Bulletin of Entomological Research (2003) **93**, 491–498 DOI: 10.1079/BER2003261

Efficacy of mosquito nets treated with insecticide mixtures or mosaics against insecticide resistant *Anopheles gambiae* and *Culex quinquefasciatus* (Diptera: Culicidae) in Côte d'Ivoire

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Abstract

Only pyrethroid insecticides have so far been recommended for the treatment of mosquito nets for malaria control. Increasing resistance of malaria vectors to pyrethroids threatens to reduce the potency of this important method of vector control. Among the strategies proposed for resistance management is to use a pyrethroid and a non-pyrethroid insecticide in combination on the same mosquito net, either separately or as a mixture. Mixtures are particularly promising if there is potentiation between the two insecticides as this would make it possible to lower the dosage of each, as has been demonstrated under laboratory conditions for a mixture of bifenthrin (pyrethroid) and carbosulfan (carbamate). The effect of these types of treatment were compared in experimental huts on wild populations of *Anopheles gambiae* Giles and the nuisance mosquito *Culex quinquefasciatus* Say, both of which are multi-resistant. Four treatments were evaluated in experimental huts over six months: the recommended dosage of 50 mg m^{-2} bifenthrin, 300 mg m^{-2} carbosulfan, a mosaic of 300 mg m^{-2} carbosulfan on the ceiling and 50 mg m^{-2} bifenthrin on the sides, and a mixture of 6.25 mg m^{-2} carbosulfan and 25 mg m^{-2} bifenthrin. The mixture and mosaic treatments did not differ significantly in effectiveness from carbosulfan and bifenthrin alone against anophelines in terms of deterrency, induced exophily, blood feeding inhibition and overall mortality, but

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were more effective than in earlier tests with deltamethrin. These results are considered encouraging, as the combination of different classes of insecticides might be a potential tool for resistance management. The mixture might have an advantage in terms of lower cost and toxicity.

Introduction

The large scale use of insecticide-treated nets is a major component of the overall strategy of malaria control, especially in Africa (Lines, 1996). Only pyrethroid insecticides are currently recommended for use on nets because of their fast knock down action, their excito-repellency, and their relative safety (Zaim *et al.*, 2000). Although resistance to pyrethroids already exists in several vectors of malaria in Africa (Chandre *et al.*, 1999a; Hargreaves *et al.*, 2000; Ranson *et al*, 2000), studies in West Africa have shown that insecticide-treated nets remain effective against *Anopheles gambiae* Giles (Diptera: Culicidae) (Darriet *et al.*, 2000) in areas of resistance due to the *kdr* mutation (Martinez-Torres *et al.*, 1998). This does not necessarily mean, however, that effectiveness will be maintained when other resistance mechanisms or other species are involved (Omer *et al.*, 1980; Malcom, 1988; Hargreaves *et al.*, 2000; Kasap *et al.*, 2000). Moreover, to be well accepted by householders, insecticide-treated nets must have a visible impact on nuisance mosquitoes or non-target arthropods (Temu *et al.*, 1999), especially *Culex quinquefasciatus* Say (Diptera: Culicidae), a mosquito which is resistant to many types of insecticide (Chandre *et al.*, 1998).

Thus the search for alternative insecticides has become a priority. New families of insecticides used in agriculture, such as neo-nicotinoids, pyrasols or mitochondrial electron transfer inhibitors are promising but these compounds, which have barely started the validation tests necessary for approval for malaria control, will not be available for several years. An alternative solution is to search among the already marketed insecticides for different modes of action. Experimental hut studies indicate that some organophosphorous and carbamate insecticides have potential for use on mosquito nets (Miller *et al.*, 1991; Fanello *et al.*, 1999; Kolaczinski *et al.*, 2000). These insecticide classes are less irritant and less excito-repellent than pyrethroids, and allow longer contact between the mosquito and the insecticide-treated nets and thus induce higher mortality (Curtis & Mnzava, 2000). On the other hand, these same characteristics enable mosquitoes to bite through the mosquito net more easily which consequently reduce the level of personal protection, particularly after the mosquito net is torn (Miller *et al.*, 1991; Kolaczinski *et al.*, 2000). Organophosphorous and carbamate insecticides are relatively slower acting and require higher doses of active ingredient than pyrethroids other than permethrin. Many are less stable and more toxic to humans (Tomlin, 2000).

