

Use of different cotton pest control strategies (conventional, organic and transgenic) had no impact on insecticide resistance in *Anopheles gambiae* s.l. populations in Burkina Faso, West Africa

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Abstract

Resistance to insecticides in the mosquito *Anopheles gambiae* s.l can jeopardize malaria vector control strategies in Africa. Previous studies have shown that the agricultural use of pesticides, particularly for cotton protection, contributes to the selection of insecticide resistance of malaria vector mosquitoes. Our study aimed at assessing the impact of three cotton pests control strategies in different ecological settings (conventional, organic and transgenic cotton growing areas) on the i) susceptibility to insecticides (Permethrin and Deltamethrin (pyrethroids), DDT (organochlorine), Bendiocarb (carbamate), chlorpyrifos-methyl (organophosphate) tested on the main malaria vectors and ii) frequencies of kdr L1014F, during rainy season of 2008, 2009, 2013 and 2014 in Burkina Faso, West Africa. From 2008 to 2014, a reduction in susceptibility to all insecticides except chlorpyrifos-methyl was detected in most populations of *An. gambiae* s.l from the three cotton growing areas. The frequency of kdr-w mutations varied depending on localities and species within the *An. gambiae* complex, but we found no relationship between the cotton pest control strategy and the frequency of kdr-w or, otherwise with phenotypic resistance evaluated from bioassays. The implementation of organic cotton and Bt transgenic cotton programs in certain areas of Burkina Faso does not change the insecticide resistance status of *An. gambiae* s.l. populations in the region concerned. These results confirm the worrying status of insecticide multi-resistance of the main malaria vector mosquito species in Burkina Faso.

1. INTRODUCTION

According to World Health Organization (WHO), vector control strategies led to major reductions in malaria cases in many areas around the world (WHO 2018). The scale-up of two principal tools, long-lasting insecticidal nets (LLINs) and to a lesser extent indoor residual spraying (IRS), have contributed up to 33 % reduction in malaria deaths in Africa, the most affected continent, during the last decade (Hemingway 2014).

For impregnating LLINs, the WHO currently recommends six pyrethroids and for IRS, twelve insecticides, belonging to four classes of insecticides (pyrethroids, organophosphates, carbamates and organochlorines) were selected. Unfortunately, malaria vectors are developing resistance to insecticides used in these tools, mainly to pyrethroids. Resistance to this insecticide class is now widespread in *Anopheles gambiae* s.l. populations across sub-Saharan Africa (Dabiré et al. 2009, Ranson et al. 2011). Mutations in *para* -type voltage-gated sodium channel gene (Hemingway and Ranson 2000) providing high levels of cross-resistance to pyrethroids and DDT that share the voltage-gated sodium channel as a target site in mosquitoes that is known to be the main mechanism resistance.

In West Africa, pyrethroid resistance in *Anopheles* mosquitoes is mainly attributed to mutation in the sodium channel target site, the L1014F *kdr* (Dabiré et al. 2009) while another L1014S mutation originated from Kenya known as *kdr* -East (Ranson et al. 2000), was recently detected in the same region, in *An. arabiensis* populations from Benin (Djègbè et al. 2011) and in *An. gambiae* s.s and *An. coluzzii* from Burkina Faso (Namountougou et al. 2013) as well as in *An. gambiae* s.s from Togo, West Africa (Djègbè et al. 2018).

Insecticide resistance in malaria vector populations in Burkina Faso has been reported as early as in the 1960s, when *An. funestus* and *An. gambiae* s.l. (thereafter, *An. gambiae* s.s) populations showed reduced mortality to dieldrin and DDT (Coz et al. 1968, Hamon et al. 1968). After, other studies have confirmed that resistance to DDT is still prevailing at a high level in *An. gambiae* s.l. populations from Burkina Faso, where cross-resistance to pyrethroids due to the *kdr* L1014F mutation was also increasingly reported (Diabaté et al. 2002, Diabate et al. 2004, Dabiré et al. 2009). Moreover, cross-resistance to organophosphates and carbamates due to *ace* -1^R G119S was also detected, although at a moderate frequency (Weill et al. 2003, Djogbénou et al. 2008, Dabiré et al. 2009). Finally, in the humid savannas regions of South-West Burkina Faso, a complex context of multiple resistance is established in *An. gambiae* s.l. populations (Dabiré et al. 2008) with the concomitant presence of *kdr* L1014F and *ace* -1^R mutation genes. This particularly alarming situation constitutes a major impendence to the success of current vector control strategies.

The use of the same chemicals (organophosphates and pyrethroids) in both agriculture and public health could lead to a more rapid development of resistance to these compounds (Gnankiné et al. 2013). It is known that the trend towards insecticide resistance in malaria vectors is partly linked to the current use of these chemicals for crop protection (Diabaté et al. 2002, Gnankiné et al. 2013, Hien et al. 2017, Chabi et al. 2018). According to Akogbéto et al. (2006), residues from insecticide applied on crops can contaminate mosquito-breeding sites, resulting in promoting insecticide resistance selection among mosquito larvae .

