Medical and Veterinary Entomology (1998) **12**, 359–366

Pyrethroid resistance in *Culex quinquefasciatus* from West Africa

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Abstract. Pyrethroid resistance was investigated in thirty-three samples of *Culex quinquefasciatus* Say from twenty-five cities in Côte d'Ivoire and Burkina Faso. Permethrin resistance ratios at LC_{50} ranged from 9.5- to 82-fold in Côte d'Ivoire and from 17- to 49-fold in Burkina Faso. For deltamethrin, resistance ratios were lower and ranged from nine to thirty-eight in both countries. A strain was selected with permethrin to investigate resistance mechanisms. After forty-two generations of selection, permethrin resistance level reached 3750-fold, but deltamethrin resistance remained unexpectedly unchanged. This indicated that a specific mechanism was involved in permethrin resistance. Synergist assays and biochemical tests indicated that resistance was partly due to P450-dependent oxidases. A target site insensitivity (*kdr*) was also involved, associated with DDT cross resistance and a dramatic loss of permethrin knockdown effect on adults. This resistance should be taken into consideration when planning the use of pyrethroid-impregnated materials in urban areas, as *Culex* is by far the main source of nuisance. Any failure in nuisance control due to resistance is likely to demotivate people in using impregnated materials.

Key words. *Culex quinquefasciatus*, resistance, pyrethroid, oxidases, knockdown resistance, West Africa, Burkina Faso, Côte d'Ivoire.

Introduction

Culex quinquefasciatus Say (Diptera: Culicidae) is a pantropical pest and urban vector of Bancroftian filariasis. Resistance to organochlorine, cyclodiene, organophosphate and carbamate insecticides is widespread and well documented for populations of this mosquito throughout the world. In contrast, only few data are available on pyrethroid resistance. The first case of pyrethroid resistance in *Cx quinquefasciatus* was obtained in California by laboratory selection of a strain with permethrin and was mainly due to a knockdown resistance (*kdr*) gene (Priester & Georghiou, 1978). *Kdr* is a common mechanism of resistance among pest insects, providing a wide spectrum of cross-resistance to DDT and pyrethroids. As a consequence, it is often stated that some species have developed pyrethroid resistance rapidly because of DDT resistance. During the 1950s and 1960s, DDT was used massively for pest control in agriculture and against public health pest and vectors in urban as well as rural areas. In West Africa, organochlorine resistance in *Cx quinquefasciatus* developed rapidly when large-scale urban control operations were launched in large cities. DDT resistance in *Cx quinquefasciatus* was first recorded in 1958 in Côte d'Ivoire and Burkina Faso (Adam *et al.*, 1958; Hamon *et al.*, 1958) and later on in Mali (Hamon *et al.*, 1961). In 1968, twelve populations of *Cx quinquefasciatus* from seven West African countries were all found to be resistant to dieldrin and most of them also to DDT (Mouchet *et al.*, 1968). Pyrethroid resistance in West Africa was first observed in 1986 in Côte d'Ivoire (Magnin *et al.*, 1988).

We have made a widespread survey of *Cx quinquefasciatus* in West Africa, with the objective of assessing the evolution and spread of insecticide resistance genes in natural populations. Large cities of Côte d'Ivoire and Burkina Faso were surveyed, covering a wide range of biogeographical areas. Samples were collected along transects, mainly the road which runs across

