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Trends in Insecticide Resistance in Natural Populations of Malaria Vectors in Burkina Faso, West Africa: 10 Years' Surveys

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1. Introduction

Malaria is a major threat in endemic regions causing at least 1 million deaths each year affecting poor and underserved populations living in tropical and sub-tropical regions. Diseases control ideally entails prevention and treatment of human infections. However, few vaccines are currently available and many pathogens are now resistant to anti-parasitic drugs. Additionally populations from endemic countries have less access to treatments due to economical impediments. Thus, the control of vectors in many instances is the only affordable measure (Beier *et al*., 2008). Mosquito control is mainly achieved by using insecticides and secondarily bio-larvicides (*Bt*-H14, *B. sphaericus*), predators (fish or copepod predators) or parasitic load (fungi), and/or by modifying the physical environment (WHO, 2006). Insecticides target a vital physiological function, leading to mosquito death. Unfortunately, due to their extremely large numbers and short generation span, mosquito populations evolve very rapidly and become resistant to insecticides, leading to repeated field control failures. Resistance results from the selection of mutant individuals able to survive and reproduce in presence of insecticide, the insecticide failing to disrupt the function of its target. In 2007, more than 100 mosquito species were resistant to at least one insecticide, some species being resistant to several compounds (Whalon *et al*., 2008). Very few classes of synthetic insecticides are available today for vector control, the most recent

has been introduced 20 years ago and none are expected in the near future (Nauen, 2007). The low availability of insecticides due to resistance is further reduced in many countries by the removal from the market of compounds for public health because for their toxicity for humans or the lack of specificity in non-target species (Rogan & Chen, 2005).
Resistance is a genetic adaptation to the modification of the environment induced by insecticides. It usually appears locally, sometimes independently in different places, but may spread rapidly through migration (Brogdon *&* McAllister, 1998; Weill *et al*., 2003). However, mosquito resistance is not only due to the insecticides used for mosquito control, but also to the many pesticide pollutions present in their environment which are generated by a large variety of human activities including insect control for agriculture and other house-hold protections. These pollutions may dramatically affect resistance genes dynamics and threaten vector control strategies. The overall pesticides pressure that select resistance in mosquitoes need to be clarified, both in terms of insecticides usage and quantity.
An. gambiae is a complex, with seven sibling species that are closely related and morphologically indistinguishable from each other by routine taxonomic methods (Gillies & Coetzee, 1987). These sibling species are however different with respect to ecological and behavioral characteristics and to vectorial competence. In West Africa, *An. gambiae s.s.* and *An. arabiensis* are the two main species of the complex that transmit malaria, with the former being the most efficient vector due to its high anthropophily (White, 1974, Lemasson *et al*., 1997). Previous study carried out on the species composition in Burkina Faso indicated that *An. gambiae s.l.* was found to be a mixture of *An. gambiae s.s.* and *An. arabiensis* across the Sudan (98.3% *vs.* 1.7%), Sudan-sahelian (78.6% *vs.* 21.4%) and the Sahel (91.5% *vs.* 8.5%) ecotypes (Dabiré *et al*., 2009a). *An. gambiae s.s.* contains two molecular forms, M and S, which co-exist in West Africa (della Torre *et al*., 2005). The M form was predominant in permanent breeding sites such as rice fields, whereas the S form was predominant in temporary habitats notably rain-filled puddles which are productive during the wet season. In Burkina Faso, genes conferring resistance to insecticides display large frequency differences in M and S forms of *An. gambiae s.s.* and *An. arabiensis*. Resistance of *An. gambiae s.l.* to DDT and pyrethroids (PYR) is especially conferred in West Africa by mutation of the sodium channel target site, the L1014F *kdr* (Chandre *et al*., 1999; Diabaté *et al*., 2002; Awolola *et al*., 2005; Nguessan *et al*., 2007). Burkina Faso is composed of three agro-climatic zones and the use of insecticides to control agricultural and human health pests varies considerably in the different zones particularly as the main cotton cropping areas are found in the south west of the country. In this last region, the intensive use of insecticides most notably for fighting the cotton *Gossypium hirsutum* L. pest is thought to have selected insecticide resistance genes in mosquitoes whose breeding sites are exposed to pesticide runoff (Diabaté *et al*., 2002; Dabiré *et al*., 2009a & b). The goal of this chapter is to summarise the resistance to insecticides status mainly in *An. gambiae s.l.* populations throughout these different agro-climatic areas and to discuss how it could limit the efficacy of malaria vector control strategies in short and long terms at the country scale. Such information is vital to determine the suitability of pyrethroids used for bednet impregnation and CX or OP based-combinations for indoor residual spraying (IRS).

2. Materials and methods

In Burkina Faso country-wide surveys associating bioassays and molecular investigations were carried out from 2000 to 2010 through 26 localities and they allow updating the

resistance status to DDT and pyrethroids and the distribution of L1014F *kdr* among *An. gambiae s.l.* into different agro climatic zones (table 1). We were also interested more recently from 2007 to perform bioassays with some insecticides among OP and CX and also to detect the *ace-1*^{R} mutation 2-3 days aged females of *An. gambiae s.l.* issued from wild larvae were exposed to several molecules such as DDT 4%, permethrin 0.75%, deltamethrin 0.05%, bendiocarb 0.1%, chlorpyriphos methyl (CM) 0.04%, carbosulfan 0.04% and fenithrotion 0.04% according to the WHO tube protocol (WHO, 1998). These active molecules were chosen as they represented each family of classic insecticides commonly used in public health. Some of these molecules such as permethrin, deltamethrin and bendiocarb are now in use in Burkina Faso through impregnated bednets and IRS application.

Study sites: Burkina Faso covers three ecological zones, the Sudan savannah zone in the south and west, the arid savannah zone (Sudan-sahelian) which extends throughout much of the central part of the country and the arid land (Sahel) in the north. The northern part of the country experiences a dry season of 6-8 months with less than 500 mm of rainfall per year. Rainfall is heaviest in the south-west (5-6 months) with a relatively short dry season. The varied ecological conditions are reflected in the different agricultural systems practiced throughout the country, from arable to pastoral lands. The western region constitutes the main cotton belt extending to the south where some new cotton areas have been cultivated. All ecological zones support the existence of *Anopheles* species that vector malaria and the disease is widespread throughout the country (Figure 1).

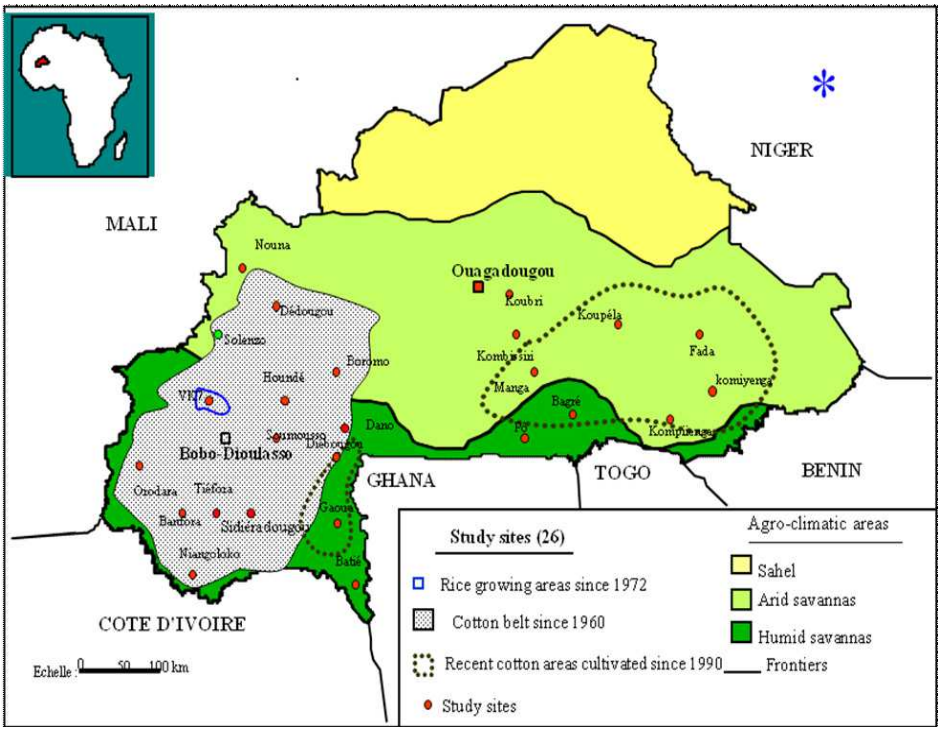

Fig. 1. Localities of the study through the three agro-climatic zones of Burkina Faso.