Indeed, the population of Africa has more than doubled over the past 30 years, increasing the demand for food and intensifying agricultural production to achieve food security (NEPAD 2013). According to the New Partnership for Africa's Development (NEPAD), 530 million of the total African population (around 48 %) depend on agriculture activities and this population expected to reach 580 million in 2020 (NEPAD 2013). Agrochemical use has increased dramatically in recent years and has resulted in an important reduction in crop losses (NEPAD 2013). At the same time, the African continent has experienced the massive deployment of long-lasting insecticidal nets (LLINs), the most widespread intervention tool against malaria vector, and to a lesser extent indoor residual spraying (IRS). In fine, the massive use of insecticides in both agriculture and public health could establish a double pressure of resistance selection in mosquito vectors. Cotton production remains a crop that requires an important use of insecticides belonging to Pyrethroids (PY) and Organophosphates (OP). Up to now, three cotton pests management strategies have been adopted in some African countries, particularly South Africa and Burkina Faso: i) conventional based on systematic use of insecticides belonging to Pyrethroids, Organophosphates and neonicotinoids classes, ii) organic when

no chemicals are used and iii) transgenic *Bt-cotton* (*Bacillus thuringiensis*) when OP and PY are not used in fields, except neonicotinoids (Gnankine et al. 2018). Transgenic cotton (*Btcotton*) has been adopted as new agricultural practice that aimed at reducing the insecticides amount and their frequency of applications but also at increasing yields (Gnankine et al. 2018). Since 2016, *Bt-cotton* cultivation has suspended by the government of Burkina Faso due to the poor quality of cotton fiber.

With respect to the insecticide resistance increase in diseases vectors, WHO launched the Global Plan for Insecticide Resistance Management in malaria vectors (GPIRM) in 2012 (WHO 2012). GPIRM comprises of five activities (described as five ‘pillars’) spanning in the short, medium and long term, aimed at controlling insecticide resistance to ensure the continued effectiveness of current and future vector control tools to prevent malaria transmission, morbidity and mortality (WHO 2012).

The current study aimed at assessing the impact of the three cotton pest’s management strategies in different ecological settings; conventional, organic and transgenic cotton growing areas on insecticide susceptibility in the main malaria vector in Burkina Faso, West Africa.

2. MATERIALS AND METHODS

2.1 Study area

The study was undertaken in fourteen (14) localities in two bioclimatic zones in Burkina Faso (Fig. 1). The bioclimatic zones include: i) Sudan-sahelian (dry savannah) which extends throughout much of the central part of Burkina Faso and ii) Sudan-savannah (humid savannah) located in the south and west part of the country. Study area is characterized by a rainy period from May to September with average annual rainfall about 1200mm (Namountougou et al. 2019) .

2.2 Cotton pests control strategies

Overall, in the western and central Burkina Faso, cotton remains the main industrial crop and around 90% of total pesticides are applied for its protection. (Ouédraogo et al. 2011). Three strategies to control cotton pests are recommended in Burkina Faso and deployed in the different study sites: i) Conventional cotton cultivation (Banfora, Diébougou, Houndé, Gaoua, Kombissiri, Koubri, Orodara, Soumouso and Vallée du Kou) with high rates of insecticide treatment (N=9 sites). Insecticide applications were done throughout the cotton season (May to September) according to three treatment windows and depending on the abundance and pest species concerned (Table S1). These treatments are based on the temporal rotation of different insecticides including PY, OP/ Carbamates (CX) and neonicotinoids; ii) Organic cotton cultivation (Dano and Tiéfora) without chemical use (N=2 sites) still known as “coton bio”. It has been introduced in Burkina Faso since 2004 but its large-scale production has been supported since 2006 by some development partners who promote this technology; organic cotton areas extend on limited areas (Table S2); iii) transgenic cotton (*Bt*) cultivation (Fada N’Gourma, Koupéla and Manga) with low insecticide use (N= 3 sites). In 2008, as regards emergency and spread of insecticide resistance in cotton pest populations, transgenic cotton was adopted and implemented into Burkina Faso to control pest caterpillars such as *Helicoverpa armigera* (Raj et al. 2002, Chaturvedi 2007), a pest species that causes severe damages. In this technology, no PY and OP are used.

2.3 Mosquito sampling and rearing

Mosquito larvae were sampled during the rainy season of the years 2008, 2009, 2013 and 2014 from their natural breeding sites in the 14 localities throughout the three cotton cultivation areas of Burkina Faso.

In each locality and each date of sampling, all instars of larvae were collected in at least 10 different breeding sites. Specimens collections were pooled per locality. Larvae were brought back to the insectarium of IRSS/Centre Muraz in Bobo-Dioulasso, reared under standard controlled conditions (temperature $25 \pm 2^\circ\text{C}$, relative Humidity: $80 \pm 10\%$ RH and 12:12h Light-Dark) and fed with Tetramin baby[®] fish food every day until adulthood. Upon emergence, we identified mosquitoes morphologically according to standard identification keys described by Gillies MT et Coetzee M (1987). Only, *An. gambiae* complex females were selected and maintained alive on 10% sugar solution until they were used for bioassays.

2.4 Insecticide susceptibility test

Bioassays were performed on 3–4 days old unfed female of *An. gambiae* complex mosquitoes using WHO test kits (WHO 1998, 2013). Insecticide-treated filter papers at the diagnostic dose as recommended by WHO (WHO 1998, 2016) were tested including: two pyrethroids (PY), Permethrin 0.75% and Deltamethrin 0.05%, one organochlorine, DDT 4%, one carbamate, Bendiocarb 0.1%, and one organophosphate, chlorpyrifos-methyl 0.4%. DDT and Permethrin were tested only in 2008 and 2009 while the three others insecticide were tested in 2008, 2009, 2013 and 2014. It is important to note that in Burkina Faso, permethrin was the reference insecticide until 2014 for long-lasting insecticide nets (LLINs) and after that date it was replaced by deltamethrin which is now the insecticide of preference for LLINs. DDT was chosen to verify the presence of cross-resistance to pyrethroids. The others insecticides belonging to the OP and CX families are used as reference insecticides for IRS. Four replicates of batches of 20–25 test mosquitoes were exposed for 1 hour to each insecticide-treated paper. The laboratory reference strain susceptible to all insecticides, *An. gambiae* Kisumu, has been tested on all insecticides impregnated papers as a control. Control mosquitoes (N=20–25 females per test population and the Kisumu strain) were tested for the same time to untreated filter papers. After exposure time, mosquitoes were transferred into insecticide-free observation tubes of the tests kit and maintained on 10% sucrose solution at 25 to 28°C. Final mortality in test and control mosquitoes was examined 24 h after exposure. For further molecular analysis, the samples of mosquitoes tested to different insecticides were kept individually at -20 °C according to their phenotypic state (dead or alive).