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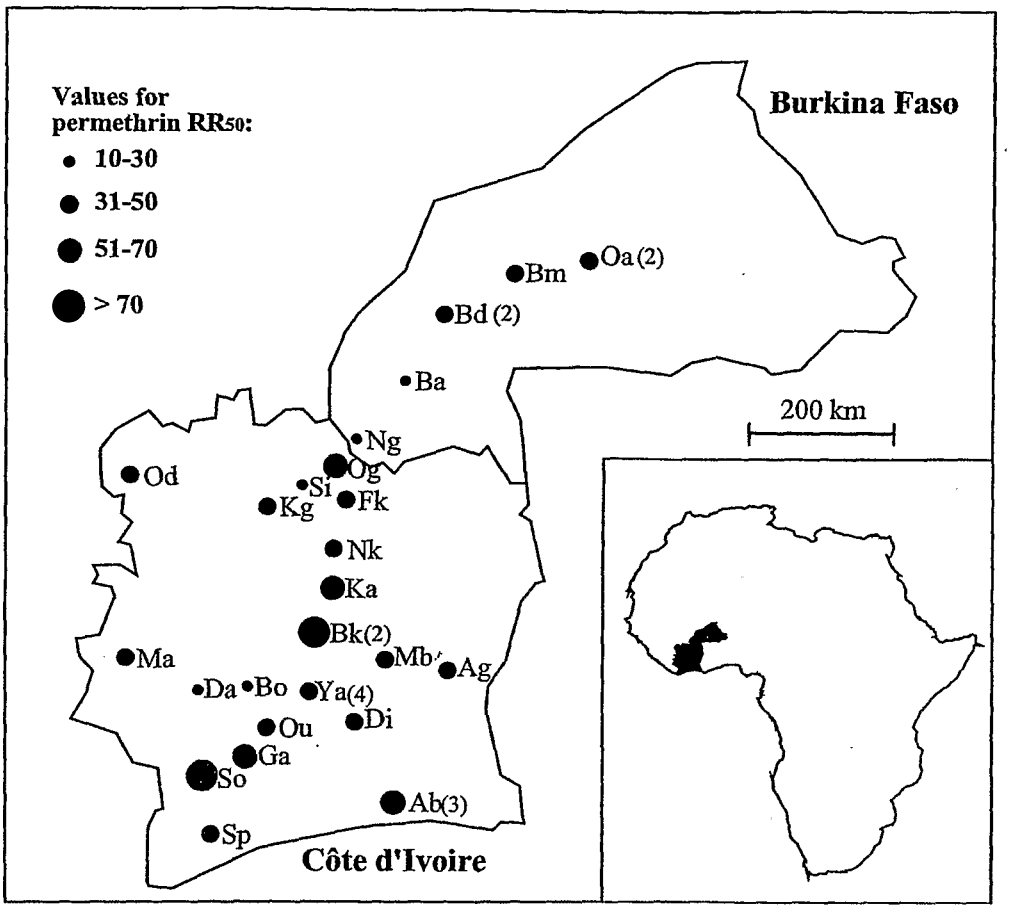

Fig. 1. Locations of the twenty-five towns surveyed for *Cx quinquefasciatus* and permethrin resistance ratios (RR_{50}) observed. Numbers of multiple samples are indicated in brackets. Abbreviations: Côte d'Ivoire: Ab, Abidjan; Ag, Abengourou; Bk, Bouaké; Bo, Bouaflé; Da, Daloa; Di, Dimbokro; Fk, Ferkessédougou; Ga, Gagnoa; Ka, Katiola; Kg, Korhogo; Ma, Man; Mb, M'bahiakro; Nk, Niakaramandougou; Od, Odienné; Og, Ouangolo; Ou, Oumé; Si, Sinématiali; So, Soubré; Sp, San Pédro; Ya, Yamoussoukro; Burkina Faso: Ba, Banfora; Bd, Bobo-Dioulasso; Bm, Boromo; Ng, Niangoloko; Oa, Ouagadougou.

Côte d'Ivoire to Burkina Faso (950 km from Abidjan to Ouagadougou). Field mosquito populations were tested systematically with organophosphates, carbamates and pyrethroids. Data on organophosphate and carbamate resistance were published previously (Chandre *et al.*, 1997) and here we report results about pyrethroid resistance.

Materials and Methods

Mosquitoes

To avoid biased sampling and/or mortality affecting the genetic composition of samples, *Cx quinquefasciatus* were collected as egg rafts in natural breeding sites. Egg rafts were transported to the laboratory on wet cotton in Petri dishes maintained at 4–10°C. Larvae were reared under standard conditions as described by Chandre *et al.* (1997). From March 1994 to June 1995, a total of thirty-three samples were collected in twenty representative cities of Côte d'Ivoire and five of Burkina Faso (Fig. 1). In five of the largest cities, multiple collections were made to compare resistance patterns between districts. Field samples were compared to 'S-Lab', a susceptible reference strain (Georghiou *et al.*, 1966) reared and tested in the same conditions.

Resistance mechanisms were investigated using strain 'Bk-Per' derived from a resistant field sample (Bk1) which was mass selected in the laboratory at every generation. Selections were made by exposing fourth instars to permethrin concentrations, providing 60–90% mortality during 24 h.

Larval bioassays

Two pyrethroids of technical grade quality were used: 93.8% permethrin 25:75, and 99.8% deltamethrin (provided by Agrevo, Berkhamsted, U.K.). Insecticide solutions were made in ethanol 95°C and stored at 4°C for less than 2 months.

Table 1. Log dose-probit mortality data for permethrin of *Cx quinquefasciatus* samples from Côte d'Ivoire and Burkina Faso. Samples are named as in Fig. 1.