Study sites	Geographic references	Climatic areas	Environment	Agricultural practices	Recent date of collection
Batié	9°80′N; 2°90′W	Sudanian	rural	cereals, cotton	20/08/09
Gaoua	10°40′N; 3°15′W	Sudanian	sub-urban	cereals, cotton	20/08/09
Diébougou	10°95′N; 3°24′W	Sudanian	sub-urban	cereals, cotton	20/08/09
Dano	11°10′N; 3°05′W	Sudanian	rural	cereals, cotton	20/08/09
Banfora	10°60′N; 4°70′W	Sudanian	sub-urban	cereals, cotton	15/08/09
Sidéradougou	10°60′N; 4°25′W	Sudanian	rural	cereals, cotton	15/08/09
Tiéfora	10°50′N; 4°50′W	Sudanian	rural	cereals, cotton	15/08/09
Orodara	11°00′N; 4°91′W	Sudanian	rural	fruits, cotton	15/08/09
Dioulassoba	11°22′N; 4°30′W	Sudanian	traditionnal-urban	swamp	15/08/09
Soumousso	11°01′N; 4°02′W	Sudanian	rural	cotton	15/08/09
VK7	11°41′N; 4°44′W	Sudanian	rural	rice, cotton	08/08/09
VK5	11°24′N ; 4°23′W	Sudanian	rural	rice	08/08/09
Pô	11°20′N; 1°10′W	Sudanian	sub-urban	cereals, cotton	28/08/09
Houndé	11°50′N; 3°55′W	Sudanian	sub-urban	cotton	10/08/09
Boromo	11°75′N; 2°92′W	Sudan-sahelian	sub-urban	cotton	16/08/09
Solenzo	12°37′N; 3°55′W	Sudan-sahelian	rural	cotton	16/08/09
Dedougou	12°50′N; 3°45′W	Sudan-sahelian	sub-urban	cotton	16/08/09
Nouna	12°70′N; 3°90′W	Sudan-sahelian	sub-urban	cotton	16/08/09
Koubri	12°35′N; 1°50′W	Sudan-sahelian	rural	vegetables	28/08/09
Kombissiri	12°05′N; 1°35′W	Sudan-sahelian	rural	vegetables, cotton	28/08/09
Manga	11°66′N; 1°05′W	Sudan-sahelian	sub-urban	cereals, cotton	28/08/09
Koupela	12°20′N; 0°40′W	Sudan-sahelian	sub-urban	cotton	30/08/09
Fada	12°05′N; 3°55′E	Sudan-sahelian	sub-urban	cotton	30/08/09
Kompienga	11°30′N; 0°40E	Sudan-sahelian	rural	vegetables, cotton	30/09/09
Komiyenga	11°70′N; 0°60E	Sudan-sahelian	rural	cotton	30/09/09
Yamtenga	12°21′N ;1°31′W	Sudan-sahelian	peri-urban	swamp	28/08/09

Table 1. Main study sites across the country from where natural populations of *An. gambiae s.l.* were collected for susceptibility tests to insecticides in Burkina Faso.

Mosquitoes sampling: To evaluate the status of resistance of *An. gambiae s.l.* to insecticides in the three ecological zones of Burkina Faso, anopheline larvae were sampled in countrywide collections during the rainy season, from September to October. Larvae were collected at each locality from breeding sites such as gutters, tires, swallow wells and pools of standing water. Larvae were brought back to the insectary and reared to adulthood. When it was not possible to collect larvae because of the distance between the sampling site and the insectary or due to sampling constraints at the site such as excessive rainfall or flooding, alternative collections of adult mosquitoes were made using indoor aerosol insecticide spraying. *An. gambiae s.l.* were identified morphologically using standard identification keys of Gillies & Coetzee (1987). The results presented here summarized those of transversal studies in whole country 2000 and 2009 with particular focus on the period from September to October 2009.

Insecticide susceptibility test: Susceptibility test was performed on 2-3-day-old *An. gambiae s.l.* females provided by larva collections using the WHO standard vertical tube protocol. Three insecticide-impregnated papers were used: DDT 4%, permethrin 0.75% (cis:trans = 25:75), deltamethrin 0.05%, bendiocarb 0.1%, CM 0.04%, carbofuran 0.04% and fenithrotion 0.04%. Mosquitoes were tested against "Kisumu" a fully susceptible reference laboratory strain. Mortality controls were carried out by exposing both the "Kisumu" strain and wild populations from each site to non-insecticidal impregnated paper. After 1 h exposure,

mosquitoes were transferred into insecticide free tubes and maintained on sucrose solution. Final mortality was recorded 24 h after exposure. The threshold of susceptibility was fixed at 98% for the four active molecules according to the protocol of WHO (1998). Are considered as susceptible, suspected resistant and resistant populations with respectively 100 to 98%, 98 to 80 %and under 80% of mortality rates. Dead and survivor mosquitoes were grouped separately and stored on silicagel at -20°C for subsequent PCR analysis.

Molecular analysis: DNA extraction and PCR identification of the *An. gambiae* M and S and *An. arabienis*: Genomic DNA was extracted from individual mosquitoes according to a slightly modified version of the procedure described by Collins *et al.* (1987). After quantification of the extracted DNA, adults of *An. gambiae s.l.* tested in bioassay were processed by PCR for molecular identification of species of the *An. gambiae* complex and molecular forms respectively (Scott *et al.*, 1993; Favia *et al.*, 2001). Those survived or dead in bioassay were after processed in other PCR analysis for the detection of *kdr* and *ace-1*^{R} mutations. For *kdr* detection, a sub-sample of 30 mosquitoes per site of the permethrin/deltamethrin-tested specimens and those collected by indoor spraying were processed by PCR for prior species identification and molecular characterisation of M and S forms of *An. gambiae s.s.* according to Scott *et al.* (1993) and Favia *et al.* (2001) respectively. The frequency of the L1014F mutation in the same samples was determined by allele-specific PCR as described by Martinez-Torres *et al.* (1998).

A*ce-1*^{R} mutation was detected using the PCR-RFLP assay described by Weill *et al.* (2004) with minor modifications. Specific primers, *Ex3AGdir* (GATCGTGGACACCGTGTTCG) and *Ex3AGrev* (AGGATGGCCCGCTGGAACAG) were used in PCR reactions (25µl) containing 2.5µl of 10X *Taq* DNA polymerase buffer, 200µM of each desoxynucleoside triphosphate (dNTP), 0.1U of *Taq* DNA polymerase (Qiagen, France), 10pmol of each primer and approximately 1 to 10ng of DNA. PCR conditions included an initial denaturation step at 94°C for 5min followed by thirty five cycles of 94°C for 30s, 54°C for 30s and 72°C for 30s, with a final extension at 72°C for 5min. Fifteen microlitres of PCR product was digested with 5U of *Alu*I restriction enzyme (Promega, France) in a final volume of 25 µl at 37°C for 3 hours. Products were then analysed by electrophoresis on a 2% agarose gel stained with ethidium bromide and visualized under UV light.