2.5 Molecular analysis

Thirty females referring to *An. gambiae* complex per site were randomly chosen within the group of bioassays specimens for molecular tests. They were identified to species member of the *An. gambiae* complex according to PCR described by Santolamazza et al. (2008), and this sampling was considered as representative of the mosquito populations being tested in each locality (Chouaïbou et al. 2008). Their genotype at the *kdr* locus was determined using the diagnostic tests described by Martinez-Torres et al. (Martinez-Torres et al. 1998).

2.6 Statistical analyses

The threshold of susceptibility was fixed at 98% mortality rate for the six active molecules according to the WHO's protocol. When the mortality rates were between 90 and 98%, the population was considered as "suspected resistant". Mortality rates below 90% indicate the presence of insecticide resistance (WHO 2016). Genotypic frequencies at the *kdr* L1014F loci in *An. gambiae* populations were compared to Hardy-Weinberg expectations using the Fischer exact test procedure implemented in GenePop (ver.4.1.4) software (Raymond and Rousset 1995).

The impact of cotton pest management practices on *kdr* L1014F frequencies within *An. gambiae* complex populations was determined using generalized mixed effect models (GLMM). Models were fitted using the glmmTMB function run in the R software (The R Development Core Team 2008). Post-hoc Tukey method was used to do multiple comparison among modalities of the fixed terms using the 'emmeans' function of

the ‘emmeans’ package (Russell et al. 2019). Also, the impact of cotton pest control practices on *kdr* L1014F frequencies in *An. gambiae*s.l. populations was assessed using a Kruskal-Wallis test and a Dunn’s post-hoc test for multiple comparisons (Dinno 2017). A difference is considered as significant when the p-value is less than 0.05.

3. RESULTS

3.1 DDT resistance status

Resistance to DDT 4% observed in all studied sites showed mortality rates ranged from 10% to 80% during 2008 and 2009 (Fig. 2). Koubri, a conventional cotton cultivation area close to the capital Ouagadougou remained an area where a high rate of mortality was detected (80%) (Fig. 2a).

3.2 Permethrin resistance status

The data recorded with permethrin in 2008 showed resistance (mortality rates less than 90%) in the most of sites investigated whatever the pest control strategy. During 2009, overall, \mortality rates were less than 90% in all study sites, confirming resistance to permethrin (Fig. 3b).

3.3 Deltamethrin resistance status

Whatever the cotton pest control strategies adopted from 2008 to 2014, mortality rates observed with deltamethrin 0.05 % in most *An. gambiae* s.l. populations were below the 90% threshold indicating high resistance status of mosquito populations. Apart from that, two sites in 2013 have a suspected resistance status with a mortality rate higher than 90% but lower than 98%: Soumouso (95%), Orodara (94%) from Conventional cotton growing area (Fig. c). Only, in 2008 and 2013, Koubri from Conventional cotton growing area, exhibited a susceptibility to deltamethrin with 100% mortality rate: (Fig. 4a).

3.4 Bendiocarb resistance status

Anopheles gambiae s.l. populations showed an increase in Bendiocarb resistance in most of the studied sites, regardless of the cotton growing area, at the scale of study. Only the populations of Koubri (a conventional cotton-growing area) showed a constant susceptibility to Bendiocarb with mortality rates ranging from 98 to 100% in 2008, 2009, 2013 and 2014.

In 2008, resistance was found in seven sites: Banfora, Diébougou, Orodara, Gaoua, Houndé, Soumouso (Conventional cotton growing area) and Dano (Organic cotton growing area) with mortality rates ranged from 29.5 to 82%. During the year 2009, resistance was observed in eleven sites namely Banfora, Orodara, Gaoua, Diebougou, Soumouso, Manga (Conventional cotton growing area), Tiefora, Dano (Biological cotton growing area) and Fada N’Gourma, Koupéla and Manga (Transgenic cotton growing area) with recorded mortality rates varying from 49.5 to 89%.

In 2013, bendiocarb resistance within *An. gambiae* populations was reported in four sites located in Conventional cotton growing area and one site in Organic cotton growing area where resistance was already observed in both previous years (2008 & 2009). Thus, the mortality rate was 74.9% in Diebougou, 79.7% in Gaoua, 71.4% in Orodara, 79.3% in Soumouso and 76 % in Dano. In 2014, bendiocarb resistance was reported in seven study sites: Vallée du Kou, Diebougou, Orodara, Soumouso, Banfora, Tiefora with mortality rates ranged from 64.7 to 79% and more significantly in Banfora and Orodara with very low mortality rate 15.7% and 37.7% respectively.

At study scale, susceptibility seemed partially restored with regard to mortality rate from 2009 to 2014 in Dano (organic cotton area) (62% in 2009; 75% in 2013 and 82 % in 2014). As for Fada N’Gourma in the transgenic area, mortality rate has risen from 90 to 100 %.

3.5 Chlorpyrifos-methyl resistance status

Anopheles gambiae s.l. populations from all study sites were susceptible to Chlorpyrifos-methyl (CM). Overall, mean mortality rates were between 98–100% in the course of the four years (Fig. 6).

