advantageous, because it causes complete kill of all three genotypes, whereas the higher dose of 470 mg m⁻² kills significantly fewer heterozygotes than homozygote susceptibles, implying incomplete recessiveness of the resistance genes and selection for them at the higher dose, on which mosquitoes rest for shorter times because of higher irritancy (Hodjati & Curtis, 1997).

Further tests with free flying mosquitoes showed that knockdown caused by doses of 1, 2, 3 and 13 mg m⁻² of the alpha-cyano pyrethroid lambda-cyhalothrin (CS formulation)

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was significantly lower for F_1 -hybrids than for susceptible homozygotes, implying incomplete recessiveness of resistance in the heterozygotes (data of Kasumba in Curtis *et al.*, 1998). However, it was not possible to obtain usable data on final mortality in that study. The knockdown data suggest that lambda-cyhalothrin, and possibly other alpha-cyano pyrethroids, may be more likely to select for resistance than permethrin at a dose of 145 mg m^{-2} .

In view of the increasing use of cheaper, low doses of alpha-cyano pyrethroids in preference to high doses of permethrin and the recently reported emergence of *kdr*-type pyrethroid resistance in *Anopheles gambiae* Giles (Diptera: Culicidae) from Côte d'Ivoire (Elissa *et al.*, 1993; Martinez-Torres *et al.*, 1998; Chandre *et al.*, 1999), the prospect of selectively killing the susceptible homozygotes, but not the resistant heterozygotes, by the use of these compounds may be a threat to the continued success of pyrethroid impregnated bednets (Curtis *et al.*, 1998).

The present study was carried out to verify whether alpha-cyano pyrethroids (such as alpha-cypermethrin, lambda-cyhalothrin and deltamethrin) are more likely to select for resistance than permethrin. Lambda-cyhalothrin and alpha-cypermethrin were tested, as both compounds performed well in a field trial of impregnated nets in an area with intense malaria transmission (Maxwell *et al.*, 1999).

Materials and methods

A detailed account of the methodology was given by Hodjati & Curtis (1997) and the following is a summary.

Mosquito strains

Anopheles stephensi strains used in the present study were:

BEECH: insecticide susceptible.

DUB 234: resistant to pyrethroids and other insecticides; from a partially resistant strain from Dubai, selected in the laboratory for permethrin resistance at the adult stage by Ladonni (1988) and Vatandoost (1996) and further selected at the London School of Hygiene and Tropical Medicine by Hodjati (1998). Pyrethroid resistance in this strain is thought to be conferred by at least three separate mechanisms; cytochrome P-450 enzymes, esterases and *kdr*-type target insensitivity (Sivananthan *et al.*, 1992; Vatandoost, 1996).

F_1 progeny from crossing virgin females from the BEECH strain with males from the DUB 234 strain. Pupae of the susceptible strain were tubed individually before emergence to ensure virginity of the females.

Mosquito netting

Bednets used in the present study were made of 100% polyester, 100 denier with 12×13 meshes per square inch and were donated by the SiamDutch Mosquito Netting Co., Bangkok, Thailand.

Insecticides and impregnation

Insecticides were impregnated into bednets at a target dose of 20 mg m^{-2} , using the formula given by Pleass *et al.* (1993) to calculate the concentration of the dipping mixture. Alpha-cypermethrin insecticide tablets (Fastac Dry 150 g kg^{-1} WG, Cyanamid) and lambda-cyhalothrin, (Icon 25 g l^{-1}

EC, Zeneca) were used. Six months after the experiment, chemical analysis of netting samples was carried out by the Department de Phytopharmacie, Centre de Recherches Agronomique de Gembloux, Belgium, for the WHO Pesticide Evaluation Scheme. Two 15 cm^2 netting pieces from each net were found to have 8.7 and 8.0 mg m^{-2} of lambda-cyhalothrin or 17.3 and 11.7 mg m^{-2} of alpha-cypermethrin. For unknown reasons the lambda-cyhalothrin residue was less than 50% of that intended and the alpha-cypermethrin residue was also considerably less. No residues were detected on samples from the untreated control net.

Procedure to test bednet efficacy with free-flying mosquitoes and a human subject under the net

The rectangular nets were hung from four points at a height such that the bottom edge was resting on the floor, to avoid entry of mosquitoes. Throughout all tests the same human subject sat under the net with his forearm resting against it. For each of the treatments 6–10 replicates were carried out with 14–30 free-flying, female mosquitoes at 28°C . Five and eight replicates were undertaken for the controls. After 30 min, mosquitoes were collected with a mouth aspirator. They were scored as fed or unfed and knocked down or not knocked down, placed in separate cups according to these criteria, and provided with cotton wool moistened with 10% glucose. Mosquitoes were re-scored for knockdown after 1 h and for mortality 24 h after the end of the 30 min exposure period.