Statistical analysis: The proportion of each species and molecular forms were compared between the study sites. The frequencies of *kdr* and *ace-1*^{R} mutations were calculated according to the formula p= 2AA+Aa/2n where AA was the number of homozygotes, Aa the number of heterozygotes and n the size of analyzed sample. It was compared between sites and between *An. gambiae* M and S molecular forms and *An. arabiensis* by chi square tests. The genotypic frequencies of ace-1R in mosquito populations were compared to Hardy-Weinberg expectations using the exact test procedures implemented in GenePOP (ver.3.4) software (Raymond & Rousset 1995)

3. Resistance to pyrethroids and organochlorine

Reports of resistance in mosquito vector populations in Burkina Faso appeared as early as the 1960 s, when *An. funestus* and *An. gambiae s.l.* populations that showed resistance to dieldrin and DDT, were described (Hamon *et al.*, 1968a; Hamon *et al.*, 1968b). More recent studies have confirmed that resistance to DDT4% is still prevailing with highest level in *An. gambiae s.l.* populations in Burkina Faso where also resistance to certain pyrethroids was increasingly reported (Diabaté *et al.*, 2002, 2004a; Dabiré *et al.*, 2009a). Indeed *An. gambiae s.l.*

populations were resistant to DDT4% in every part of the country and mortality rates below 60 % were observed at the country scale (Fig. 2). They were found also resistant to permethrin 0.75% in the Sudan climatic zone in the western region and also in several sites in the central part of Burkina Faso (Fig. 3). Surprisingly, except for the areas with a very long history of cotton cropping, the tested populations of *An. gambiae* remained susceptible to deltamethrin 0.05%, although decreased mortality values lead to suspect an emergence of resistance in the ongoing years (Dabiré *et al.*, 2009a). However, this result should be interpreted with caution as the resistance is a progressive process and recent data recorded in 2009-2010 showed 5 sites mostly located in the central region remained susceptible foci (Fig. 4).

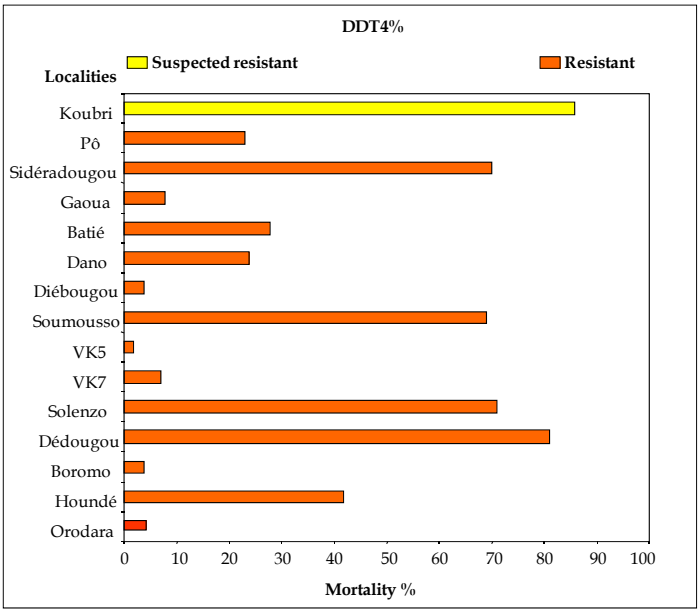

Fig. 2. Mortality rates of *Anopheles gambiae s.l.* populations to DDT 4% from Sudan, Sudan-sahelian and sahelian areas in Burkina Faso.

Resistance is the result of a limited number of physiological mechanisms; it is often monogenic and due to point mutations in a structural gene, gene amplification or changes in transcriptional regulation (Hollingworth & Dong, 2008). It results from the selection of mutant individuals able to survive and reproduce in presence of insecticide, the insecticide failing to disrupt the function of its target (Whalon *et al.*, 2008). The resistance phenotype to pyrethroids and DDT 4% observed in natural populations of *An. gambiae s.l.* was already attributed to a *kdr* mutation as it is the major mechanism involved in cross resistance to pyrethroids and DDT4% in West Africa (Chandre *et al.*, 1999; Diabaté *et al.*, 2002). Until recently, it was assumed that this mutation was the L1014F substitution in West Africa while the L1014S substitution was found in the East (Ranson *et al.*, 2000). However, we now know that both mutations coexist in some countries and are widely distributed throughout sub-Saharan continent and also in Benin and Burkina Faso (Verhaeghen *et al.*, 2006; Etang *et al.*, 2006; Djegbe *et al.*, 2011; Dabiré *et al.*, unpublished).

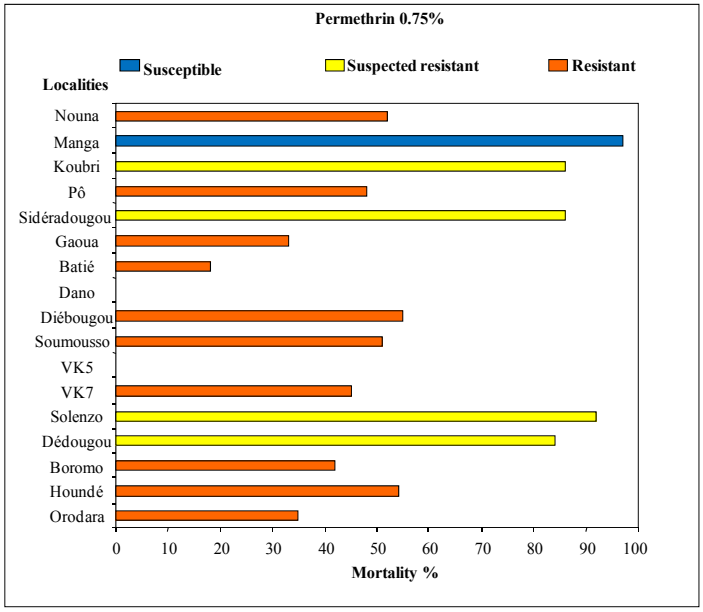

Fig. 3. Mortality rates of *An. gambiae s.l.* populations to permethrin 0.75 % from Sudan, Sudan-sahelian and sahelian areas in Burkina Faso.

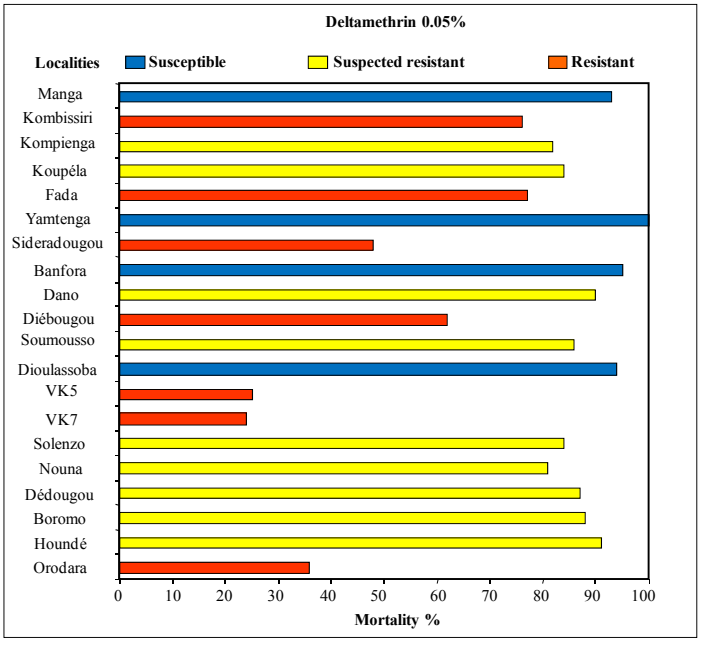

Fig. 4. Mortality rates of *An. gambiae s.l.* populations to deltamethrin 0.05 % from Sudan, Sudan-sahelian and sahelian areas in Burkina Faso.