Samples	LC_{50}(mg/l)		LC_{95}(mg/l)		RR_{50}
S-Lab	0.0021	(0.0019–0.0022)	0.0054	(0.0047–0.0064)	–
Côte d'Ivoire					
Ab1	0.114	(0.106–0.124)	0.302	(0.258–0.370)	54
Ab2	0.137	(0.126–0.150)	0.413	(0.355–0.498)	65
Ab3	0.122*	(0.083–0.180)	0.336	(0.151–0.752)	58
Ya1	0.071	(0.063–0.080)	0.331	(0.266–0.442)	33
Ya2	0.087	(0.074–0.101)	0.546	(0.411–0.809)	41
Ya3	0.097	(0.084–0.112)	0.675	(0.496–1.031)	46
Ya4	0.073	(0.065–0.083)	0.382	(0.302–0.524)	35
Bk1	0.138	(0.117–0.166)	1.508	(0.928–3.216)	66
Bk2	0.173	(0.154–0.196)	1.033	(0.801–1.426)	82
Ka	0.112	(0.101–0.125)	0.494	(0.405–0.635)	53
Kg	0.082	(0.073–0.092)	0.426	(0.336–0.580)	39
Si	0.020	(0.012–0.028)	0.150	(0.114–0.238)	9.5
Fk	0.082	(0.072–0.093)	0.477	(0.370–0.666)	39
Nk	0.099	(0.086–0.114)	0.777	(0.562–1.208)	47
Og	0.136	(0.118–0.157)	0.886	(0.642–1.377)	65
Bo	0.050	(0.043–0.057)	0.243	(0.197–0.320)	24
Da	0.051	(0.046–0.056)	0.136	(0.119–0.162)	24
Ma	0.075	(0.050–0.110)	0.179	(0.079–0.440)	36
Od	0.065*	(0.046–0.092)	0.215	(0.104–0.450)	31
Ou	0.069	(0.049–0.097)	0.257	(0.122–0.569)	33
Ga	0.113*	(0.076–0.168)	0.724	(0.261–2.111)	54
So	0.149	(0.136–0.165)	0.445	(0.370–0.568)	71
Sp	0.103	(0.091–0.116)	0.537	(0.417–0.753)	49
Mb	0.077	(0.068–0.088)	0.430	(0.337–0.596)	37
Di	0.067	(0.062–0.072)	0.140	(0.123–0.168)	32
Ag	0.085	(0.079–0.092)	0.207	(0.177–0.257)	40
Burkina Faso					
Ng	0.048	(0.044–0.053)	0.133	(0.116–0.158)	23
Ba	0.036*	(0.013–0.101)	0.192	(0.026–1.504)	17
Bd1	0.102	(0.092–0.113)	0.347	(0.277–0.472)	49
Bd2	0.085	(0.076–0.095)	0.366	(0.297–0.483)	41
Bm	0.067*	(0.042–0.105)	0.229	(0.084–0.632)	32
Oa1	0.051	(0.043–0.059)	0.343	(0.228–0.681)	24
Oa2	0.087	(0.076–0.100)	0.442	(0.346–0.609)	41

* Rejection of linearity of log dose-probit mortality response ($P < 0.05$). $LC_{50/95}$ = lethal concentrations and their 95% confidence intervals in brackets. RR_{50} = resistance ratio at LC_{50} (LC_{50} sample tested/LC_{50} of S-Lab).

Bioassays were made with late third and early fourth instars (4 days after hatching). Batches of twenty larvae were assayed in 99 ml of distilled water, adding one ml of insecticide solution at the required concentration. Five replicates per concentration, and five to eight concentrations, providing between 0 and 100% mortality, were used for each bioassay. Mortality was recorded after a 24-h exposure. Temperature was maintained at 27 ± 1°C during bioassays. Controls were made with 1 ml of ethanol and mortality never exceeded 4%.

The effect of two classical synergists, DEF (S,S,S-tributyl phosphorotrithioate; Interchim, Asnières, France), an inhibitor of esterases and glutathione-S-transferases, and PBO (piperonyl butoxide; Fluka, St-Quentin, France), an inhibitor of oxidases, was tested on the permethrin-resistant strain 'Bk-Per' after twenty generations of selection. Concentrations used were 0.008 mg/l for DEF and 1 mg/l for PBO, the maximum sub-lethal concentrations for 'S-Lab'. Larvae were exposed to synergist 4 h prior to insecticide, and maintained for 24 h in insecticide plus synergist solution.

Adult bioassays

Adult bioassays were made with the 'Bk-Per' strain to investigate possible resistance to knockdown effect of pyrethroids. Permethrin-impregnated papers were prepared according to the WHO protocol (1970), using silicone oil (Dow Corning 556) as the carrier. Impregnation was made on the basis of 3.6 mg of permethrin oil solution per cm^2. Four replicates of twenty-five unfed females (3–5 days old) were

Table 2. Log dose-probit mortality data for deltamethrin of *Cx quinquefasciatus* samples from Côte d'Ivoire and Burkina Faso.