3.6 Distribution of *Anopheles gambiae* complex species according to cotton pest control strategy

Overall, identification of sibling species within the *An. gambiae* complex by PCR analysis shown that mosquito populations were composed of a mixture of *An. coluzzii*, *An. gambiae* s.s and *An. arabiensis* with an alike distribution throughout the three settings of cotton cultivation (transgenic, organic and conventional cotton growing area) (Table S3). *Anopheles gambiae* s.s (55.14%, n=1608/2916) predominated in the three areas of cotton cultivation (transgenic, organic and conventional) following by *An. coluzzii* (25.06%, n=731/2916) and *An. arabiensis* (19.78%, n=577/2916) (Table S3).

The statistical analysis did not show any significant difference regarding the distribution of the *Anopheles gambiae* sl complex species across the three settings of cotton cultivation (Kruskal-wallis, Chi-squared test, $\chi^2=1.41$, df= 2, p-value = 0.49).

3.7 Impact of cotton pest management strategies on *kdr* L1014F frequency

The frequencies of *kdr* L1014F were analyzed according to the members of the *An. gambiae* complex in the three cotton growing areas (Table S4) and show different patterns of variation from 2008 to 2014 (Fig.7). The three cotton pest control strategies recommended in Burkina Faso did not affect the dynamics of *kdr*L1014F frequencies in *An. gambiae* sl populations, nor their evolution over the time of the study (Tukey’s tests, p-values > 0.13).

In the conventional cotton growing area, the frequencies of *kdr*L1014F mutation were almost close to fixation in *An. gambiae* sl populations with values reaching 0.95 at most sites located in the western part of the country as Orodara, Banfora, Tiefora and Houndé. However, in Gaoua and Koubri the frequencies were relatively low in *An. gambiae* s.l in 2008, with values of 0.31 and 0.33 respectively, and then much higher values (0.98) were found in 2014.

As for the transgenic cotton growing area, the frequency of the *kdr* mutation in *An. gambiae* sl populations was 0.61 in Fada N’Gourma, 0.5 in Koupéla and 0.66 in Manga at the beginning of the study (2008). In 2014, an increase in the *kdr* frequency was found at these sites with maximum values of 0.98 in both Koupéla and Fada N’Gourma.

In the organic cotton growing area (Tiefora where data were available from 2008 to 2014), the *kdr* L1014F frequencies were already higher in 2008 with values of 0.92. These frequencies increased in subsequent years to a maximum of 0.98 in 2014.

4. DISCUSSION

Previous studies undertaken in Burkina Faso established that the use of pesticides against crop pests, especially for cotton protection, contributes to the selection of insecticide resistance in malaria vector mosquitoes (Diabate et al. 2002, Gnankiné et al. 2013, Hien et al. 2017).

This original study aims to provide the impact of introduction of a new method of cotton cultivation, transgenic cotton, that is expected to reduce the amount of insecticides and the number of their treatments used against the pests of this crop (Gnankiné et al. 2018) compared to pre-existing cotton cultivation technologies, essentially conventional and to a lesser extent organic, on the insecticide susceptibility of *Anopheles* mosquitoes. This study represents a great opportunity to further our knowledge on the effect of cotton growing systems on the insecticide resistance dynamics of the main malaria vector mosquitoes, belonging to the *An. gambiae* complex, in connection with the eco-climatic regions of Burkina Faso.

Consecutive years of intensive use of chemicals in both agriculture and public health have led to the selection of resistant pests and vector populations as observed for *An. gambiae* in Burkina Faso (Gnankiné et al. 2013). Previous studies have also showed the presence of resistance in the tomato bollworm *Helicoverpa armigera* to Pyrethroids (Martin et al. 2000, 2005, Raj et al. 2002), the whitefly *Bemisia tabaci* to Pyrethroids, Organophosphates and neonicotinoids due to intensive use of PY and OP for crop protection (Gnankiné et al. 2013).

In regard to this situation, the cotton strategy has been modified in conventional cotton growing areas that consisted in reduction of PY and OP use and re-introduction of endosulfan insecticide (Martin et al. 2005). At the same time, nationwide surveys have shown phenotypic and genotypic resistance of *An. gambiae* s.l. populations to PY and OP in agricultural settings including cotton growing areas in central and western Burkina Faso (Chouaïbou et al. 2016, Chabi et al. 2018).

In 2008, genetically modified cotton plants expressing both Cry1Ac and Cry2Ab toxins were implemented in Burkina Faso (Gnankiné et al. 2018). For transgenic cotton, pesticides belonging to the OP and PY families are not used in fields but pesticides from neonicotinoids class are used at the end of the cotton phenological stages (Gnankiné et al. 2018). As far as species of the *An. gambiae* complex concerned, *An. gambiae* s.s., *An. coluzzii* and *An. arabiensis* were identified and the cotton pest strategies do not affect the distribution of these species. Moreover, according to the previous studies investigated in the same climatic zones, the geographical distribution of these species did not change over the past 10 years (Diabate et al. 2004, Dabiré et al. 2012).

In this current study, the proportions of *An. gambiae* s.l. complex populations in Burkina Faso shows that *An. gambiae* s.s. predominates in the west and southwest parts of the country and still lives in sympatry with *An. coluzzii* in proportions of 70% and 30% respectively. In central and east-central parts, *An. coluzzii* was prevalent and was found in sympatry with *An. arabiensis*, being most frequent in the northwest and east-central part of the country with frequencies between 16.7% and 51.5%.

Human activities leading to the creation of permanent streams and ponds have also favoured the emergence of *An. coluzzii*, a species typical of irrigated areas (Pages et al. 2007) as reported in the current study in the rice growing area of the Vallée du Kou. So far, *An. arabiensis* has changed in Soumoussou (rural area) where its proportion has increased significantly in recent years (Namountougou et al. 2019) compared to the present study (from 2008 to 2014) during the same sampling period (e.g. rainy season). The expansion of *An. arabiensis* in this locality is attributed to global ecological changes (such as climate change) or local human activities (such as deforestation) that promote the colonization of this species.