Another tactic for managing insecticide resistance consists of simultaneous use of insecticides having different modes of actions (Denholm & Rowland, 1992). The use in combination, on the same mosquito net, of a pyrethroid and a non-pyrethroid (carbamate or organophosphate) has the potential to eliminate the insects which are genetically susceptible to either or both of the compounds (Curtis *et al.*, 1993) and thus to delay the appearance of double resistance (Curtis, 1985). The term 'mosaics' has been used for the use of two insecticides separately: for example the ceiling of the net, being further from the sleeper could be treated with a more toxic insecticide, in this case a non-pyrethroid, and the sides could be treated with a pyrethroid (Guillet *et al.*, 2001). With mixtures, the pyrethroid and non-pyrethroid insecticides would be used to treat the entire net. If potentiation occurs it may be possible to lower the dosage of each component without losing the necessary high mosquito mortality, thereby reducing the cost of impregnation. Potentiation between insecticides has often been observed in agricultural pests (Koziol & Witkowski, 1982; Campanhola & Plapp, 1989) and, more recently, in insecticide susceptible *A. gambiae* in contact with netting impregnated with a mixture (Corbel *et al.*, 2002). This has encouraged us to test mixtures and mosaics in experimental huts against wild populations of *A. gambiae* and *C. quinquefasciatus* showing one or more mechanisms of resistance.

Material and methods

Insecticides

Bifenthrin (Talstar® 80 gl^{-1} SC, FMC, Philadelphia, USA) is a non alpha-cyano pyrethroid formulated as a suspension concentrate which is characterized by weak knock-down and irritant effects (Hougard *et al.*, 2002). Preliminary studies in huts showed that it was effective at 50 mg m^{-2} against mosquitoes with a high frequency of the *kdr* gene (Guillet *et al.*, 2001). Carbosulfan (Marshall® 25 gl m^{-1} CS, FMC, Philadelphia, USA) is a carbamate effective at 300 mg m^{-2} on nets (Kolaczinski *et al.*, 2000; Guillet *et al.*, 2001). The formulation used was a microcapsule suspension. For simplicity, treatments will be referred to by their common chemical names rather than the optimized formulations product names. Mixtures of the diluted formulations of the two products were entirely compatible.

Impregnation of mosquito nets

Mosquito nets were made of knitted polyester, 100 denier, 156 meshes per square inch, measuring 2 m long, 1.9 m wide and 1.8 m high (Vestergaard Frandsen A/S, Copenhagen, Denmark). A total of 20 intact mosquito nets were tested, using alternatively two replicate treated nets per hut to compensate for variability between individual net treatments. Each net was treated by soaking in a test solution and drying horizontally. Untreated mosquito nets were used as controls. The four treatments were 50 mg m^{-2} bifenthrin, 300 mg m^{-2} carbosulfan, a mosaic of 300 mg m^{-2} carbosulfan and 50 mg m^{-2} bifenthrin (the impregnation was carried out in two stages, first with carbosulfan on the ceiling and then, after drying, with bifenthrin on the side walls), and a mixture using doses that gave the best potentiation under laboratory conditions (6.25 mg m^{-2} of carbosulfan and 25 mg m^{-2} of bifenthrin – Corbel *et al.*,

2002). The mosquito nets were first used one week after impregnation. The study was double blind (to the volunteers under the nets and the experimenter) and carried out over six months.

Mosquito susceptibility

The susceptibility of wild *A. gambiae* and *C. quinquefasciatus* to technical material of bifenthrin and carbosulfan was compared with that of the two susceptible reference strains, *A. gambiae* (Kisumu) and *C. quinquefasciatus* (S-Lab). Tests were carried out using WHO susceptibility test kits on adult mosquitoes (WHO, 1998), with filter papers impregnated with diagnostic concentrations of 0.25% bifenthrin and 0.4% carbosulfan (i.e. 91 mg and 145.6 mg of active ingredient per m^2 filter paper, respectively) (Hougard *et al.*, 2002; N'Guessan *et al.*, 2003). Batches of 25 females (not blood fed and aged 2–5 days), were exposed in the tube, placed vertically, for 1 or 2 h. The number knocked down was recorded up to 60 min. The time at which 50% and 95% of mosquitoes were knocked-down (KDT_{50} and KDT_{95}) with their 95% confidence limits were calculated using log-probit analysis (Raymond *et al.*, 1997). Mortality was recorded 24 h after the end of the exposure, corrected for control mortality (Abbott, 1925). Each test was repeated four times.