Samples	LC_{50}(mg/l)		LC_{95}(mg/l)		RR_{50}
S-Lab	0.00016	(0.00014–0.00017)	0.00045	(0.00039–0.00055)	–
Côte d'Ivoire					
Ab1	0.0059	(0.0054–0.0064)	0.0186	(0.0158–0.0231)	37
Ab2	0.0060	(0.0056–0.0065)	0.0156	(0.0137–0.0185)	38
Ab3	0.0060	(0.0056–0.0064)	0.0125	(0.0111–0.0148)	38
Bk1	0.0054	(0.0049–0.0060)	0.0173	(0.0146–0.0213)	34
Bk2	0.0057	(0.0052–0.0062)	0.0175	(0.0150–0.0215)	36
Ka	0.0047	(0.0043–0.0051)	0.0113	(0.0099–0.0135)	29
Kg	0.0044	(0.0040–0.0049)	0.0177	(0.0147–0.0224)	28
Bo	0.0037	(0.0033–0.0041)	0.0107	(0.0092–0.0132)	23
Da	0.0046	(0.0042–0.0051)	0.0139	(0.0117–0.0178)	29
Ma	0.0015	(0.0009–0.0027)	0.0043	(0.0017–00116)	9.4
Od	0.0039	(0.0036–0.0043)	0.0096	(0.0085–0.0113)	24
Ga	0.0028	(0.0025–0.0032)	0.0095	(0.0079–0.0121)	18
Sp	0.0034	(0.0034–0.0039)	0.0083	(0.0073–0.0099)	21
Burkina Faso					
Bd1	0.0029	(0.0026–0.0032)	0.0068	(0.0057–0.0089)	18
Oa1	0.0039	(0.0031–0.0045)	0.0234	(0.0142–0.0556)	28

$LC_{50/95}$ = lethal concentrations and their 95% confidence intervals in brackets. RR_{50} = resistance ratio at LC_{50} (LC_{50} sample tested/LC_{50} of S-Lab).

exposed for 1 h to impregnated papers in WHO test kits maintained in vertical position. The knockdown rate (mosquitoes lying on their side or unable to fly) was checked periodically during insecticide exposure. After exposure, mosquitoes were kept in observation tubes and supplied with 10% honey solution food. Mortality was checked 24 h after exposure. Control tests used adult females exposed to untreated papers and mortality never exceeded 5%.

P450-dependent oxidase assays

Preliminary investigations of P450-dependent monooxygenase activities were carried out with the 'Bk-Per' and 'S-Lab' strains using a microplate assay developed for *Drosophila* (De Sousa *et al.*, 1995). This microfluorimetric method measured the ethoxycoumarin-deethylase (Ecod) activity in single individuals. Adult mosquitoes were anaesthetized using carbon dioxide and killed by separating head, thorax and abdomen. For each mosquito, the body parts were incubated together in a well of a microtitre plate containing 100 µl of phosphate buffer (pH = 7.2) with 7-ethoxycoumarin 0.04 mM. After 4 h at 30°C, production of 7-hydroxycoumarin was stopped by adding 100 µl of glycine-ethanol buffer (pH = 10.4). Microtitre plates were read on a Dynatech fluorimeter (Fluorolite 1000) fitted with 390 nm excitation and 450 nm emission filters. Fluorescence values were adjusted to a standard curve for 7-hydroxycoumarin, to evaluate the amount of this metabolite produced by each individual. Comparisons were based on the means of 7-hydroxycoumarin in picograms produced by mn and mg of mosquito.

Data analysis

Mortality and knockdown data were analysed using a log-probit software (Raymond *et al.*, 1993), based on Finney (1971). Samples were considered as having the same susceptibility when parallelism of their probit lines was not rejected ($P > 0.05$) and the ratio did not differ significantly from the value 1. As multiple simultaneous tests were made, levels of significance of each test were adjusted to take others into account (Rice, 1989).

Results

Pyrethroid resistance in field samples

All samples of *Cx quinquefasciatus* collected in Côte d'Ivoire and Burkina Faso were resistant to permethrin (Table 1, Fig. 1). In Côte d'Ivoire, resistance ratios at LC_{50} (RR_{50}) ranged from 9.5- to 82-fold, but most (21/26) were within the narrower range from 31- to 66-fold, indicating homogeneous levels of resistance among samples. In Burkina Faso, permethrin RR_{50} ranged from 17 to 49. The samples were also resistant to deltamethrin (Table 2), but to a lower extent (RR_{50} from 9.4 to 38). All dose–mortality curves (except five with permethrin) were well fitted by a straight line ($P > 0.05$), suggesting that samples were usually homogeneous for permethrin and deltamethrin resistance.