In Burkina Faso, the frequency of the L1014F *kdr* mutation was first described in the S form of *An. gambiae s.s.* in high frequencies especially in the Western part of the country where the use of insecticides is intensive in agriculture (Chandre *et al*., 1999, Diabaté *et al.,* 2002). But few years later it had been also found within the M form and was suspected to be the result of an introgression from the S form *An. gambiae s.s.* (Weill *et al*., 2000; Diabaté *et al*., 2003). Up to day the distribution of this mutation at the country scale is variable, ranging from 0.5 to 0.97 for the S form in the Sudanian region and decreasing in the Sudano-sahelian and Sahelian areas with averaged values fluctuating between 0.1 and 0.6. Compared to 2000 data (Diabaté *et al*., 2004a), the frequency of L1014F *kdr* mutation increased notably from 2004 to 2006 before getting stable around the fixation level in some localities (Fig. 5). As mentioned above, no *kdr* was detected in 1999 in the M form (Chandre *et al*., 1999). But early in 2000, the L1014F mutation was identified from few specimens of M form from rice growing area, peaking maximally at 0.04 (Diabaté *et al*., 2003). Nowadays the L1014F *kdr* has increased drastically in the M form with varying frequencies between climatic areas, and reaching high frequencies (0.93) in cotton growing belts with a geographic expansion to the sudano-sahelian region where it was formerly absent (Dabiré *et al*., 2009a). It has also increased in *An. arabiensis* (0.28) where it was formerly reported only from one specimen in 2002 (Diabaté *et al*., 2004b) (Fig. 6).

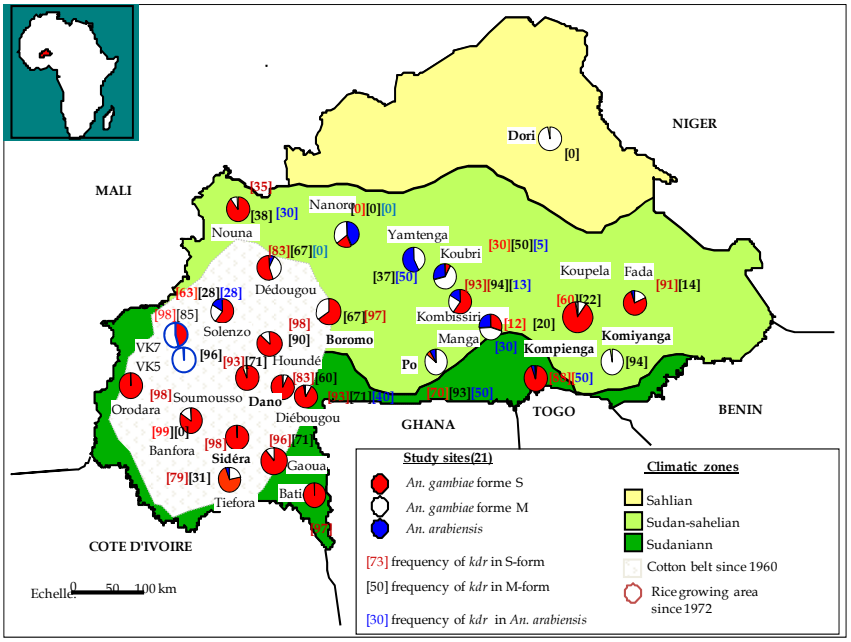

Fig. 5. Geographic distribution of L1014F *kdr* allele in *An. gambiae s.l.* populations inducing pyrethroids and DDT resistance profile in Burkina Faso in 2009 [numbers in bracket represent frequency of L1014F *kdr* allele frequencies].

Globally the distribution of DDT and pyrethroids resistance in regions of intensive cotton cultivation suggests that indirect selection pressures from the agricultural use of insecticides may be responsible for the development of resistance in *An. gambiae s.l.* populations. The

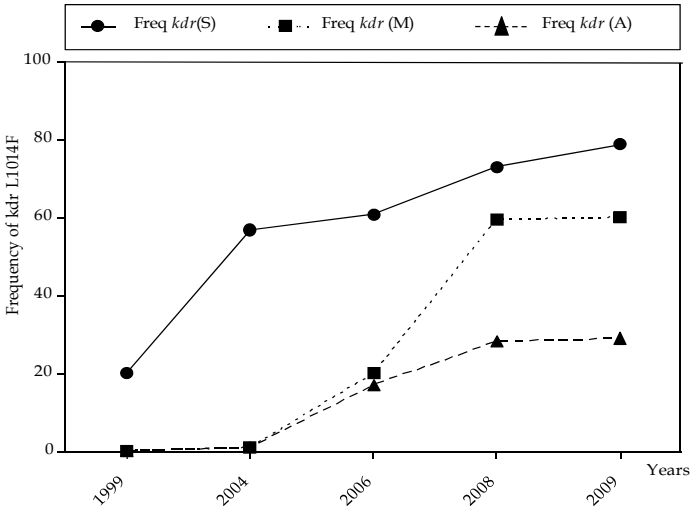

Fig. 6. Evolution of allelic frequencies (in percentages) of L1014F *kdr* in natural populations of *An. gambiae s.l.* from 1999 to 2009 in Burkina Faso[S: *An. gambiae* S form; M: *An. gambiae* M form, A: *An. arabiensis*].

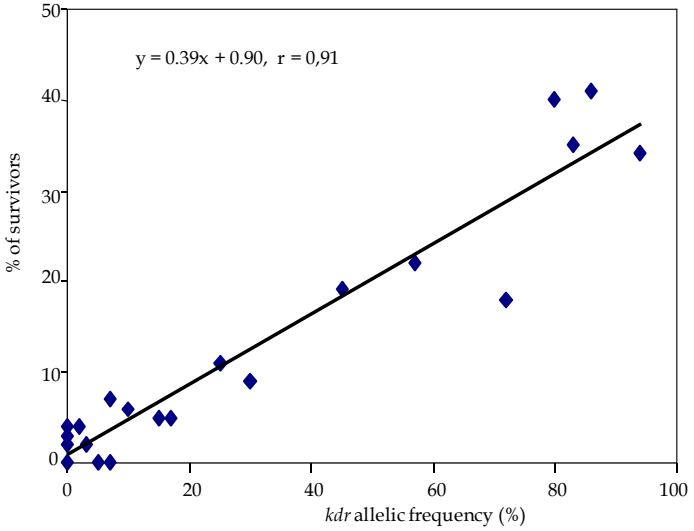

Fig. 7. Correlation between *kdr* frequency and mortality rates of *An. gambiae s.l.* tested with permethrin 0.75%.

mean frequency of the *kdr* allele was the highest in populations from the Sudanian zone where the lowest mortality rates to pyrethroids and DDT 4% were seen in bioassays. By contrast, the *kdr* allele frequency was lower in *An. gambiae s.s.* populations is central and eastern sites where cotton cultivation is recent. A majority of susceptible phenotypes were observed in wild populations of *An. gambiae* from these areas. In addition, the results of this

study suggest that the domestic use of insecticides may also exert a selection on *An. gambiae* populations that is secondary to that from the agricultural insecticides. Indeed all collections made in cities located outside the cotton belt showed high mortality rates and a relative low frequency of *kdr* compared to those of cotton belt. The correlation between the high frequencies of L1014F *kdr* mutation and the proportion of surviving individuals after DDT/pyrethroids exposures (figure 7) suggests that this mutation is the main mechanism of resistance to these insecticides.