Our phenotypic data exhibited resistance to DDT and permethrin (from 2008-2009) as well as deltamethrin (2008-2014) in all populations of *An. gambiae* s.l. sampled regardless of cotton pest control. The situation is quite different with Bendiocarb. Although resistance to this insecticide has been observed in populations collected in conventional growing areas, the level of susceptibility appears to have been partially restored from 2009 to 2014 at Dano (organic cotton area) and completely restored in Fada N'Gourma (transgenic cotton area) where the mortality rate recorded by bioassays has increased from 90 to 100 %.

The first studies on the resistance of *An. gambiae* s.l. to pyrethroids in West Africa and more particularly in Burkina Faso began in the late 1990s when Chandre et al. (1999) reported cross-resistance to permethrin and DDT in *An. gambiae* s.l. populations (but these populations were then susceptible to deltamethrin) in south-western Burkina Faso. Three years later, this resistance was subsequently confirmed by the works

undertaken by Diabaté et al. (2002) in several parts of the country. Other studies covering different regions of the country have shown that the *kdr* L1014F mutation is the main mechanism involved in cross-resistance to DDT and pyrethroids in *An. gambiae* s.l. populations from Burkina Faso. This resistance mechanism was detected firstly in *An. gambiae* s.s and then *An. coluzzii* inherited it by genetic introgression from *An. gambiae* s.s (Diabate et al. 2004). Recent phenotypic and genotypic analysis have shown that resistance to both DDT and pyrethroids is now widespread among the three species of the *An. gambiae* complex occurred in Burkina Faso (Dabiré et al. 2014, Hien et al. 2017). These malaria vectors, although present in sympatric in most of areas, have divergent ecological preferences and behaviours (Diabaté et al. 2008, Coulibaly et al. 2016). Thus, *An. Arabiensis*, due to its exophagic and exophilic biting behaviours, would spare to the selective insecticide pressure from the main vector control tools (essentially LLINs) as exerted on *An. gambiae* s.s and *An. coluzzii*. Beyond the use of insecticides in public health, particularly through LLINs, the use of insecticides in cotton growing areas also favoured the emergence of this cross-resistance to DDT/pyrethroids in *An. gambiae* s.l. populations (Diabate et al. 2002). This causality may be explained by intense and permanent selection pressure throughout the rainy season, that is exerted on the entire mosquito population at the larval stage in breeding sites and not only on the proportion of biting females. In fact, an increase of insecticide resistance in mosquito vectors was observed during insecticide treatments periods in cotton growing areas (Dabiré et al. 2012). However, by comparing the frequencies of *kdr* L1014F by cotton growing area (transgenic, organic and conventional), no significant differences were found during the four years follow-up, except in the *transgenic* cotton growing area in 2008 and 2013. Indeed, a slight reduction in the frequency of the *kdr* L1014F mutation in this area was detected (from 0.6 to 0.45 in *An. gambiae* s.l. between 2009 and 2013) coupled with the bednet campaign. This reduction should improve the susceptibility of *An. gambiae* s.l. to both DDT and pyrethroids as the *kdr*L1014F mutation is known to be the main mechanism of crossed resistance to DDT/pyrethroids. Unfortunately, this reduction was observed during a very short period of time since an increase of the frequency of the *kdr* L1014F mutation was immediately observed the following year (2014). The high levels of pyrethroids resistance in malaria vectors maintained in transgenic and organic cotton growing areas, both characterized by low insecticide use, are believed to be due to the fact that the *kdr* alleles are widespread in all areas of southern and central Burkina Faso and almost fixed. If the *kdr* mutation is fixed, its frequency will not vary even though the selection pressure of pyrethroid treatments in cotton fields decreases due to its low genetic cost; except in the case of a large influx of migrants who can bring new genetic variants, which seems unlikely given its expansion throughout the West African region.

Moreover, the transgenic and especially organic cotton fields investigated are close to conventional cotton growing areas, or even vegetable areas where agricultural insecticide applications are also practiced. Insecticides residues from conventional or vegetable areas can retain significant selection pressure on neighboring *An. gambiae* s.l. populations. Furthermore, it is not excluded that some cotton farmers by fear of seeing developing some secondary pests not targeted by transgenic *Bt*, treat in secret their fields by OP and PY chemicals except those recommended by the technical route.

In addition to molecular resistance mechanism (target site modification), other mechanism of insecticide resistance like metabolic resistance may be involved in *Anopheles* s.l. populations where *kdr* gene was almost fixed as shown recently by Namountougou et al. (2019). These authors found that most of populations of *An. gambiae* s.s from cotton growing areas (Conventional cotton) of western part of Burkina Faso exhibited high level of GSTs and NSEs enzymes with a level of enzymes might vary in connection with cotton growing areas.

This resistance status is worrying as cotton farmers are heavy consumers of insecticides that have been and continue to be used at large scale in the country that may have an negative impact on the vector control strategies based on insecticides applications (LLINs or IRS). This situation of insecticide resistance requires regular annual monitoring for a better vector control management. This could already lead to predict a possible temporal rotation of the insecticidal classes, especially in IRS, but also the need to find new insecticides or innovative methods to prevent and control vector-borne diseases as recommended by WHO (WHO 2012, 2019).

5. CONCLUSION

The current study on the multi-year monitoring of insecticide resistance in *An. gambiae s.l.* populations in different cotton growing areas of Burkina Faso showed widespread pyrethroids resistance in the main malaria vector species. Vector control strategies must take into account cotton pest control strategies. Bioassays conducted with diagnostic doses according to the WHO protocol indicate widespread cross-resistance to DDT and pyrethroids due mainly to the *kdr*L1014F mutation, regardless of the cotton growing system.