Multiple samples were tested from three cities of Côte d'Ivoire (Ab, Bk, Ya) and two cities of Burkina Faso (Bd, Oa). For *Cx quinquefasciatus* from each city, permethrin probit lines were parallel ($P > 0.8$) and the ratios of lethal concentrations between districts (after slope correction) were not significantly different from 1, except for Oa. This indicates that there was no significant difference in permethrin resistance level within cities. A similar observation was made in Bk and Ab with deltamethrin.

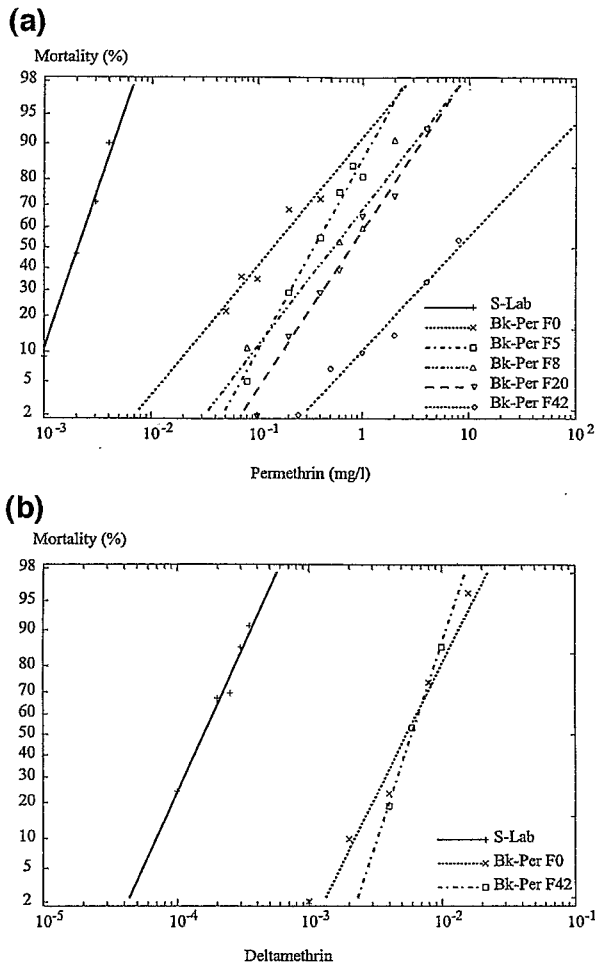

Fig. 2. Evolution of (a) permethrin and (b) deltamethrin resistance of the 'Bk-Per' strain of *Cx quinquefasciatus* through permethrin selection. 'S-Lab': susceptible reference strain.

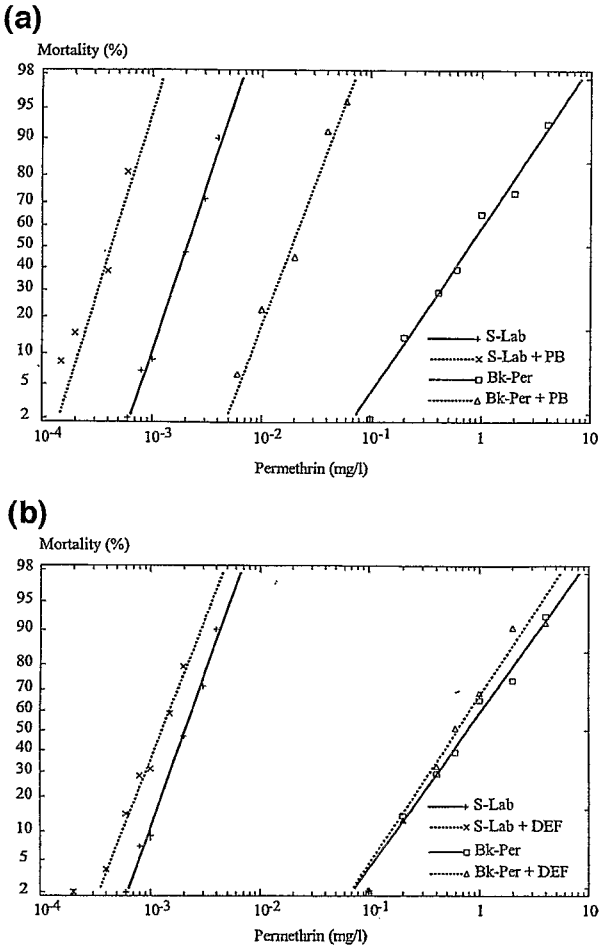

Fig. 3. Synergist modification of concentration–mortality lines with the susceptible reference strain 'S-Lab' and the resistant strain 'Bk-Per' of *Cx quinquefasciatus* in the presence of (a) PBO and (b) DEF.