Insecticide persistence

Knockdown and mortality of the four treatments were measured in the huts one month after impregnation of the nets, then every month for five consecutive months. Mosquitoes 2–5 days old which had not been blood fed were exposed for 3 min in cones attached to the nets and the mosquitoes were then transferred to holding cups (WHO, 1998). The temperature during exposure was 25°C ± 2 under subdued light and only five mosquitoes were tested at a time. The mosquitoes were held at 27°C ± 2 and 80% ± 10 rh with honey solution provided. For each sample tested 50 mosquitoes were used. The percentage of mosquitoes knocked down was recorded after 60 min and percentage mortalities after 24 h.

Experimental huts

The mosquito nets were evaluated in experimental huts (Darriet *et al.*, 2002) in two areas of Côte d'Ivoire. The M'bé valley station is located near a rice growing area. The Yaokoffikro station is located in a peri-urban area near to rice and vegetable cultivation. *Anopheles gambiae* accounts for 68% of the mosquitoes caught in experimental huts at M'bé and for 30% of those at Yaokoffikro (Darriet *et al.*, 2000). *Culex quinquefasciatus* is not encountered at M'bé but accounts for nearly 60% of the total number caught at Yaokoffikro (Darriet *et al.*, 1998). Five experimental huts were used in each station. Each was 2.5 m long, 1.75 m wide and 2 m high, with the huts spaced at 5 m intervals. The walls were made of breeze blocks, the floor of cement, the roof of corrugated iron. A plastic cover was stretched under the roofing sheets to facilitate hand catching of mosquitoes. Each hut was surrounded by a water-filled moat to exclude ants and spiders. Entry by mosquitoes was only possible through four windows slits (1 cm wide) located on three sides of the hut, the slits being designed to inhibit exiting of mosquitoes. Mosquitoes could exit into a large screened verandah trap located on the fourth side. Preliminary catches showed there was no significant difference in the attractiveness of huts to mosquitoes.

With the ethical approval of the health ministry of Côte d'Ivoire, five adult male volunteers, who gave their informed consent and were given chemoprophylaxis, slept in the huts from 20:00 to 05:00 h each night. To reduce the effect of variation in individual attractiveness to mosquitoes, sleepers were rotated between huts on successive nights. Preliminary catches using this rotating sleepers method showed no significant difference (by using the Kruskal-Wallis test – see 'data analysis') in attractiveness of different huts. Each treatment was randomly allocated to one of the huts for the duration of the trial to reduce the risk of cross-contamination between huts. Wild mosquitoes entered each hut via the window slits and could exit into the verandah trap. Mosquito collections were made three times per week for six months, starting one week after introduction of the nets. At 05:00 h the sleeper closed the windows and lowered a curtain separating the room from the verandah and all mosquitoes were collected from each hut (inside and outside the net) at 08:00 h and recorded 12 h after the beginning of the experiment as either alive or dead, and unfed or blood-fed. Surviving mosquitoes were provided with 10% honey solution and held for 24 h before scoring delayed mortality (i.e. 36 h after the beginning of the experiment). The effects of each treatment on mosquitoes was expressed relative to the control in terms of:

1. Deterrency effect: percentage reduction in the number of mosquitoes caught in a treated hut compared to the number caught in the control hut.
2. Exophily: percentage of the total mosquito collection caught in the verandah trap.
3. Blood feeding rate: percentage of mosquitoes collected in the hut that were blood fed.
4. Overall mortality: total number of mosquitoes found dead immediately and after 24 h observation.

When the differences between the treated and control percentages were significant at the 5% level, mortality and exophily due to the treatments were corrected for mortality and exophily in the control (Abbott, 1925). Inhibition of blood feeding was corrected for feeding rate in the control as follows: 1 – (% fed treated / % fed control).

Data analysis

Statistical analysis of data was carried out on a fortnightly basis, a period of time which allowed for a complete rotation of sleepers between huts, thus avoiding any bias due to individual variation in attractiveness. The non-parametric Kruskal-Wallis test was used as the data from different fortnights were not normally distributed (Sofware packages used: Statistica Visual Basic, version 6.0). Significance of difference in hut attractiveness between each treatment (including the control) was based on the number of mosquitoes collected during each fortnight in the hut concerned. Significance of difference in exophily, blood feeding and mortality were expressed as porportions of the total catch in each fortnight.