4. Metabolic resistance

Most studies conducted in Burkina Faso have focused on the modification of target sites by mutation and did investigate the occurrence and the role of metabolic resistance in the observed resistance of *An. gambiae s.l.* Recent tests performed in Dioulassoba (an old central district of Bobo-Dioulasso crossed by the Houet river) showed that *An. arabiensis* from urban polluted breeding sites was resistant to DDT 4% but fully susceptible to pyrethroids and OP/CX, suggesting an existence of metabolic resistance probably GST which is more specific to DDT acting as the main resistance mechanism (Dabiré *et al*., unpubl.). Even more recently, preliminary results gathered only in VK7 (a sample from a rice growing area surrounded by cotton fields) showed an overexpression of detoxifying enzymes such as glutation-S-transferases, cytochrome P450 oxygenases in populations of *An. gambiae s.s.* with high *kdr* frequencies suggesting the existence of multi-resistance mechanisms to pyrethroids (Fig. 8A,B&C). But more investigations are needed to better address the role of metabolic components on the expression of resistance phenotypes observed in natural populations of *An. gambiae s.l.* especially in areas where insecticide pressure is high.

5. Resistance to organophosphates (OP) and carbamates (CX) and geographic distribution of *ace-1^R* mutations and duplicated *ace-1^D* allele

In Burkina Faso the resistance to OP/CX has been monitored since 2002 only in few sites of the Western areas of the country, and lately extended to the country scale since 2006. Although fenithrotion 0.4%, chlorpyriphos methyl (CM) 0.4%, carbosulfan 0.4% and bendiocarb 0.1 % were tested, the monitoring was well sustained only with bendiocarb 0.1% which was expected to be used in Burkina Faso indoor residual spraying to supplement the efficacy of ITNs especially in localities where *An. gambiae* is resistant to PY. Except for CM 0.4% for which *An. gambiae* populations were fully susceptible irrespectively of the locality, the other OP/CX mentioned above showed mortality rates ranging from 5% to 100% (Fig. 9). The lowest mortality rates were obtained with carbosulfan 0.4% (5%) and bendiocarb 0.1% (20%) especially in areas located in cotton belt such as Houndé, Orodara, Tiefora and Banfora. The susceptibility to bendiocarb 0.1% was also recorded in the central areas where cotton growing is recent (Fig. 10). From 2005 on, the detection of *ace-1^R* mutation involved in OP/CX resistance allowed to evaluate the distribution of this allele in field populations of *An. gambiae s.l.* The characterisation of this allele was based on a PCR-RFLP diagnostic (Weill *et al*., 2004) that allow the identification of the amino-acid substitution, from a glycine to a serine at the position 119, in the AChE1 catalytic site (G119S). In *Culex pipiens*, there is direct and indirect evidence that the resistance allele (*ace-1^R*) entails a large fitness cost, probably due to the mutated AChE1 having a much lower level of activity. Homozygous *ace-1^R* mosquitoes survive in the presence of insecticide, but are rapidly outcompeted in the

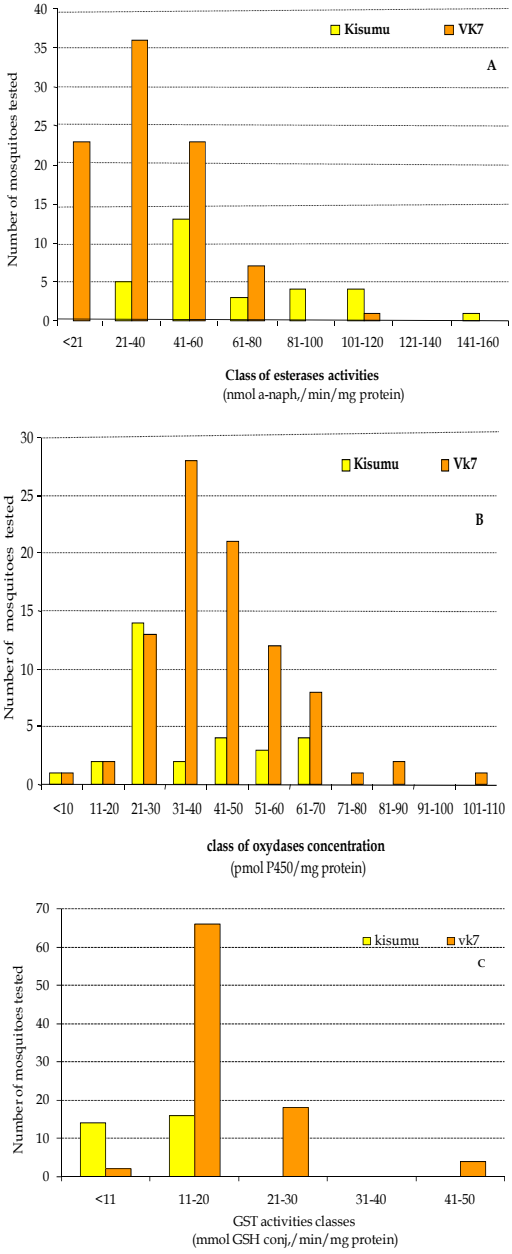

Fig. 8. Activity of detoxifying enzymes such as esterases (A), oxydases (B), and GST (C) in natural populations of *An. gambiae* from Vallée du Kou (VK7) compared to that of *An. gambiae* "Kisumu" (susceptible reference strain). Note the over-expression of oxydases and GST in the VK7 sample.

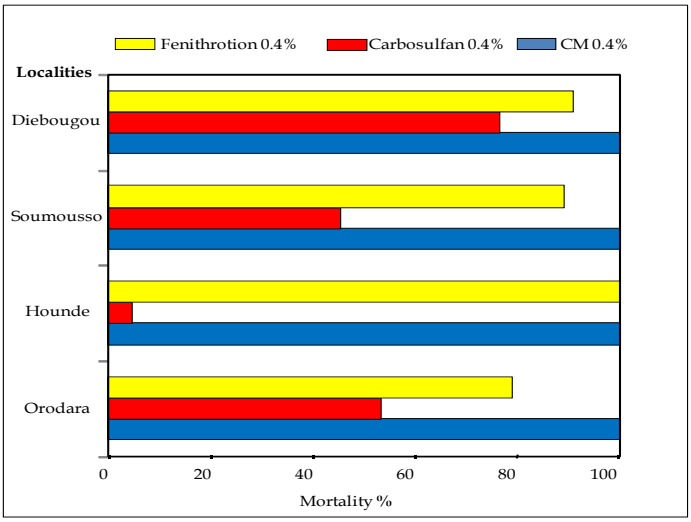

Fig. 9. Mortality rates of *An. gambiae s.l.* populations exposed to Chlorpyriphos methyl (CM) 0.04 %, carbofuran 0.04% and fenithrotion 0.04% from four sites located on the cotton belt in South west of Burkina Faso [100-98%=susceptible; 98-80%= suspected resistance; <80%=resistant].

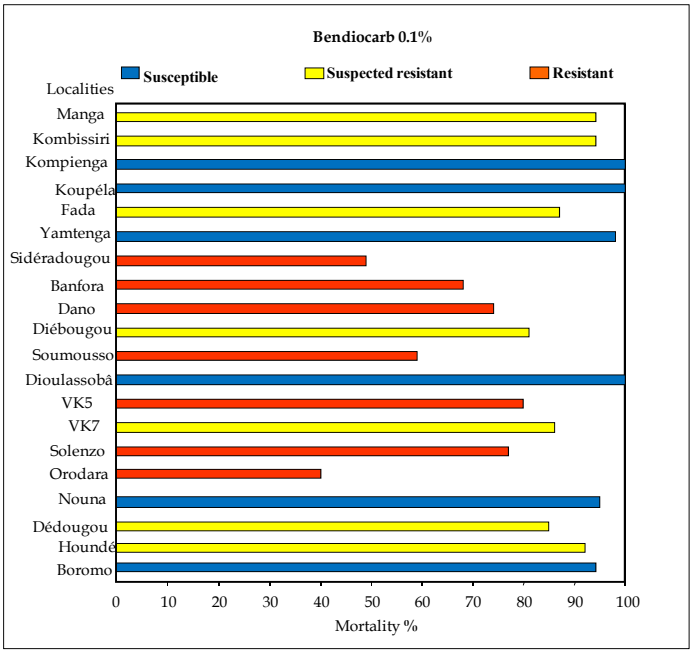

Fig. 10. Mortality rates of *An. gambiae s.l.* populations exposed to bendiocarb 0.1% from Sudan, Sudan-sahelian and sahelian areas in Burkina Faso.

absence of insecticide. There are evidences that the same phenomenon exists also in *An. gambiae s.s* (Djogbenou *et al*., 2010). Even though the results of bioassays were more recent (2009) we presented only the *ace-1*^{R} frequencies from 2006 to 2008 samples.