Data analysis

Statistical analysis was carried out on the proportions of fed, knocked down and killed mosquitoes in each replicate. Proportions were arcsin transformed and submitted to two way analysis of variance using SPSS for Windows. The 95% confidence intervals were calculated using arcsin transformed proportions, which were back-transformed for presentation as histograms.

Results and Discussion

Feeding, knockdown and mortality of the three genotypes of *A. stephensi* are summarized in figs 1–4.

Both lambda-cyhalothrin and alpha-cypermethrin impregnated mosquito nets provided personal protection from host seeking female *A. stephensi* (fig. 1). For all three genotypes the proportion of bloodfed mosquitoes was reduced significantly compared to controls. However, neither treatment performed as well as permethrin in the same type of experiment (Hodjati & Curtis, 1997). At a dose of $145 \text{ mg permethrin m}^{-2}$ bloodfeeding of both pyrethroid susceptible and F_1 -hybrid *A. stephensi* mosquitoes was almost completely prevented.

Significantly better personal protection by a permethrin target dose of 500 mg m^{-2} than by lambda-cyhalothrin (25 mg m^{-2}) impregnated holed bednets has been reported by Lindsay *et al.* (1991) who obtained a 91% reduction in bloodfeeding of *A. gambiae* with the permethrin impregnated nets. Further evidence for a better reduction in bloodfeeding by permethrin was given by Curtis *et al.* (1996), when comparing different doses of lambda-cyhalothrin to a target dose of $200 \text{ mg permethrin m}^{-2}$ in experimental huts. Target doses of 5, 10 and $15 \text{ mg lambda-cyhalothrin m}^{-2}$ seemed to

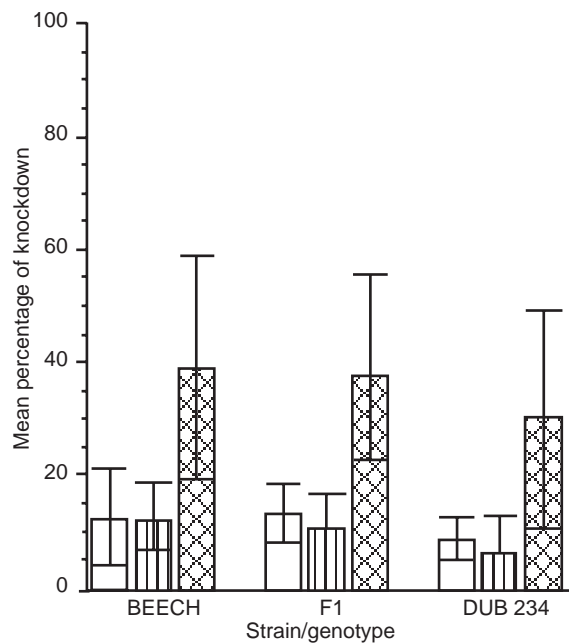


Fig. 1. Percentage bloodfed of *Anopheles stephensi* DUB 234 (resistant strain), BEECH (susceptible strain) and the F₁-hybrid, after 30 min of flying freely in a room containing a net with a human subject inside and treated with a target dose of 20 mg lambda-cyhalothrin m⁻² (□) or 20 mg alpha-cypermethrin m⁻² (▨) or an untreated net (⊠).

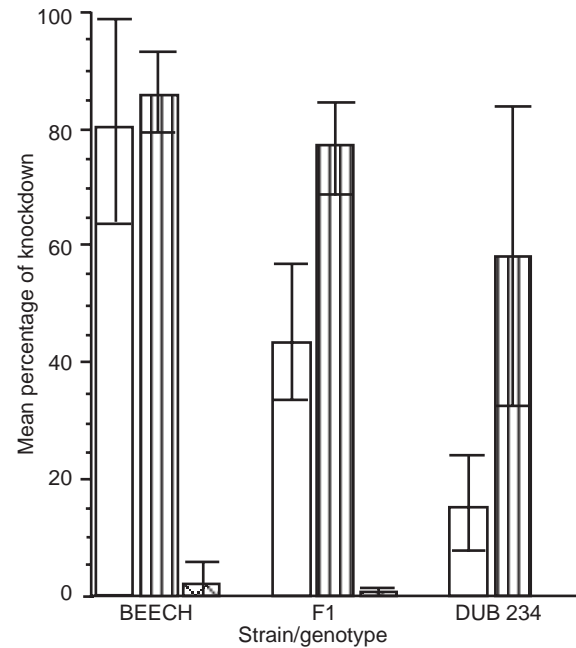


Fig. 2. Percentage knockdown of *Anopheles stephensi* DUB 234 (resistant strain), BEECH (susceptible strain) and the F₁-hybrid, after 30 min of flying freely in a room containing a net with a human subject inside and treated with a target dose of 20 mg lambda-cyhalothrin m⁻² (□) or 20 mg alpha-cypermethrin m⁻² (▨) or an untreated net (⊠).