Characterization of the resistance mechanisms in the 'Bk-Per' strain

The initial LC_{50} of 'Bk-Per' strain (sample Bk1) was 0.138 mg/l with permethrin (RR_{50} = 66). Selection with permethrin gradually increased the resistance level to 370-fold and 3750-fold after twenty and forty-two generations, respectively (Fig. 2a). During selection, log dose–probit mortality responses were statistically fitted by straight lines ($P > 0.05$), indicating that resistance may be polyfactorial.

The initial deltamethrin LC_{50} of 'Bk-Per' was 0.0054 mg/l (RR_{50} = 34). After forty-two generations of permethrin selection, surprisingly, the deltamethrin resistance level (LC_{50} = 0.0059 mg/l) had not changed significantly (Fig. 2b). Pyrethroid resistance in 'Bk-Per' was associated with a high level of DDT resistance in larvae of the twentieth generation ($RR_{50} > 100$, data not shown).

Synergism studies were made with the twentieth generation of 'Bk-Per'. The use of PBO significantly decreased tolerance to permethrin in both strains 'S-Lab' and 'Bk-Per' (Fig. 3a). The higher decrease of permethrin LC_{50} for 'Bk-Per' (41-fold) than for 'S-Lab' (fivefold), indicated an increase of oxidative metabolism. However, resistance was incompletely synergised and the RR_{50} remained high (39-fold) when using PBO. The use of DEF did not change the toxicity of permethrin for strain 'Bk-Per', indicating that esterases were not involved (Fig. 3b).

P450-dependent oxidase assays

Preliminary investigations of Ecod activities were carried out with about fifty males and thirty females of the two strains (Table 3). The amount of 7-hydroxycoumarin produced by male and female mosquitoes was significantly higher in 'Bk-Per' than in 'S-Lab' (ANOVA, $P < 0.01$). For each strain, Ecod activity in females was also significantly higher than in males (ANOVA, $P < 0.05$).

Adult bioassays

The high resistance level observed in larvae of the 'Bk-Per' strain was associated with decreased mortality of adults exposed

to permethrin impregnated papers (Table 4). After 1 h of exposure to the WHO diagnostic concentration for permethrin (0.25%), the mortality of adults was 61% for 'S-Lab' vs. 3% for 'Bk-Per'. With a dose tenfold higher (2.5%), the mortality reached 98% in 'S-Lab' but remained low in 'Bk-Per' (13%). Percentages of mosquitoes knocked down by different exposure times were analysed with log-probit units to estimate the Kd_{50} and Kd_{95} times (Table 4). Data were well fitted by a straight line ($P > 0.05$). At 0.25%, there was no knockdown of the 'Bk-Per' strain, whereas the time of Kd_{50} for 'S-Lab' was 39 min. At higher dosage (2.5%), Kd_{50} time was tenfold longer in 'Bk-Per' than in 'S-Lab'.

Discussion

High levels of pyrethroid resistance were observed in all thirty-three samples of *Cx quinquefasciatus* collected from Côte d'Ivoire and Burkina Faso, suggesting that they had been submitted to strong insecticide selection pressure under field conditions. Attempts made to correlate resistance levels and areas where pyrethroids are widely used in agriculture were not conclusive. Because no organized control campaign against *Culex* based on pyrethroids has been undertaken so far in both countries, it is likely that selection resulted mainly from the domestic use of pyrethrins and pyrethroids in aerosols, coils and flit sprays. This selection pressure is likely to be fairly homogeneous in different districts within cities, which might explain why resistance levels were quite similar. The similar conclusion was reached for the same cities in a study of organophosphate and carbamate resistance (Chandre *et al.*, 1997).

Table 3. ECOD activities of adults of *Cx quinquefasciatus* for a susceptible reference strain (S-Lab) and a resistant strain (Bk-Per).

Strain	Sex	7-hydroxycoumarin (pg/min/mg) ± CI_{95}	*n*
S-Lab	Female	37.2 ± 6.5	31
	Male	15.9 ± 2.8	52
Bk-Per	Female	59.9 ± 12.9	29
	Male	40.3 ± 6.4	55

CI_{95} = 95% confidence intervals. *n* = number of mosquitoes assayed.

Already in 1988, a sample of *Cx quinquefasciatus* collected in Bouaké (i.e. the Bk samples of the present study) showed cross-resistance between deltamethrin and DDT (Magnin *et al.*, 1988). That population was heterogeneous for deltamethrin resistance with a RR_{50} of 14-fold. Those authors showed that resistance was due to cytochrome P450 oxidases, then named mixed function oxidases, and to an unsynergised component, probably a target site mutation. Since 1988, the deltamethrin resistance level has more than doubled to $RR_{50} \approx 35$ and populations appeared homogeneous, indicating an increase in the frequency of resistance genes. The role of P450 oxidases was confirmed by bioassays in presence of PBO and by measuring the Ecod activity.