Table 1. Mortality and knock-down of a susceptible strain of *Anopheles gambiae* (Kisumu) and wild populations of *A. gambiae* (M'bé station) and *A. gambiae* and *Culex quinquefasciatus* (Yaokoffikro station) exposed to diagnostic doses of technical material of bifenthrin and carbosulfan 0.25 and 0.40%, i.e. 91 mg and 145.6 mg of active ingredient per m^2 filter paper, respectively).

Insecticide		*A. gambiae*			*C. quinquefasciatus*	
		Kisumu	M'bé	Yaokoffikro	S-Lab	Yaokoffikro
Bifenthrin (0.25%)	% mortality 24 h	100 (105)	96 (103)	79 (104)	100 (99)	41 (105)
	KDT_{50} in min (95% CI)	24 (22.3–25.4)	40 (38.8–41.8)	NE	32 (31.4–34.1)	NE
	KDT_{95} in min (95% CI)	55 (48.4–66.1)	69 (64.9–75.6)	NE	49 (45.9–52.9)	NE
Carbosulfan (0.40%)	% mortality 24 h	99 (102)	63 (103)	53 (112)	100 (103)*	90 (99)*

Number tested in parentheses (4 replicates). NE, no detectable effect. * Two hours exposure time. $KDT_{50/95,}$ times required to achieve 50% and 95% knockdown of mosquitoes, respectively.

Results

Mosquito susceptibility

Mortality values were not corrected owing to low control mortality (< 5%). At the diagnostic dose of 0.25% (table 1) bifenthrin induced 100% mortality after 1 h exposure of the Kisumu and S-Lab strains, with a KDT_{95} of less than 1 h. The wild strain of *A. gambiae* from M'bé was susceptible to bifenthrin with 96% mortality and KDT values similar to those recorded with the laboratory strains. *Anopheles gambiae* from Yaokoffikro was, however, resistant to bifenthrin with a mortality of less than 80% and no knock-down after 60 min exposure. *Culex quinquefasciatus* from Yaokoffikro was highly resistant to bifenthrin with only 41% mortality and no knock-down after 60 min exposure. Carbosulfan exposure induced > 99% mortality at the Kisumu strain after 1 h exposure, and S-Lab strain after two hours (table 1). The wild populations of *A. gambiae* were both resistant to carbosulfan (mortalities of 63% at M'bé and 53% at Yaokoffikro after one hour of exposure) and the population of *C. quinquefasciatus* at Yaokoffikro also showed slight resistance (90% mortality after 2 h of exposure).

Insecticide persistence

Mortalities recorded after six months of evaluation of each of the treated mosquito nets varied from 96 to 100% (table 2). They were as high or even higher than the values recorded at the beginning of the experiments (from 72 to 100%), for all the treatments tested. The same trend was noted for bifenthrin and the mixture with the percentages of knocked down mosquitoes after 60 min (no KD was noted with carbosulfan).

Efficacy of nets

The trial continued for 78 hut-nights per site (26 weeks × 3 nights per week). The efficacy of the various treated nets is summarized in table 3.

The deterrent effect due to insecticide was apparent for *A. gambiae* (52–71%) but not detectable for *C. quinquefasciatus*. There were no differences between treatments in the anopheline population.

The levels of exophily of *A. gambiae* varied between 22% and 56%. There was evidence for induced exophily with most of the treatments (21–37%, i.e. 1.3 –2.3 times the rate of natural exophily) but not for the mosaic and carbosulfan

Table 2. Residual efficacy under WHO cones against a susceptible strain of *Anopheles gambiae* (Kisumu) of different mosquito net treatments maintained for six months in experimental huts at M'bé and Yaokoffikro stations.