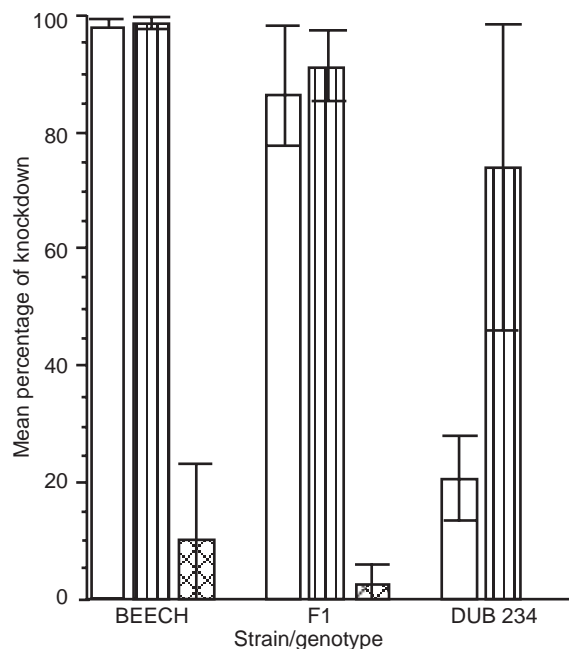


Fig. 3. Percentage knockdown of *Anopheles stephensi* DUB 234 (resistant strain), BEECH (susceptible strain) and the F₁-hybrid, 1 h after the end of the 30 min free-flying period. Results are shown for nets impregnated with a target dose of 20 mg lambda-cyhalothrin m⁻² (□) or 20 mg alpha-cypermethrin m⁻² (▨) or an untreated net (⊠).

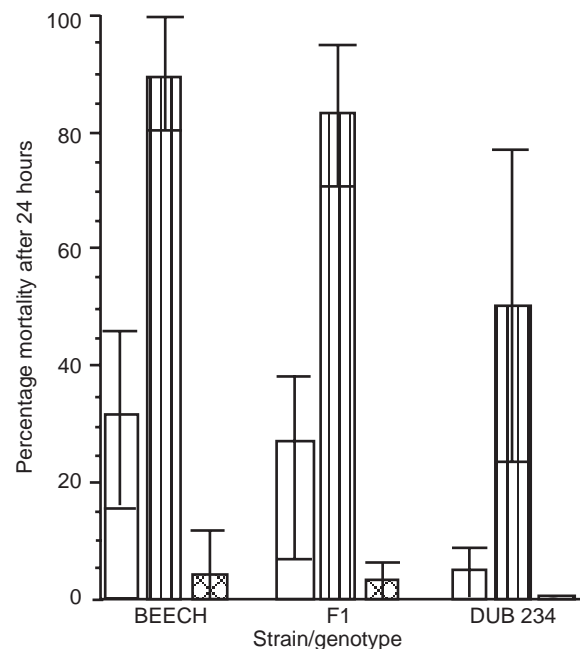


Fig. 4. Percentage mortality of *Anopheles stephensi* DUB 234 (resistant strain), BEECH (susceptible strain) and the F₁-hybrid, 24 h after the end of the 30 min free-flying period. Results are shown for nets impregnated with a target dose of 20 mg lambda-cyhalothrin m⁻² (□) or 20 mg alpha-cypermethrin m⁻² (▨) or an untreated net (⊠).

cause less reduction in bloodfeeding of *A. gambiae* and *Anopheles funestus* Giles (Diptera: Culicidae) than permethrin, though the difference was significant only for the 5 mg m⁻² EC and the 10 mg m⁻² CS formulations.

Figure 1 shows that in the present trial no significant difference was found in the percentage of female bloodfed mosquitoes with the lambda-cyhalothrin and alpha-cypermethrin treatments indicating that both compounds provide similar protection against female *A. stephensi* of all three genotypes. This is consistent with Maxwell *et al.* (1999) who, in an area where the *A. gambiae* s.s. were pyrethroid susceptible, compared a CS formulation of lambda-cyhalothrin with an SC formulation of alpha-cypermethrin impregnated into holed nets, at a target dose of 20 mg m⁻², in experimental huts, and showed a reduction in bloodfeeding of 47% and 55% respectively. Overall alpha-cypermethrin gave slightly but significantly less bloodfeeding, while lambda-cyhalothrin gave slightly but significantly higher percentage kill.

