RESEARCH ARTICLE

Resistance of *Culex quinquefasciatus* to selected chemical and biological pesticides.

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Abstract.

Resistance of 3 organophosphorous insecticides (malathion, temephos, and chlorpyrifos), one carbamate (propoxur), and one pyrethroid (permethrin and permethrin + piperonyl butoxid), DDT and two microbial insecticides: *Bacillus thuringiensis* and *B. sphaericus* was investigated on *Culex quinquefasciatus* in Ougadougou, Burkina Faso, in 1996-97. The results showed that there was little or no resistance to the bio-larvicides, to the OP-compounds and the carbamate, but significant resistance to the pyrethroid and very high resistance to DDT. OP compounds were used in the 70s in less extensive campaigns, whereas DDT was used in Ouagadougou in the malaria campaign in the 60s. The data provide background for a follow-up on resistance measurements in *C. quinquefasciatus* that like *Anopheles gambiae* bites humans by night and therefore are expected to be impacted by and impact the LLIN campaigns realized in the town since 2004.

Keywords: *C. quinquefasciatus*, resistance, pyrethroids, DDT, Bacillus.

Introduction

Culex quinquefasciatus (Say) (Diptera, Culicidae), is the dominating mosquito species in many tropical towns, including towns in West Africa. Contrary to what is found in East Africa, *C. quinquefasciatus* is not a vector of filariasis in West Africa, but the major nuisance problem in urban environments. In other tropical regions, it is a vector of Japanese encephalitis and West Nile virus that have pigs and birds, respectively as target hosts. It breeds in organic rich water as septic tanks, open sewage drains and blocked drain channels along roads. Several studies have shown that bed nets are used when nuisance of mosquitoes are at high levels by night and this is assured in urban environment by this species and not by the much less common malaria transmitting *Anopheles* species or the day biting *Aedes* species (1, 2). In most tropical towns, there are no national programs that aim to control *C.*

quinquefasciatus, but since it bites by night and rests indoors, it will also be exposed to insecticides that target the malaria mosquitoes like Long Lasting Insecticidal Nets (LLIN) and Intradomicile Residual Spraying (IRS). In Ouagadougou, small companies exist that provide IRS applications to individual houses as paid service and these target *C. quinquefasciatus* since this is the species bothering most people. We observed that these sprayings mostly contained malathion, temephos or permethrin. Individual households spent an important part of their income on aerosol cans and smoking spirals for mosquito control (3, 4, 2004). In the 70s, the town provided mass campaigns for a short period with these two Organo-Phosphorous (OP)s. In the WHO global campaign against malaria in the 60s, DDT was used for IRS at large scale guided by the local WHO office. In other countries, *Culex* is targeted with larvicides. Most larvicides are fast deteriorated in the organic and soap rich water in the sewage systems, but the synthetic chlorpyrifos and the bacteria *Bacillus sphaericus* are known to provide control for days or weeks (5). In this study, we tested the susceptibility of larvae and adults of *C. quinquefasciatus* to the insecticides that was used before the current WHO guided campaign against malaria in tropical countries including Burkina Faso. Since *C. quinquefasciatus* also bites indoors and by night, it will be exposed to the insecticides used on bednets. We interviewed people and found that they could not distinguish between mosquito species, but thought that blood fed, unfed or blood fed with digested blood were different species, where indeed they were all *C. quinquefasciatus*, the species that consisted 98 % of all mosquitoes caught by light traps or on humans (6). Since people evaluate bed net efficacy from the biting they are exposed to, lack of control of *Culex* will be experienced as bed net failure and possible discard even it may still be effective against the much fewer *A. gambiae*. The resistance levels of *C. quinquefasciatus* are thus becoming a parameter for the use rate of LLIN

Methods

Eggs of *C. quinquefasciatus* were collected in open cesspits in the older part of the town of Ouagadougou on two occasions in October 1996 and October 1997, giving the strains named below as Ouaga1 and Ouaga2, each based on more than 2000 larvae. Larvae and adults from F_0 to F_3 were tested for susceptibility to the following insecticides, technical grades: Malathion (Cheminova), Temephos (Agro-evos), Propoxur (Bayer), Chlorpyrifos (Sigma), Permethrin (Agroevos), and Permethrin + piperonyl butoxide (Sigma), *Bacillus thuringiensis israelensis* (the standard IPS82, Institut Pasteur) and *B. sphaericus* (the standard SPH84, Institut Pasteur). The susceptible reference strain *C. quinquefasciatus* S-Lab originating from Prof. Georghiou, University of Riverside, California, was provided by Dr. N. Pasteur, University of Montpellier II, France.