The incomplete synergism of PBO and the absence of DEF effect on larval mortality indicated the possible involvement of a non-detoxicative component, such as target site insensitivity. This was investigated by studying resistance to the knockdown effect of permethrin on unfed female adult *Cx quinquefasciatus*. The use of knockdown time for resistance detection (Privora, 1975) is a good indicator of resistance in mosquitoes using WHO test cones (Kang *et al.*, 1995) or WHO test tubes (Elissa *et al.*, 1993; Chandre *et al.*, in press). The complete loss of knockdown from 0.25% permethrin exposure and the increase of knockdown times with 2.5% permethrin indicated the presence of a *kdr*-type mechanism in the 'Bk-Per' strain, consistent with the high level of DDT resistance in larvae (data not shown).

In several insects, *kdr* is associated with a single mutation on the sodium channel sequence coding for the target site of pyrethroids and DDT, e.g. the housefly *Musca domestica* (Williamson *et al.*, 1996), the cockroach *Blatella germanica* (Miyazaki *et al.*, 1996) and *Anopheles gambiae*, the main malaria vector in Africa (Martinez-Torres *et al.*, 1997). A preliminary investigation of *Cx quinquefasciatus* indicates that the same mutation is also present in the resistant 'Bk-Per' strain (Martinez-Torres, pers. comm.).

Usually, when a resistant strain is selected with an insecticide, the resistance extends to other compounds of the same class of insecticides. For *Cx quinquefasciatus* this was observed with pyrethroids in a Californian strain (Priester & Georghiou, 1980; Halliday & Georghiou, 1985). Unexpectedly, permethrin selection of our 'Bk-Per' strain did not affect its resistance level to deltamethrin, suggesting that permethrin resistance was partly governed by a specific factor. This is an unusual

Table 4. Comparison of knockdown time and mortality for permethrin with a susceptible reference strain (S-Lab) and a resistant strain (Bk-Per) of *Cx quinquefasciatus*.

	KDT_{50}	CI_{95}	KDT_{95}	CI_{95}	Mortality (%)
Permethrin 0.25%					
S-Lab	38.7	35.7–41.8	88.1	77.1–105.3	61
Bk-Per	No kd	–	No kd	–	3
Permethrin 2.5%					
S-Lab	10.6	9.8–10.7	24.5	23.1–26.3	98
Bk-Per	108.7	97.1–127.8	287.2	220.2–426.8	13

$KDT_{50/95}$ = knockdown time in minutes for 50 or 95% of adult mosquitoes after one hour of exposure to permethrin-impregnated papers in WHO test kits. CI_{95} = 95% confidence intervals.

phenomenon. In one case, decreased sensitivity of the central nervous system to permethrin, but not to cypermethrin, was observed in a resistant strain of *Spodoptera littoralis* (Gammon, 1980). A strain of *Cx quinquefasciatus* from Saudi Arabia was found to be resistant to DDT and permethrin, but electrophysiological testing for nerve insensitivity using lambda-cyhalothrin revealed no evidence of *kdr* (Amin & Hemingway, 1989). Further investigations on this strain confirmed the presence of a target site insensitivity which was selective to some but not all pyrethroids (Hemingway, pers. comm.). Further studies are needed to identify which mechanism, oxidases or *kdr*, provided specific resistance in 'Bk-Per' strain.

Although the domestic use of pyrethroids for pest control may be considered as the most likely cause of the resistance in *Cx quinquefasciatus* observed in this study, it may also have originated partly from DDT selection pressure during past decades. As was DDT resistance (Mouchet *et al.*, 1968), pyrethroid resistance is probably widely distributed throughout West Africa. This resistance should be taken into consideration when planning the use of pyrethroid-impregnated materials for malaria vector control in urban areas. Impregnated bednets are an important new tool recommended by the WHO for malaria prevention but, to be efficient, the active participation of people at community level is required. In urban areas, without a significant impact on mosquito nuisance caused essentially by *Cx quinquefasciatus*, there is a risk that bednet programmes will be disregarded by the local people. In this regard, we think it necessary to evaluate the impact of *Cx quinquefasciatus* resistance on the efficacy of impregnated materials. Nuisance mosquito control by impregnated bednets may not have the intended efficacy because of pyrethroid resistance, in addition to the innate tolerance of *Culex* when exposed to residual insecticide deposits (Brown & Pal, 1973), possibly due to the special empodium beneath *Culex* tarsi. Therefore, it may be wise to supplement the implementation of bednet programmes by *Culex* control measures based on larviciding and sanitation in urban areas.