In the future, it would be interesting to use the topical method to better assess the level of resistance in these different vector populations as well as the evolution of this resistance over time and space according to the agricultural use of insecticides. There is no doubt that for better control strategies, studies on the enzymatic activity generally involved in insecticide metabolism (oxidases esterases and glutathion-S-transferases) associated with the involvement of other target modification mechanisms such as the *ace-1* mutation would be useful to improve knowledge on resistance mechanisms, their respective role and their distribution within vector populations according to different agricultural practices.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHORS' CONTRIBUTION

MN, DDS and RKD participated in the study design and supervised the field study. MN and DDS participated in conducting bioassays performed in the laboratory and molecular analysis. NM, DDS and PSS participated in sample collection in field. MN, DDS and OG analyzed the data and drafted the manuscript which was revised by the co-authors. All authors approved the final version of the manuscript.

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DATA AVAILABILITY STATEMENT

Data underlying this paper will be available on Dryad, with

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Figure Legends

Figure 1. Geographic map of sampling sites in Burkina Faso

Figure 2. DDT 4% mortality rates in *Anopheles gambiae*s.l. test populations from Burkina Faso collected in the 2008 and 2009 rainy season

Figure 3. Permethrin 0.75% mortality rates in *Anopheles gambiae* s.l. test populations from Burkina Faso collected in the 2008 and 2009 rainy season.

Figure 4 . Deltamethrin 0.05% mortality rates in *Anopheles gambiae* s.l. test populations from Burkina Faso collected in the 2008, 2009, 2013 and 2014

Figure 5. Bendiocarb 0.1% mortality rates in *Anopheles gambiae* s.l. test populations from Burkina Faso collected in the 2008, 2009, 2013 and 2014 rainy season.

Figure 6. Chlorpyrifos-Methyl mortality rates in *Anopheles gambiae* s.l. test populations from Burkina Faso collected in the 2008, 2009, 2013 and 2014 rainy season.

Figure 7. Variations of *kdr* L1014F frequency according to the cotton pest management scheme

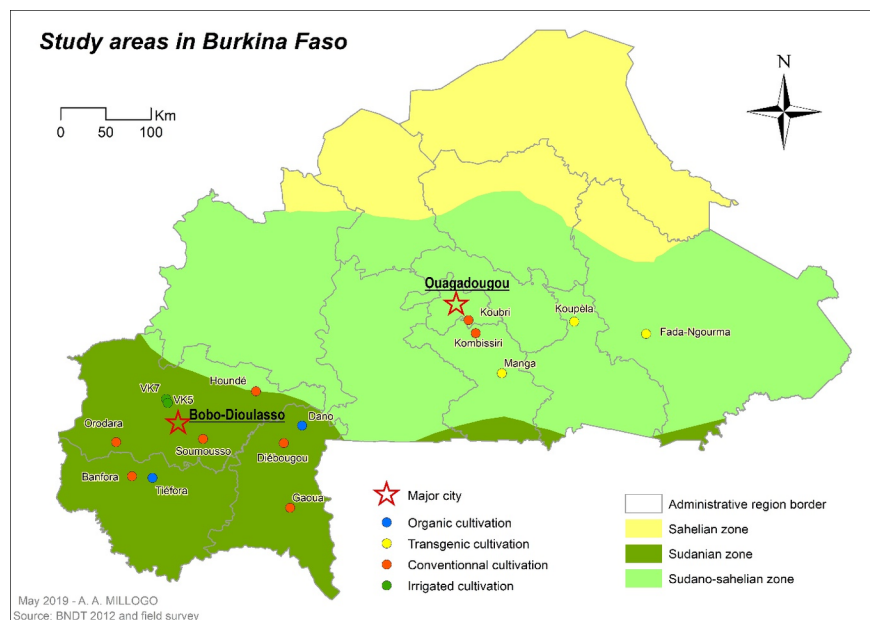


FIGURE 1 Geographic map of sampling sites in Burkina Faso

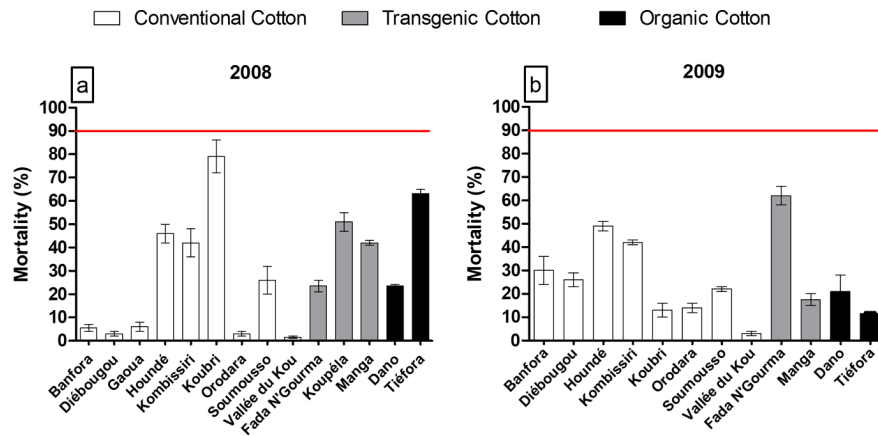


FIGURE 2 DDT 4% mortality rates in *Anopheles gambiaes.l.* test populations from Burkina Faso collected in the 2008 and 2009 rainy season