Three cups with 100 ml water and 20 early 4rth instars were used per concentration of pesticide. Six to eight dilutions were used per pesticide. The dilution rate was adapted in a pre-test to obtain that at least 5 concentrations gave mortality between 5 and 95 % mortality (in absence of resistance), at least 2 below 50 and 2 above 50 % mortality. Chemical insecticides were first dissolved in alcohol (alternatively in acetone if not possible), then serial

dilutions 1:10 were created in alcohol. From this series, a pre-calculated measure was transferred to the cups with larvae and 99 ml water with a 1000 µl pipette (Gibson) and alcohol was added to make the total addition 1000 µl. Biocides were homogenised and further diluted in water as described by Skovmand et al (7) and 10 ml transferred to cups to make a total of 100 ml. Mortality was read after 24 hr for bioassays with chemicals and *B. thuringiensis israelensis* and 48 hr for those with *B. sphaericus*. 5 repeats were made per serial dilution. The method as used for testing the bio-larvicides is described in details by Skovmand and Becker (7).

Two- to three-day old females were tested in WHO test tubes with exposure to the insecticide impregnated Whatman paper for one hour and reading of the mortality after 24 hours (8, updated in 9). Papers were prepared at the laboratory following the protocol described in the same report, but with a serial dilution of the insecticide. During the exposure hour with Permethrin, knock down rates of mosquitoes were measured at 5 min interval in the period succeeding the first knockdown. KT_{50} and LC_{50} could thus be obtained from the same series of tests. 3 repeats were made for the serial dilutions except for some extreme values.

Resistance ratio was calculated on LC_{50} values based on log-probit analysed data (10). When data indicated a resistance level, as seen on the graphic presentation as a plateau at successive pesticide concentrations, the transformations suggested by Finney (ibid) were used to calculate the log-probit line. Slope of dose mortality curves obtained from the Culex Ouaga strains and S-lab were compared for parallelism. For statistical reasons, where curves are not parallel, the dose responses cannot be compared and a resistance ratio cannot be calculated (10, 11). Nevertheless, there is a tradition in insecticide resistance studies to calculate ratios anyhow. In this work, ratios are calculated in all cases, but marked in Table 1, when the dose response curves were not parallel.

Results

Larval tests showed that the susceptibility of the Ouaga test strains toward the OPs (malathion, chlorpyrifos and temephos) and the carbamate (propoxur) were only slightly lower or not significant different from that of the reference strain, S-Lab (Table 1). Contrary to that, the susceptibility to DDT was 1405 lower in the Ouaga strains, making it impossible to kill the larvae with the amount of DDT that can be dissolved in water. The larvae literally swim between recrystallized DDT. The susceptibility to permethrin was in the two years 22 and 17 times lower than for the Ouaga strains than for S-lab. A student t-test shows that the susceptibility levels for the two years was not different ($P>0.10$), mean resistance level is thus 20 times. Further, when piperonyl butoxide was added, the resistance ratio was reduced only slightly, indicating that the mixed function oxidases (also called cythochrome or mono-oxidases) did not play an important role in this resistance. Test with the biocide standards for *B. thuringiensis israelensis* and *B. sphaericus* showed that the Ouaga strains were as susceptible as S-lab (Table 1).

Table 1. Susceptibility and resistance of *Culex quinquefasciatus* larvae to various insecticides.