The difference in the proportion of the three genotypes knocked down after exposure (fig. 2) shows the protective effect of the resistance gene, with the highest proportion of knockdown occurring amongst the susceptible homozygotes, the lowest amongst the resistant homozygotes and the heterozygotes being intermediate. Performance of the nets treated with the two insecticides was significantly different for the heterozygote and the resistance homozygote, with the alpha-cypermethrin nets causing higher knockdown. One hour later the difference in knock-down between the nets was less pronounced, a significant difference being registered only with the resistance homozygote (fig. 3). However, 24 h after exposure the difference in the performance of the two compounds was highly significant for all three genotypes, with the alpha-cypermethrin net causing at least three times more kill than the lambda-cyhalothrin net (fig. 4).

The difference in performance of the two insecticides may be partially explained by the under-dosing of the lambda-cyhalothrin treated net compared to the alpha-cypermethrin one, as noted above. Alternatively lambda-cyhalothrin may not be as effective against *A. stephensi*. Lambda-cyhalothrin (25 mg m⁻²) did not perform as well in bioassays with a susceptible strain of *A. stephensi* when compared to 500 mg permethrin m⁻², while results with *A. gambiae* were comparable (Hodjati, 1998).

It may be misleading to extrapolate results from bioassays, with mosquitoes confined in a small space (cone/WHO test kit) close to an insecticide impregnated surface, to tests with free-flying mosquitoes. Curtis *et al.* (1993) point out that 'it is the dosage experienced by the insect allowed to behave naturally which matters', and the present study indicates that with free-flying *A. stephensi*, the alpha-cypermethrin net performed significantly better in killing mosquitoes than the lambda-cyhalothrin net with a somewhat lower dose.

There were significant differences between the susceptible and heterozygous mosquitoes in knockdown immediately and 1 h after exposure, indicating reduced efficacy of both insecticides against the heterozygotes. This is consistent with the observations of Kasumba (data in Curtis *et al.*, 1998) suggesting that pyrethroid resistance would be selected by a range of dosages of lambda-cyhalothrin on the basis of knockdown results.

In the present study, the proportion killed by the insecticides after 24 h was not significantly different for

these two genotypes. Therefore our data do not give reason to favour permethrin in preference to alpha-cyano pyrethroids to minimize the risk of selection for pyrethroid resistance. However, both insecticides caused lower kill than previously reported for 145 mg permethrin m⁻² and the overall better reduction in blood feeding and complete mortality of both resistant genotypes at 145 mg permethrin m⁻² (Hodjati & Curtis, 1997) suggests that permethrin may still have advantages for malaria vector control despite its probable higher cost per net treated (Feilden, 1996).

Despite the obvious importance of choosing the most effective compound and dose for impregnation of bednets to minimize the risk of selecting for resistance, there is as yet only one area in which bednets appear to have selected for pyrethroid resistance (Vulule *et al.*, 1994, 1996; Curtis *et al.*, 1998). The lack of other reports of resistance selected by impregnated bednets might be due to pyrethroid resistance genes being effectively recessive or almost recessive, as demonstrated for *A. stephensi* in response to permethrin by Curtis *et al.*, (1990), Ladonni *et al.* (1990), Hodjati & Curtis (1997) and Hodjati (1998), depending on dose and type of exposure.

Potentially more dangerous for the selection of resistance in mosquitoes seems to be the use of pyrethroids for agricultural pest control. Large-scale application of these compounds on crops may exert selection pressure on non-target organisms, such as mosquitoes (Lines, 1988) and was suggested to be the cause for the high frequency of *kdr*-resistance currently observed in some *A. gambiae* populations from Côte d'Ivoire and Burkina Faso (Elissa *et al.*, 1993). As the frequency of the selected resistance gene increases, impregnation of bednets with a dose that reliably kills resistance heterozygotes will cease to be sufficient to prevent effective selection for resistance (Curtis, 1987). Homozygotes for the resistance gene will appear at appreciable frequency and, due to their greater resistance to treated nets than that of heterozygotes (see figs 2–4), it can be assumed that resistance will increase steadily to a very high frequency.

We conclude that, even if pyrethroids are carefully tested to ensure a minimal risk for selection of resistance heterozygotes by bednets, prior selection of heterozygotes by large-scale agricultural application of pyrethroids may select for resistance, which may go to completion by selection with nets. This may have occurred in Côte d'Ivoire, where the *kdr* gene frequency in certain populations is almost 90% (Martinez-Torres *et al.*, 1998; Chandre *et al.*, 1999). There was evidence for a higher frequency of the resistance gene among those surviving exposure to pyrethroid-treated nets and a switch to unrelated compounds, such as one of the organophosphate or carbamate compounds of low human toxicity, may be desirable (Fanello *et al.*, 1999). In East Africa, however, tests of *A. gambiae* from cotton growing areas have so far not revealed any resistance (Curtis *et al.*, 1999, and unpublished data) and use of a pyrethroid treatment which reliably kills heterozygotes may be sufficient there to indefinitely postpone a resistance problem.

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Invertebrates as Webmasters in Ecosystems

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