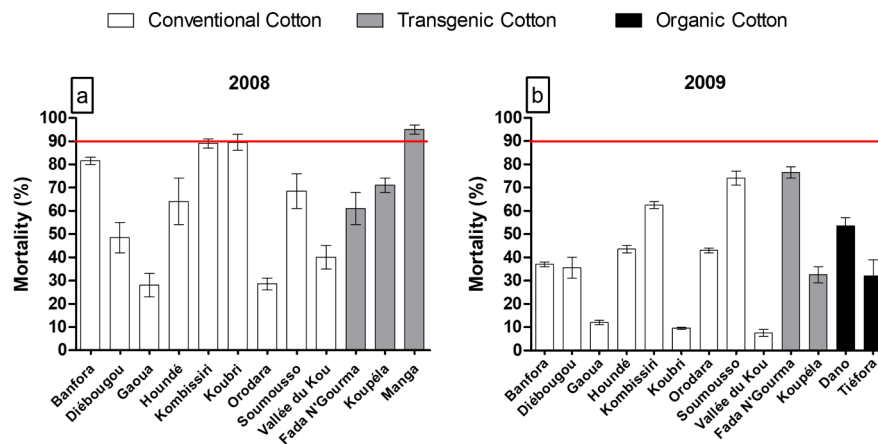


FIGURE 3 Permethrin 0.75% mortality rates in *Anopheles gambiae s.l.* test populations from Burkina Faso collected in the 2008 and 2009 rainy season.

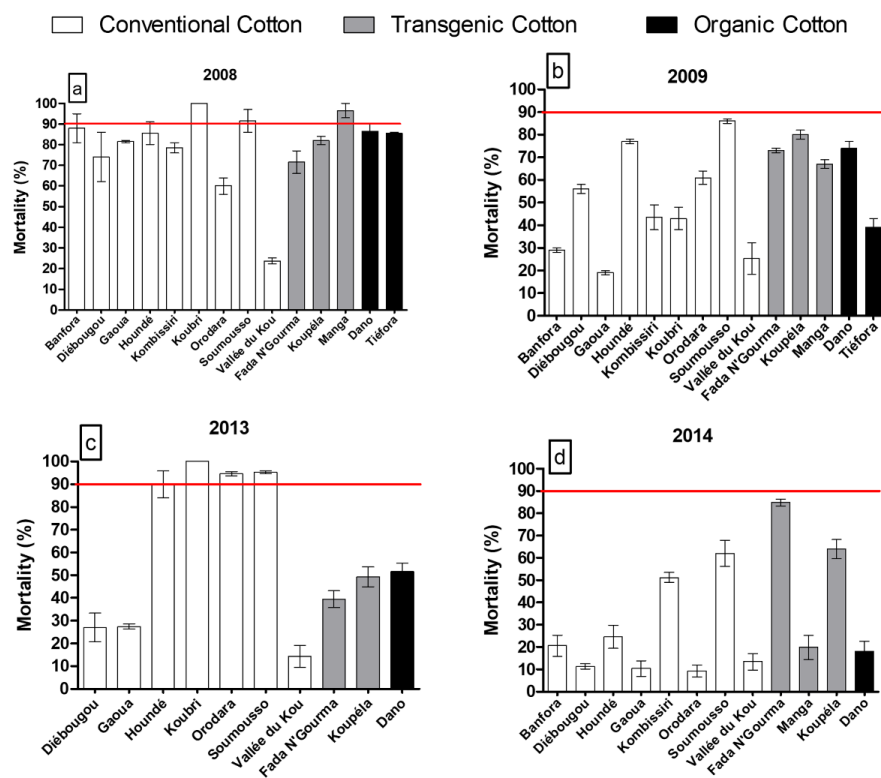


FIGURE 4 Deltamethrin 0.05% mortality rates in *Anopheles gambiae* s.l. test populations from Burkina Faso collected in the 2008, 2009, 2013 and 2014

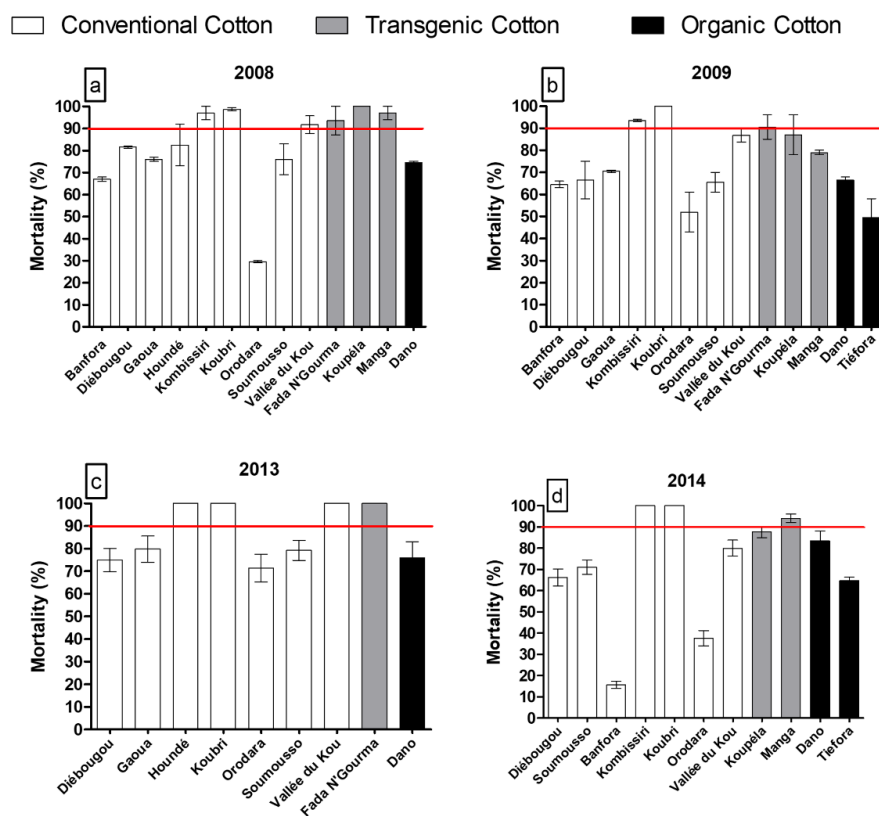


FIGURE 5 Bendiocarb 0.1% mortality rates in *Anopheles gambiae* s.l. test populations from Burkina Faso collected in the 2008, 2009, 2013 and 2014 rainy season.