Pesticide/Strain		Ouaga		S-lab		R/S
DDT	LC_{50}	2.67	± 0.16	0.0019	±0.007	1,405*
Ouaga1, F_{1-2}	slope	2.60	± 0.11	5.23	±1.88	
Permethrin	LC_{50}	0.041	± 0.012	0.0019	±0.0005	22*
Ouaga1, F_{1-2}	slope	3.28	± 0.53	5.75	±2.72	
Ouaga2, F_{1-2}	LC_{50}	0.033	± 0.009			17*
	slope	2.91	± 1.10			
Permethrin + Piperonylbutoxide	LC_{50}	0.0022		0.0002	±0.00013	15*
Ouaga2,F_2	slope	1.67		1.76	±0.77	
Chlorpyrifos,	LC_{50}	0.0056	± 0.0026	0.0015	± 0.0008	4
Ouaga1, F_{1-2}	slope	3.34	± 1.37	5.57	±2.33	
Malathion	LC_{50}	0.043	± 0.0015	0.024	±0.004	2
Ouaga1, F_3	slope	5.32	± 0.61	6.45	±1.62	
Temephos,	LC_{50}	0.0011	± 0.0004	0.0012	±0.0003	1
Ouaga1, F_3	slope	6.31	± 2.07	7.14	±0.91	
Ouaga2, F_1	LC_{50}	0.0068	± 0.0001			2
	slope	7.22	± 0.95			
Propoxur	LC_{50}	0.116	± 0.028	0.075	±0.018	2
Ouaga1, F_{1-2}	slope	4.05	± 0.80	4.91	±1.22	
Ouaga2, F_1	LC_{50}	0.389	± 0.205			5
	slope	4.86	± 0.75			
Bt israelensis	LC_{50}	0.0139	± 0.0047	0.0139	± 0.0047	
IPS 82 (24 h)	slope	3.06	± 1.36	3.06	± 1.36	
B. sphaericus	LC_{50}	0.0088	± 0.0025	0.0089	±0.0045	1
SPH 84 (48 h)	slope	2.63	± 1.01	2.31	± 0.65	

The data for Ouaga1 and 2 on the bacterial standards are pooled since they were not different
Resistance ratios marked * are significant different ($P<0.05$).

Test with adult, non-engorged females on permethrin impregnated paper showed that adult females of the Ouaga strain were less susceptible than females of S-Lab (Table 2). Mortality and KT-50 data showed plateaus that may indicate that a single resistance mechanism dominates. Observations of paralysed mosquitoes (i.e. knocked down) showed that at 0.25 % and 0.50 % permethrin, KT_{50} was 37 and 29 min for S-lab. No knock down was obtained at the lower concentration with the Ouaga strain and it was nearly 2 hr between dosages of 0.5 to 2 % showing a plateau.

Table 2. Two measures of susceptibility and resistance of *Culex quinquefasciatus* adults to Permethrin

Permethrin %	Measure	Culex Ouaga	Culex S lab
0,0625	KT50	NK	NK
	Mort 24hr	0	8%
0,1250	KT50	NK	NK
	Mort 24hr	2%	41±18 %
0,2500	KT50	NK	37 min
	Mort 24hr	14±14%	64±7 %
0,5000	KT50	106±22 min	29±4 min
	Mort 24hr	13±10%	92±5 %
1,0000	KT50	120±14 min	20±3 min
	Mort 24hr	21±20%	97±3 %
2,0000	KT50	120±29 min	
	Mort 24hr	43%	
4,0000	KT50	66 min	
LC 50 Mort 24hr		1,06%	0,09%
Slope		1,44	1,07
Resistance factor		11	

NK indicates No Knockdown within the 5 min measurement. 1 % permethrin on impregnated paper corresponds to 360 mg/m².

Plotting the KT_{50} against logarithmic concentrations of Permethrin for Ouaga2 and the reference strain gives two non-parallel curves, indicating that a ratio of resistance is not really measurable. If calculated nevertheless for the KT_{50}, the ratio is 37 and 11 for 24hr mortality (Table 2).

Discussion

The very high level of DDT resistance found with adults and larvae is astonishing since this compound has not been used for more than 20 years in Ouagadougou at the time of our measurements (Staff at the Centre National de Recherche et de

Formation sur le Paludisme in Ouagadougou, pers. comm.). Accordingly, this resistance is either very stable or sustained by the use of pyrethroids since both insecticides target the voltage dependent sodium channel in the neural axons. The permethrin resistance level was 20 times for larvae and 11 to 37 times (measured by mortality or knock-down time) for adults. In the larval test, there was little or no effect of adding piperonyl butoxide. This phenomenon of reduced or delayed knock down is described as the knock-down resistance (KDR: 12). It is more common to-day to measure KDR by identifying some genetic mutations that are involved in this resistance mechanism, but the resistance level can be very different in the presence of these mutations indicating that other genes contribute. Physiological measure of KDR as done here is therefore a better start point for knowing the importance of this resistance mechanism. Whereas this mechanism also provides resistance to DDT, it cannot explain the high level of DDT resistance. Accordingly, that level is probably caused by the previous intensive use of DDT and genetic stability of a specific mechanism as DDTase, however we did not test for this.