Mosquito net treatment (concentration)		Months after treatment					
		1	2	3	4	5	6
Untreated	% mortality 24 h	5.8 (52)	2.1 (48)	6.4 (50)	3.8 (52)	5.9 (51)	7.7 (52)
Bifenthrin (50 mg m^{-2})		98.2 (60)	100 (51)	98.0 (52)	100 (51)	100 (46)	100 (53)
Carbosulfan (300 mg m^{-2})		71.9 (49)	81.5 (54)	95.7 (50)	91.7 (48)	100 (52)	95.8 (52)
Mixture bifenthrin–carbosulfan (25 – 6.25 mg m^{-2})		100 (55)	100 (41)	100 (49)	98.1 (53)	100 (45)	100 (49
Untreated	% KD 60 min	0 (52)	0 (48)	0 (50)	0 (52)	0 (51)	0 (52)
Bifenthrin (50 mg m^{-2})		85.0 (60)	96.1 (51)	100 (52)	100 (51)	84.6 (46)	100 (53)
Mixture bifenthrin–carbosulfan (25 – 6.25 mg m^{-2})		100 (55)	100 (41)	100 (49)	100 (53)	95.5 (45)	100 (49)

Number tested in parentheses (10 replicates).

Table **3.** Efficacy of four mosquito net treatments in experimental huts against wild populations of *Anopheles gambiae* (M'bé station) and *A. gambiae* and *Culex quinquefasciatus* (Yaokoffikro station). Total mosquitoes collected from 78 hut-nights, August 2001-January 2002.

Station	Species		Mosquito net treatment			
		Untreated	Mixture bifenthrin–carbosulfan (25 – 6.25 mg m^{-2})	Mosaic bifenthrin–carbosulfan (50 – 300 mg m^{-2})	Bifenthrin (50 mg m^{-2})	Carbosulfan (300 mg/m^2)
M'bé	*A. gambiae*	Number caught	1763 [a]	846 [b]	613 [b]	629 [b]	511 [b]
Yao	*A. gambiae*	(after 12 h)	347 [a]	106 [b]	121 [b]	103 [b]	106 [b]
	C. quinquefasciatus		305 [a]	248 [a]	236 [a]	254 [a]	250 [a]
M'bé	*A. gambiae*	% deterrency	–	52.0 [1]	65.5 [1]	64.3 [1]	71.0 [1]
Yao	*A. gambiae*		–	69.4 [1]	66.1 [1]	70.3 [1]	69.4 [1]
	C. quinquefasciatus		–	NS [2]	NS [2]	NS [2]	NS [2]
M'bé	*A. gambiae*	% observed exophily	39.5 (696) [a,1]	56.1 (475) [b,1]	38.3 (238) [a,1]	52.6 (331) [b,1]	43.0 (220) [a,1]
Yao	*A. gambiae*		21.9 (76) [a,2]	47.2 (50) [b,2]	50.4 (61) [b,2]	40.8 (42) [b,2]	40.6 (43) [b,1]
	C. quinquefasciatus		36.4 (111) [a,1]	44.8 (111) [a,2]	36.0 (85) [a,1]	39.8 (101) [a,2]	43.6 (109) [a,1]
M'bé	*A. gambiae*	% induced exophily	–	27.5	NS	21.7	NS
Yao	*A. gambiae*		–	32.3	36.5	24.2	23.9
	C. quinquefasciatus		–	NS	NS	NS	NS
M'bé	*A. gambiae*	% blood feeding	14.0 (247) [a,1]	7.6 (64) [a,1]	4.7 (29) [b,1]	8.7 (55) [a,1]	8.4 (43) [a,1]
Yao	*A. gambiae*		43.2 (150) [a,2]	24.5 (26) [a,b,3]	23.1 (28) [a,b,2]	15.5 (16) [b,1]	12.3 (13) [b,1]
	C. quinquefasciatus		5.6 (17) [a,1]	2.4 (6) [b,2]	3.4 (8) [a,1]	3.5 (9) [a,2]	0.8 (2) [c,2]
M'bé	*A. gambiae*	% blood feed. inhibition	–	NS	66.2	NS	NS
Yao	*A. gambiae*		–	NS	NS	64.1	71.6
	C. quinquefasciatus		–	57.1	NS	NS	85.6
M'bé	*A. gambiae*	% overall mortality	7.0 (124) [a,1]	68.0 (575) [b,1]	72.3 (443) [b,1]	63.1 (397) [c,1]	77.5 (396) [b,1]
Yao	*A. gambiae*	(after 36 h)	6.3 (22) [a,1]	71.7 (76) [b,1]	67.8 (82) [c,1]	79.6 (82) [b,2]	85.5 (91) [b,1]
	C. quinquefasciatus		9.8 (30) [a,1]	82.3 (204) [b,1]	83.0 (196) [b,1]	87.4 (222) [b,2]	89.6 (224) [b,1]
M'bé	*A. gambiae*	% corrected mortality	–	65.5	70.2	60.3	75.8
Yao	*A. gambiae*		–	69.8	65.6	78.2	84.9
	C. quinquefasciatus		–	80.3	81.2	86.0	88.5