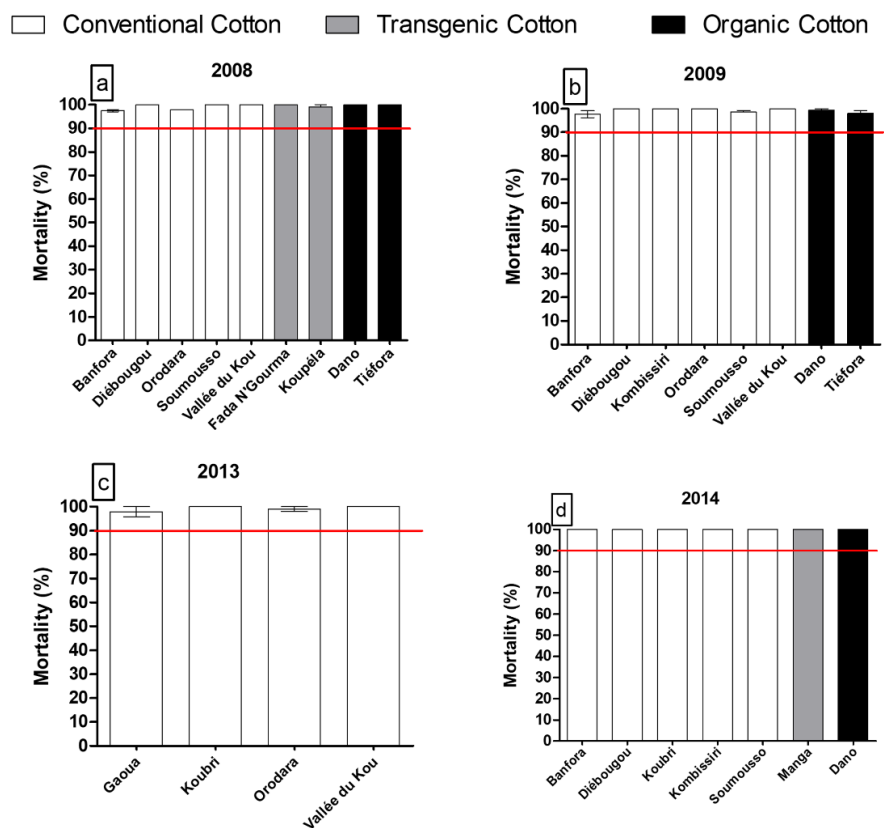


FIGURE 6 Chlorpyrifos-Methyl mortality rates in *Anopheles gambiae* s.l. test populations from Burkina Faso collected in the 2008, 2009, 2013 and 2014 rainy season.

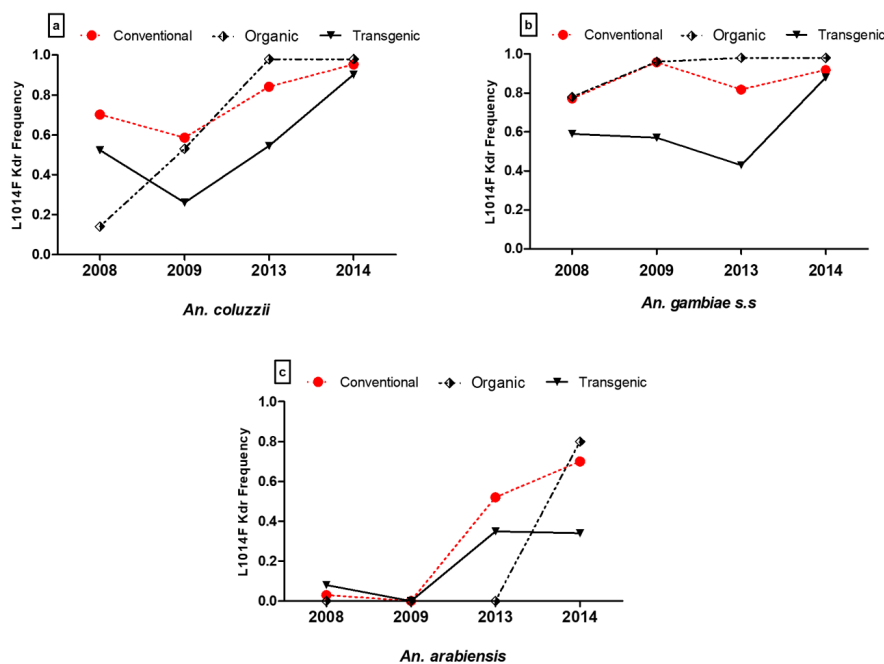


FIGURE 7 Variations of *kdr* L1014F frequency according to the cotton pest management scheme

SUPPORTING INFORMATION

Table S1. Main chemical insecticides (active ingredient) used in conventional cotton growing areas in Burkina Faso

Table S2. Cotton cultivation area according to the three pest control strategies in Burkina Faso from 2007 to 2014

Table S3. Geographical distribution of species of *An. gambiae* s.l in Burkina Faso according to the three cotton pest management strategies

Table S4. Allelic and genotypic frequencies at the *kdr* locus in *An. gambiae* s.l. populations in Burkina Faso from 2007 to 2014

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Graphical figure.jpg available at <https://authorea.com/users/302142/articles/432157-use-of-different-cotton-pest-control-strategies-conventional-organic-and-transgenic-had-no-impact-on-insecticide-resistance-in-anopheles-gambiae-s-l-populations-in-burkina-faso-west-africa>