This mutation was distributed throughout the Sudan and Sudan-Sahelian localities reaching relative high frequencies (0.6) in the South-West, moderate frequencies (<50%) in the central region, and being absent in the Sahel. It was far more frequent in the S form than in the sympatric M mosquitoes (averaging in mean 0.32 for the S form *vs.* 0.036 for the M form) (Djogbenou *et al*., 2008a). Even though the *ace-1*^{R} mutation was spread across two climatic zones, it was recorded mostly in the cotton growing areas (Dabiré *et al*., 2009b). Although the *ace-1*^{R} mutation was less spread within the *An. gambiae* s.s. M form, the highest frequency (0.63) was recorded in this form at Houndé located just on the limit of the Sudan region in 2008 (Fig. 11). The observed genotypic frequencies were not significantly different from Hardy-Weinberg expectations at the 95% confidence level in populations from any site except in the *An. gambiae* s.s. S form population from Orodara, where an excess of heterozygotes was observed. S-form samples from a number of other sites also showed a higher than expected number of heterozygous genotypes including Banfora (expected 10, observed 13), Diebougou (expected 11, observed 16) and VK7 (expected 12, observed 17) although Hardy–Weinberg equilibrium was not rejected. These results suggest that a fitness cost is associated to this mutation (Labbé *et al*., 2007), but see the next paragraph. No *An. arabiensis* was detected up today carrying the *ace-1*^{R} mutation.

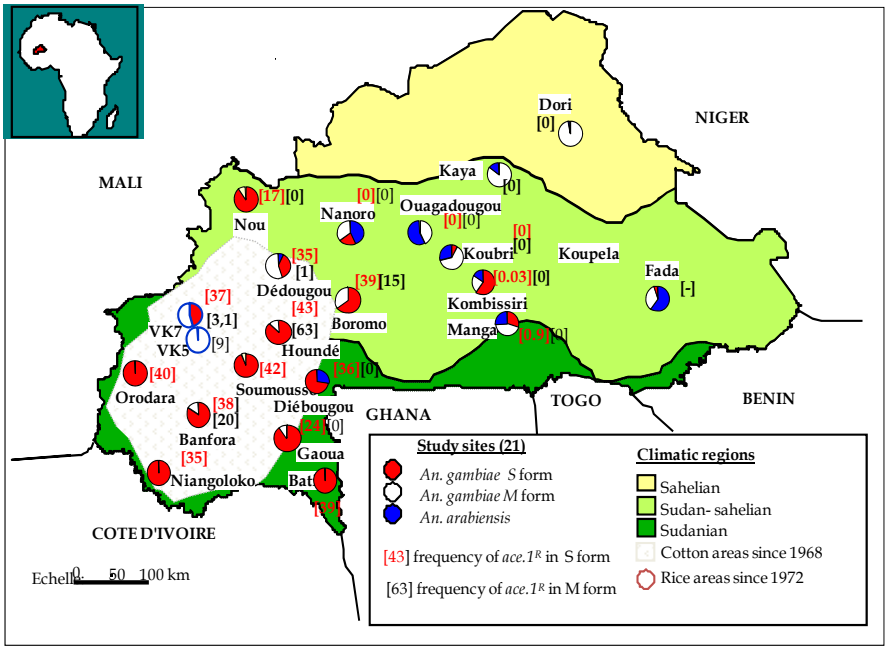

Fig. 11. Geographic distribution of *ace-1*^{R} allele in *An. gambiae s.l.* populations inducing OP/CX resistance profile in Burkina Faso in 2008 [numbers in bracket represent percentage of *ace-1*^{R} allele frequencies]. No *An. arabiensis* was found carrying ace-1R allele so we did not represent its frequency for this species.

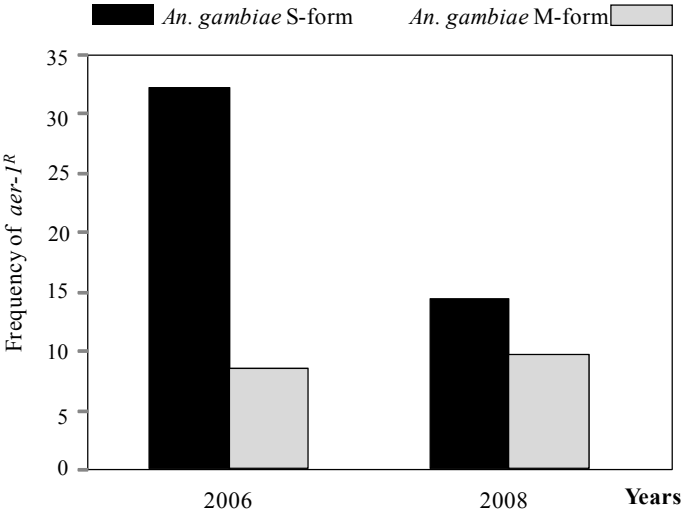

Fig. 12. Evolution of allelic frequencies of *ace-1*^{R} in natural populations of *An. gambiae s.s.* in Sudan area (cotton belt) from 2006 to 2008 in Burkina Faso.

The frequency evolution of this allele during the two years is not regular (fig.12), but considering only the Sudan area, it seems to decrease within the S form and increase slightly in the M form from 2006 to 2008 (Fig. 12). As no data existed before 2006, we did not know the trait of evolution of this allele in the past. That should greatly contribute to explain the inverse tendency of this allele within the two forms because the resistance pattern is complex in this area where excess of heterozygous for *ace-1*^{R} allele should probably co-exist with the duplication allele Ag-*ace-1*^{D} (see the next paragraph). However the allele frequencies in the two forms need to be compared statistically from a solid sample sizes. Regular monitoring of the same localities with the same protocols should give a better insight of the evolution of the G119S mutation of the *ace-1* gene. Data will have to be analyzed in relation with the possible coexistence of other resistance mechanisms such as *kdr* mutation or metabolic based resistance as well as with the existence of the duplicated allele Ag-*ace-1*^{D} which may decrease the fitness cost of this mutation (Berticat *et al*., 2008).

6. Duplication of *ace-1*^{D} allele in *An. gambiae s.s.* from Burkina Faso

The G119S mutation conferring resistance to organophosphates and carbamates was distributed throughout the Sudan and Sudan-sahelian correlated with the cotton growing areas. This mutation has been identified in the *ace-1*^{R} allele, and recently in a "duplicated allele" (*ace-1*^{D}), putting in tandem a susceptible and a resistant copy of *ace-1* on the same chromosome. The ace-*1*^{D} has been recorded in field populations of *An. gambiae* M and S forms and was shown to have come from the same duplication event in both forms (Djogbenou *et al*., 2008b). A unique *ace-1*^{D} allele has been observed in Côte d'Ivoire and Burkina Faso, with an estimated frequency >50% in some populations (Djogbenou *et al*., 2009).