[a,b,c] In each row, values not sharing a superscript letter are significantly different at the 5% level.
[1,2,3] In each column, for a given parameter, values not sharing a superscript number are significantly different at the 5% level.
Number of mosquitoes in parentheses. NS, no significant difference at the 5% level from results with the untreated hut.

treatments at M'bé. The level of exophily of *C. quinquefasciatus* observed in the treatments was not significantly different from natural exophily observed in the control.

The rate of blood feeding by *A. gambiae* at M'bé was less than 9% in all treatments, compared with 14% in the control. However, the apparent treatment-induced reduction was only significant for the mosaic (66%). The blood feeding rate was higher at Yaokoffikro (12–25%) but the unusually high blood feeding rate in the control (43%) should be noted. The lowest blood feeding rate was recorded at Yaokoffikro with *C. quinquefasciatus* (0.8–3.5%). These rates were not significantly different from the control rate, except with carbosulfan and, to a lesser extent, the mixture (respectively 2.4 and 0.8% feeding which correspond to 85.6 and 57.1% inhibition, respectively).

The treatment-induced mortality (corrected for control) was consistently high and of the same order of magnitude, confirming the efficacy of the nets in controlling mosquitoes. Few significant differences were observed between treatments, except with bifenthrin and the mosaic which proved to be less effective against *Anopheles* at M'bé and Yaokoffikro, respectively. Conversely, bifenthrin was more effective at Yaokoffikro than at M'bé. Delayed mortality was negligible for all treatments (3.5–6.4%) and thus represented a minor component of overall mortality.

Discussion

Mosquito susceptibility

The relative susceptibility of the wild strain of *A. gambiae* from M'bé to bifenthrin is explained by the frequency of the *kdr* gene which occurred at only 4% in this population but, at Yaokoffikro, exceeded 96% (Chandre *et al.*, 1999b). Nevertheless, as shown in table 3, there was very little difference in the performance of nets treated with bifenthrin against *A. gambiae* between these two locations. Insensitive acethylcholinesterase was present in both wild populations of *A. gambiae* but its level seemed to be higher in the population from Yaokoffikro than from M'Bé (N'Guessan *et al.*, 2003). However, as already reported by Kolaczinski *et al*. (2000) the carbamate carbosulfan was extremely effective in killing *A. gambiae* at Yaokoffikro. At Yaokoffikro, the high resistance of *C. quinquefasciatus* to bifenthrin confirms the findings of Chandre *et al*. (1998) who showed the involvement of P450-dependent oxidases and *kdr* mechanisms in this species. The relative susceptibility of these mosquitoes to carbosulfan accords with Chandre *et al*. (1997) who reported an allelic frequency of only 6.25 % for insensitive acethylcholinesterase. Despite the evidence for pyrethroid resistance in the huts, 87–90% mortality of *C. quinquefasciatus* was recorded with bifenthrin as well as with carbosulfan.

Insecticide persistence

The high level of efficacy of bifenthrin against these mosquitoes after six months agrees with the high persistence shown by other types of pyrethroid on polyester nets unless they are washed many times (Njunwa *et al.*, 1991; Curtis *et al.*, 1996). The comparable results obtained with carbosulfan accords with those obtained with house spraying which showed a persistence equivalent to that of pyrethroids (Darriet, 1998). Mixing the two insecticides did not appear to decrease insecticidal persistence, thus indicating an acceptable degree of compatibility between the SC and CS formulations.

Efficacy of nets

The deterrent effect recorded against the susceptible populations of *A. gambiae* from M'bé confirms the findings of Miller *et al.* (1991) and Darriet *et al.* (2000). No deterrency was noted in our experiments with the resistant *C. quinquefasciatus*, which is known to be much less responsive to the repellent effect of pyrethroids (Guillet *et al.*, 2001). The deterrent effect of pyrethroids recorded on the resistant population of *A. gambiae* was, on the other hand, less marked than recorded by some authors, but was higher than 40% (Darriet *et al.*, 2001; N'Guessan *et al.*, 2001). Carbosulfan induced an excito-repellent effect in *A. gambiae* at least as great as that of pyrethroids, which confirms the observations made by Darriet *et al.* (1998) with house spraying. The frequency of insensitive acethylcholinesterase therefore does not appear to affect deterrency or any of the other parameters measured in the huts at these sites.