The use of pyrethroids and the natural pyrethrum in Ouagadougou was only on private initiatives in the 90ies and included mostly coils, wall spraying on demand arranged privately, aerosol cans and a very low coverage with impregnated bed net (3). Seen in the light of these sporadic control interventions, the resistance ratios of 11 to 36 (for adults and larvae, respectively) against pyrethroids are high. It is assumed that the insecticide resistance found in many *Anopheles* species before the second global malaria campaign is mostly caused by the application of pesticides in irrigated fields in agriculture (13, 14), where the *Anopheles* larvae live. But *C. quinquefasciatus* larvae in Ouagadougou are found in cesspits, septic tanks, and polluted puddles and these are not treated with insecticides (5). The resistance level of 22-36 times to permethrin identified here as including Knock Down resistance and no or little P450 based resistance may indicate a link to the old malaria campaigns with DDT, since DDT and pyrethroids have the same target in the nervous system, the voltage gated sodium channel. *C quinquefasciatus* bites and rest indoors and will thus be exposed to control measures against *Anopheles* mosquitoes.

A later study in the second biggest town in Burkina Faso, Bobo Diaolasso, a few years later and part as follow-up program, showed similar levels of resistance to DDT, OPs and the pyrethroids permethrin and deltamethrin. It further included Fipronil as a larvicide and found no resistance (15). Bobo Dioulasso is in the middle of the cotton growing area and it has been shown that pyrethroid resistance in this area developed much earlier for *Anopheles gambiae* than north of this cotton area where Ouagadougou is situated. This underlines that resistance in *C. quinquefasciatus* is not influenced by agricultural practices for larger crops as *Anopheles* (14).

Guillet *et al.* (16) found that pyrethroid impregnated bednets had low efficacy on *C. quinquefasciatus* in a study area with pyrethroid resistance in this species, whereas the carbamate carbosulfan was effective. This can be explained by our findings with a high resistance to pyrethroids and no resistance to the carbamate. Chandre *et al.* (17) found that knock down resistance for *Anopheles*

gambiae resulted in less irritancy-repellence action, and that the mosquitoes remained for longer periods on a treated net. This is also the case for *C. quinquefasciatus* tested in the same way (Skovmand, unpublished results). Accordingly, *C. quinquefasciatus* is not easily controlled with pyrethroid impregnated bed nets and the addition of piperonylbutoxide seems not to make any difference based on the bioassays of this study. Since people evaluate bed net efficacy from the personal experience of being protected (18), the lack of effect against *C. quinquefasciatus* may lead them to discard use of LLIN that targets *Anopheles* even these are improved by adding piperonyl butoxide. A recent study of spatio-temporal distribution of mosquitoes caught in and out door all over Rwanda showed that between 75 and 80 % of all mosquitoes caught by human landing or pyrethrum sprays were culicine (19). It is likely that the biting behaviour and resistance of these mosquitoes are more important for bed net use rate and attrition than the 20-25 % Anophelines. In Ghana, a resistance patterns like the one we found in Burkina resulted in reduced efficacy against C quinquefasciatus to two LLIN and it was suspected that this may result in reduced bed net use (20).

There was no resistance to the two larval biocides tested, *B. thuringiensis israelensis* and *B. sphaericus*. Accordingly, these may be used at standard rates for *C. quinquefasciatus* control in polluted water. Experimental application of a granule and fluid products based on the two bacteria showed good control of both, but long residual impact of the *B. sphaericus* was only obtained in a sustained-released granular formulation granule (5, 12). Operational scale use of this product showed it to be effective and a cheap alternative to the traditional house hold products (4). Application of the biocide *B. sphaericus* every second week in combination with environmental management and targeting *C. quinquefasciatus* and *A. gambiae* was shown to be effective (6).

A follow-up study now nearly 20 years later with several campaigns using LLIN and IRS against *A gambiae* would be expected to have effect on *C. quinquefasciatus* resistance patterns and levels also and such a study is in the planning.

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