Acknowledgements

We thank Agrevo for providing technical insecticides. We are grateful to F. Fumoux (Director of Centre Muraz, Bobo-Dioulasso) for his helpful support in Burkina Faso, R. N'guessan and B. Salou for mosquito rearing and technical assistance, S. Ouedraogo, A. Cisse, B. Koffi, A. Konate, and N. Chandre for technical assistance, and all staff members from IPR and ORSTOM in Bouaké. This study was supported, in part, by OCCGE, ORSTOM, and MESR (Grant no. 92-058; ACC-SV3 no. 95.03.037).

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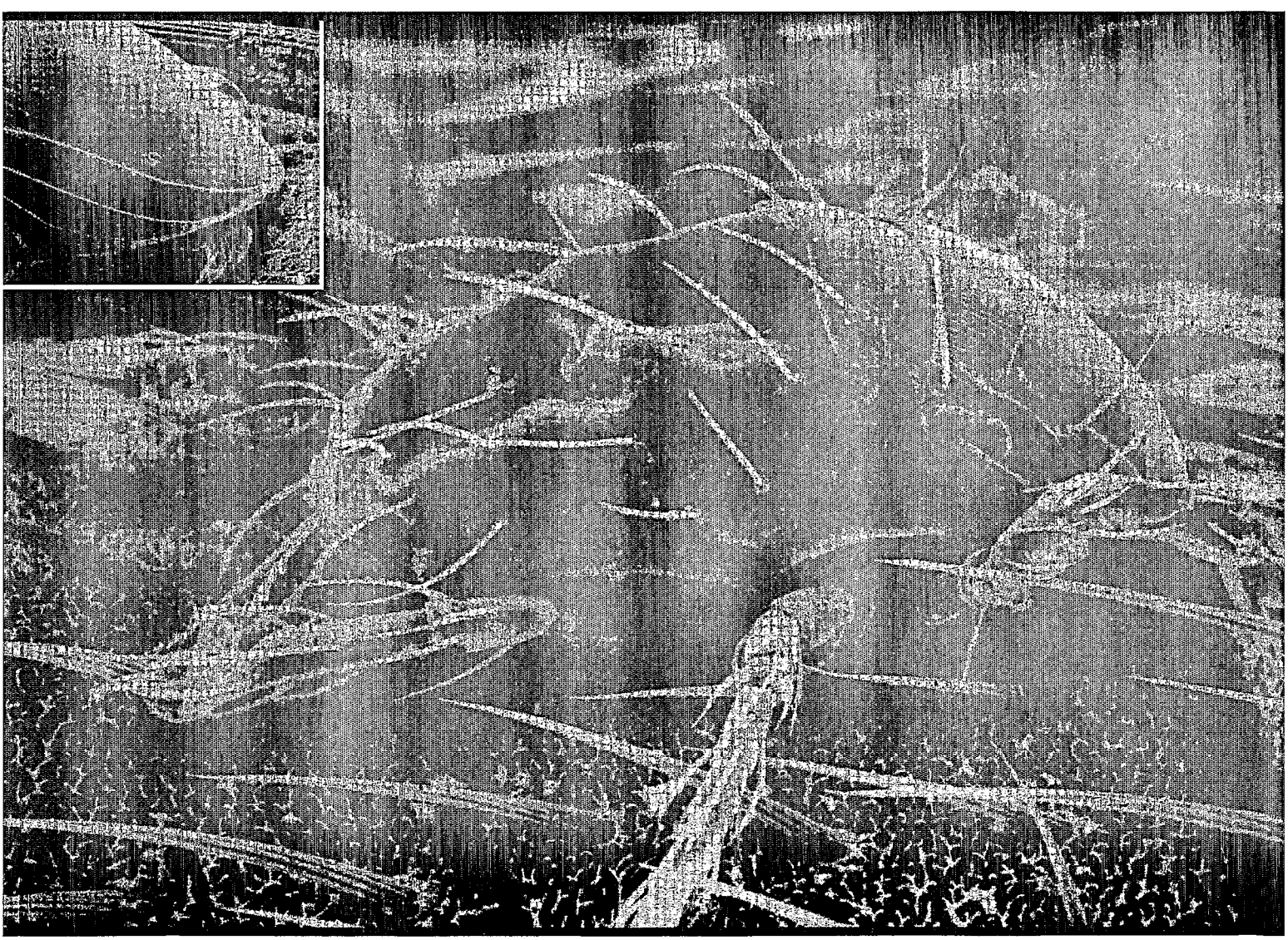
Accepted 26 April 1998

Volume 12 • Number 4 • October 1998 CODEN MVENE4 ISSN 0269-283X

Medical and Veterinary Entomology

Editors: G. B. White (Medical) and A. R. Walker (Veterinary)



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