In Burkina Faso, the *ace-1*^{D} allele frequency could reach 50% and is mainly present in the S form principally in the old cotton belt in the South West. The duplicated allele was also

observed on the littoral of Ivory Coast with high frequencies in the M form, and may be present at a low frequency in Benin and Togo (Djogbenou *et al*., 2010) (Fig. 13).
If, as suspected by Labbé *et al.* (2007), *ace-1*^{D} allele has a lower fitness cost than *ace-1*^{R}, it would increase dramatically the diffusion of OP and CX resistance in *An. gambiae* s.s. natural populations.
Presently, there is no simple test to characterized the duplicated allele *ace-1*^{D} as diagnostic tests do not discriminate between heterozygotes *ace-1*^{S}/*ace-1*^{R} and genotypes with the *ace-1*^{D} allele. Formal identification of *ace-1*^{D} in a field female thus necessitate to cross this female with a susceptible male, and screen its offspring for CX resistance (see Labbé *et al*. 2007 for detailed procedure). This is clearly not possible on a large number of specimens and to better address the role and the impact of the *ace-1* duplication in resistance schemes, it appears urgent to build an *ace-1*^{D} homozygous laboratory strain to investigate how the duplication modifies the fitness of its carriers.

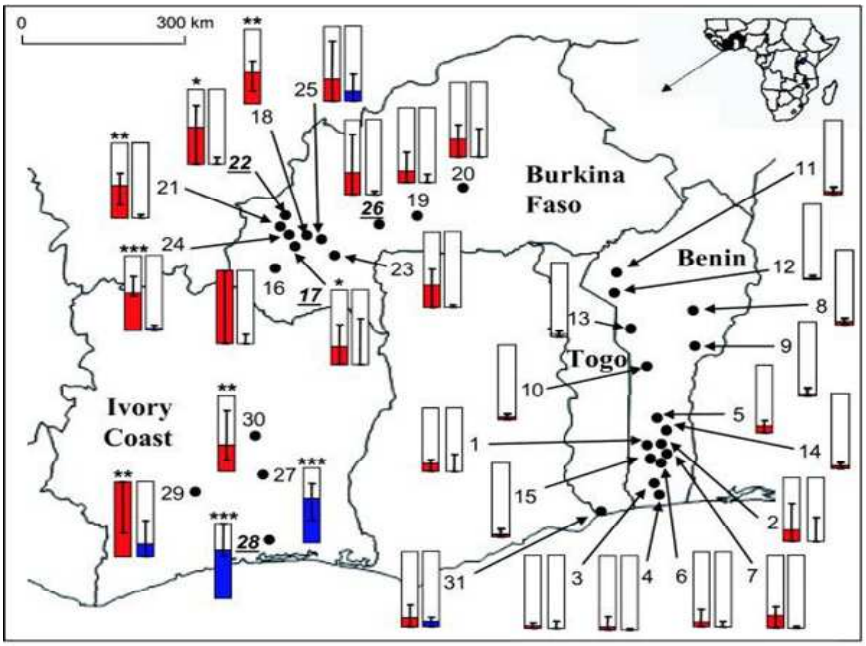

Fig. 13. Ag- *ace-1*^{D} frequency in Western Africa. The frequency is given for each *An. gambiae* molecular form: S (red) and M blue). Samples in which *ace-1*^{D} was detected by molecular analysis are bolded and underlined. Significant presence of the duplicated allele (before Bonferroni correction) is given with * for $P < 0.05$, ** for $P < 0.01$ and *** for $P < 0.001$.
(Figure from Djogbenou *et al*., 2009 in Malaria Journal)

7. Multiresistance status in natural populations of *An. gambiae s.l.*: the coexistence of *kdr* and *ace-1^R* mutations

It was in 2005 when the detection of *ace-1^R* (G119S) mutation was systematically performed for the first time in *An. gambiae s.s.* natural populations from Burkina Faso (Dabiré *et al*., 2008). This mutation was found together with *kdr* mutation within the same populations. That suggests the existence of multiresistance mechanisms occurring in the same populations. The same individuals were found carrying the two genes but the *kdr* always appeared as homozygous (*kdr*^{R}/*kdr*^{R}). The functional links of the two genes needed to be further investigated (Dabire *et al*., 2008). Indeed as indicated by the results of bioassays, the occurrence of such multiresistance mechanisms should explain why *An. gambiae s.s.* populations are becoming resistant to all classes of insecticides especially in the South west parts of the country. The individuals carrying the two genes appeared to be phenotypically more resistant to pyrethroids and bendiocarb than those carrying only *kdr* or *ace-1^R*, respectively. Such a synergy between *kdr* and *ace-1^R* has been observed in *Culex pipiens* (Berticat *et al*., 2008). Assuming that the *ace-1^R* is associated to a high fitness cost (Djogbenou *et al*., 2010), it should be interesting to investigate how the *kdr* mutation influences the fitness cost related to *ace-1^R*. Also, it was previously shown in *Culex pipiens* that mosquitoes carrying both *kdr* and *ace-1^R* mutations suffer less cost than the one carrying only *ace-1^R* (Berticat *et al*., 2008). Both this synergy between pyrethroids and OP/CX resistance mechanisms and the spread of the *ace-1^D* allele in natural populations of *An. gambiae s.s.* could largely hamper the expected results of using OP/CX as alternative insecticides to the PYR becoming ineffective by the presence of *kdr*. It is crucial to build laboratory colonies carrying the two mutations from which the benefit of changing insecticides could be properly tested. More recently, in 2011, we also recorded metabolic based resistance in *An. gambiae* from Burkina Faso. Even though these results are preliminary, they further complicate the pattern of resistance in this country, and may represent a dramatic threat for malaria vector control in the near future.

In conclusion, all these aspects need to be properly addressed by fine fundamental research to decrypt the link between the resistance schemes and to give sense in vector control point of view.

8. Other malaria vectors

Anopheles funestus belongs to a group of no less than nine species that are difficult to distinguish based solely on morphological characters of a single life stage (Gillies and Coetzee 1987, Harbach 1994). Species identification difficulties have been recently addressed by molecular techniques based on the polymerase chain reaction (PCR) by using a cocktail of species-specific primers permitting the identification of the six most common species of the group (Koekemoer *et al*., 2002). Recent analyses of rDNA sequences (Cohuet *et al*., 2003) revealed the occurrence, in West and Central Africa, of a new taxon morphologically related to *An. rivulorum* Leeson, which is provisionally named *An. rivulorum*-like, thereby enlarging the number of members of the *An. funestus* group to 10 species. Among all the members of the *funestus* group, *An. funestus* s.s. is the most anthropophilic species, and it is considered as the only major malaria vector (Coetzee & Fontenille, 2004), although in a Tanzanian village the circumsporozoite protein of *Plasmodium falciparum* was detected by immunological techniques in some *An. rivulorum* specimens (Wilkes *et al*. 1996).

An. funestus like other malaria vectors is controlled by the use of insecticides such as insecticide treated materials or as indoor residual spraying (IRS). Unfortunately, *An. funestus* is increasingly developing resistance across Africa to different classes of insecticides used in public health, such as PYR, CX and DDT (Brooke *et al*., 2001; Casimiro *et al*., 2006; Cuamba *et al*., 2010; Morgan *et al*., 2010). There are alternative agrochemicals, such as fipronil that could be introduced but the potential for cross-resistance from existing mechanisms segregating in field populations needs to be more investigated.