The percentages of natural exophily observed in *A. gambiae* from M'bé and *C. quinquefasciatus* at Yaokoffikro were of the same order of magnitude as those observed by other authors under the same experimental conditions (Guillet *et al.*, 2001; N'Guessan *et al.*, 2001). On the other hand, the unusually low level of natural exophily observed *A. gambiae* from Yaokoffikro did not allow us to interpret the differences of exophily induced by each treatment. Carbosulfan did not induce exophily in *A. gambiae* from M'bé, as was the case with house spraying (in Darriet, 1998). It is also possible that the presence of carbosulfan on the net in the mosaic inhibited induced exophily. This phenomenon was not observed with the mixture, perhaps because of the low dose of carbosulfan present in the mixture. The absence of induced exophily noted in *C. quinquefasciatus*, even with bifenthrin alone, is probably related to the high resistance of this strain to pyrethroids.

The rate of inhibition of blood feeding obtained with carbosulfan on *C. quinquefasciatus* confirms the results of Kolaczinski *et al.* (2000) who obtained a higher rate of inhibition with this compound, compared to an organophosphate or two pyrethroids. These authors also obtained similar results to ours with the anopheline population from Yaokoffikro.

Generally, the good results obtained with carbosulfan and bifenthrin confirm those obtained by other authors, both in the laboratory and in the field (Curtis *et al.*, 1999; Kolaczinski *et al.*, 2000; Guillet *et al.*, 2001; Hougard *et al.*, 2002). This reinforces the validity of the choice of these two compounds as 'pilot' insecticides to evaluate the effectiveness of mosquito nets impregnated with a mixture as compared with a mosaic or singly impregnated intact nets. The fact that the mixture and the mosaic were as effective as in earlier studies with deltamethrin-treated nets (Darriet *et al.*, 2000; Guillet *et al.*, 2001) is encouraging, but in the present trial the mixture and mosaic did not differ in effectiveness from bifenthrin or carbosulfan treated nets. Moreover, the doubly-treated mosquito nets may possibly be a potential tool for the implementation of resistance management, as they combine different classes of insecticides.

In the absence of data on the effect of 25 mg m^{-2} of bifenthrin alone, the fact that a mixture has any real advantage over a lowered dose of bifenthrin without any admixture of carbosulfan has yet to be confirmed. If additional hut studies confirm a synergistic effect, as already described under laboratory conditions by Corbel *et al.* (2002), this could confer an advantage in terms of cost. There may also be an advantage in reducing human toxicity (WHO, 1986), provided that a synergistic effect on human toxicity does not occur (a warning that this might occur was given some years ago by safety advisers to the World Health Organization). Morever, as carbosulfan degrades to a more toxic metabolite (carbofuran), the use of a less toxic compound remains preferable and studies are under way with chlorpyrifos-methyl, an organophosphorous insecticide with low toxicity for mammals (acute oral toxicity to rats ranging between 1630 and 2140 mg kg^{-1}). Preliminary tests carried out in the laboratory against adult *A. gambiae* (susceptible strain) have shown that it is active at relatively low dosages (close to those of pyrethroids) and that, between bifenthrin and this compound, there is a level of potentiation at least as high as that with carbosulfan (Darriet, *et al.*, 2003). The use of mixtures and mosaics of compounds from different chemical classes with different modes of action and effects therefore offer great promise as a practical means of controlling multiresistant strains of mosquitoes.

Acknowledgements

This study was financially supported by the programme of the Ministère Français de la Recherche on malaria and associated communicable diseases for developing countries (PAL^+ programme) and the French Institut de Recherche pour le Développement (IRD). The authors thank FMC Corporation for providing the formulations of bifenthrin and carbosulfan and Vestergaard Frandsen A/S for the mosquito nets. The authors are grateful to Mark Rowland for reading the manuscript and Luc Finot for his useful contribution in the impregnation process.

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(Accepted 13 June 2003)