The insecticide resistance in *An. funestus* populations was early recorded from Burkina Faso, where resistance was found to dieldrin, a cyclodiene abundantly used in Africa in the 1960s for cotton crop protection but also for malaria vector control (Hamon *et al*., 1968b). Dieldrin resistance was also reported in *An. funestus* from Cameroon, Benin, Nigeria and Mali (Service, 1960; Toure, 1982; Brown, 1986). Recent studies have shown that *An. funestus* remains fully susceptible to all tested insecticides (DDT, PYR, OP/CX) except to dieldrin for which resistance remains high despite the fact that cyclodienes are no longer used in public health control programs (Dabire *et al*., 2007). But the distribution of this resistance across the rest of the continent is unknown and need to be clarified. The understanding of factors explaining the persistence of high levels of resistance against cyclodienes in *An. funestus* as well as the geographical distribution of this resistance across the continent has been recently addressed by Wondji *et al*., 2011. These studies indicated that Rdl^R mutation extensively reported in West and Central Africa should sustain dieldrin resistance in such *An. funestus* populations.

9. Pesticide pressures on disease vectors are from multiple origins

The question that remains to be clearly identified is the origin of insecticide pressures that select the resistances observed in mosquitoes from sub-Saharan Africa. In Burkina Faso, the emergence of the $ace\text{-}1^R$ mutation in *An. gambiae s.s.* populations is also associated with the insecticide treatment history with OP and CX of cotton. Since the mid 1990s and until recently a pest management strategy including four windows of treatment per cropping cycle using pyrethroids, OP/CX (such as chlorpyrifos, profenofos and trizophos) and organochlorines had been adopted in order to manage the pyrethroid-resistance of *Helicoverpa armigera* and *Bemisia tabaci* that emerged throughout the cotton belt. Some bioassays performed in 2003 on *An. gambiae* populations from four sites located in the cotton belt of western Burkina Faso revealed early resistance against CX and OP insecticides pre-empting the discovery of the genetic resistance mechanism revealed in further studies. In a previous study the agricultural use of insecticides was already implicated in the development of resistance to pyrethroids in *An. gambiae s.l.* populations. Then the geographical distribution of resistance decreased in *An. gambiae s.l.* populations from the Sudan savannah to Sahelian areas and the highest levels of resistance were found in sites of cotton cultivation. The areas under cotton cultivation have expanded dramatically in the last ten years (210,000 ha in 1996 to more than 520,000 ha in 2005). A corresponding increase in the level of insecticide use has also been reported reaching more than $3x10^6$ litres of pesticide per cropping campaign. Furthermore, a clear knowledge concerning the practices of populations regarding the uses of insecticides in Africa is required.

10. Insecticide resistance and malaria vector control

Although several studies in Ivory Coast and Burkina Faso had shown that ITNs may still achieve good control of PYR-target resistant *An. gambiae s.s.* populations (Darriet *et al*., 2000; Henry *et al*., 2005; Dabiré *et al*., 2006), recent results from experimental hut trials conducted in Southern Benin with lambdacyhalothrin (PYR) suggested that such ITNs may fail to control these field populations (Ngessan *et al*., 2007). Failure of indoor residual house spraying (IRS) with deltamethrin (PYR) had also occurred in South Africa where the malaria vector *An. funestus* had developed PYR-metabolic resistance. A recent study in Bioko Island (Equatorial Guinea) reported a failure of indoor residual spraying with deltamethrin on *An. gambiae* populations of the M molecular form carrying the Leu-Phe *kdr* mutation at a high frequency. These *kdr*-pyrethroid resistant populations were controlled after the introduction of a carbamate insecticide in IRS (Sharp *et al*., 2007). Thus the malaria outbreak in this country was only brought under control after reversion to DDT spraying (organochlorine insecticide).

A concern for the potential use of OP and CX as alternative for PYR is that target and metabolic resistances to these insecticides are already present in some *An. gambiae s.s.* populations in West Africa especially in Burkina Faso. Several studies have suggested that the use of agricultural pesticides, especially for cotton but also for vegetable crops, favored the emergence and facilitated the spread of insecticide resistance within mosquito populations. Other studies have given evidence for the selection of *kdr* alleles associated with the use of pyrethroids in ITNs and other domestic strategies of personal protection, especially in Kenya and Niger (Czeher *et al*., 2008). In countries supported by PMI (President Initiative against Malaria) such as Ghana, Senegal, Mali, Benin and Liberia in West Africa the large-scale pilot interventions implemented with bendiocarb 400mg/m^2 in IRS could also contribute to select the OP/CX resistance. However, as no global health control program in Africa used OP and CX for mosquito control, it is necessary to clearly identify the origin of insecticide pressure that select these resistances.

11. Conclusions

Reports of insecticide resistance in malaria vectors in West Africa especially in Burkina Faso indicate that insecticide resistance increases year after year and highlight the threat to the effectiveness of vector control strategies. In fact *An. gambiae s.l.* populations in Burkina Faso, and more broadly in West Africa, have evolved resistance to many of the insecticides classes used for vector control. Resistance may be conferred by target-site insensitivity such as *kdr* and *ace-1^R*, other metabolic mechanisms or a combination of all as *kdr* and *ace-1^R* resistance mechanisms occur concomitantly in the same populations of *An. gambiae* s.s. in the South-Western region of the country.

In conclusion the geographical distribution of insecticide resistance in *An. gambiae s.l.* populations was found in sites of cotton cultivation and vegetable in urban settlement that has expanded dramatically in the last ten years. But the role of agriculture in the selection of resistance in natural mosquito's populations needs to be clarified, both in terms of insecticides usage and quantity in order to devise strategies that may help to reduce the extension of resistance. Until the discovery of new insecticides or using new formulations of existing insecticides and also the use of genetically modified mosquitoes (GMM) and sterilised males techniques (SIT), it is crucial to integrate the regional vector resistance status

in the implementation of control interventions that will preserve a long term efficacy of these vector control tools.

Unfortunately reports of insecticide resistance in vector populations increase year by year and could jeopardize malaria vector control based on the use of insecticides. The use of insecticides for bednets impregnation or for IRS represents the primary means for malaria prevention worldwide. However the efficacy of such tools has been evaluated in areas where vectors are susceptible to insecticides. Moreover, mosquito resistance is not only due to the insecticides used for mosquito control, but to the many pesticide pollutions present in their environment which are generated by a large variety of human activities necessitating insect control for agriculture (large cultures, fruits and vegetables), animal and other household protections. These pollutions may dramatically affect resistance genes dynamics and threaten these strategies.

The overall pesticide pressures that select resistance in mosquitoes need to be clarified, both in terms of insecticides usage and quantity. It is also crucial to improve our knowledge on the practices of people regarding the use of insecticides and the reasons underlying their decision process based on social and cultural contexts.

Malaria vector control programs require up-to-date information on the distribution and composition of mosquito vector populations and the susceptibility of these populations to the insecticides used for control.

12. Acknowlegements

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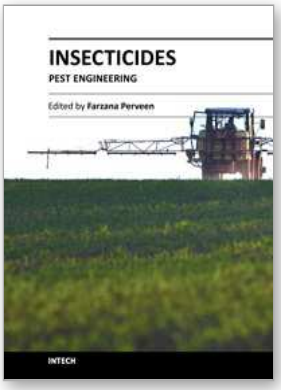

Insecticides - Pest Engineering

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This book is compiled of 24 Chapters divided into 4 Sections. Section A focuses on toxicity of organic and inorganic insecticides, organophosphorus insecticides, toxicity of fenitrothion and permethrin, and dichlorodiphenyltrichloroethane (DDT). Section B is dedicated to vector control using insecticides, biological control of mosquito larvae by Bacillus thuringiensis, metabolism of pyrethroids by mosquito cytochrome P40 susceptibility status of Aedes aegypti, etc. Section C describes bioactive natural products from sapindacea, management of potato pests, flower thrips, mango mealy bug, pear psylla, grapes pests, small fruit production, boll weevil and tsetse fly using insecticides. Section D provides information on insecticide resistance in natural population of malaria vector, role of Anopheles gambiae P450 cytochrome, genetic toxicological profile of carbofuran and pirimicarp carbamic insecticides, etc. The subject matter in this book should attract the reader's concern to support rational decisions regarding the use of pesticides.

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