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THE ROLE OF AGRICULTURAL USE OF INSECTICIDES IN RESISTANCE TO PYRETHROIDS IN *ANOPHELES GAMBIAE* S.L. IN BURKINA FASO

ABDOULAYE DIABATE, THIERRY BALDET, FABRICE CHANDRE, MARTIN AKOGBETO, T. ROBERT GUIGUEMDE, FRÉDÉRIC DARRIET, CÉCILE BRENGUES, PIERRE GUILLET, JANET HEMINGWAY, GRAHAM J. SMALL, AND JEAN MARC HOUGARD

Laboratoire d'Entomologie et de Parasitologie, Centre Muraz, Bobo-Dioulasso, Burkina Faso; Institut Pierre Richet Bouaké, Bouaké, Côte d'Ivoire; Centre de Recherches Entomologiques de Cotonou, Cotonou, Bénin; Laboratoire de Lutte Contre les Insectes Nuisibles (Institut de Recherché pour le Développement/Montpellier), Montpellier, France; World Health Organization, Geneva, Switzerland; School of Biosciences, Cardiff University, Main College, Cardiff, United Kingdom

Abstract. Agricultural use of insecticides is involved in the selection of resistance to these compounds in field populations of mosquitoes in Burkina Faso. *Anopheles gambiae* s.l. was resistant to permethrin and DDT in cotton-growing and urban areas, but susceptible in areas with limited insecticide selection pressure (rice fields and control areas). Nevertheless, resistance to these insecticides was observed in a village on the outskirts of the rice fields at the end of the rainy season, suggesting that the latter population of mosquitoes had migrated from the surrounding cotton villages into the rice fields. A seasonal variation of resistance observed in the cotton-growing area is related to the distribution of the molecular M and S forms of *An. gambiae*, since resistance to pyrethroids has so far only been reported in the S form. Pyrethroid resistance in west African *An. gambiae* was conferred by target site insensitivity through a knockdown resistance (kdr)–like mutation, which was present at high frequencies in mosquitoes in the cotton-growing and urban areas.

INTRODUCTION

Resistance to insecticides has become a limiting factor in the use of these compounds in the control of many insect pests. Mosquito control has focused on the use of insecticides (initially organochlorines, followed by organophosphates and carbamates) through indoor residual spraying. By 1990, more than 500 species of insects and mites had developed resistance to one or more classes of insecticides.[1] The use of insecticide-treated bed nets (ITNs) for both individual and collective protection against malaria has shown potential, reducing childhood malaria morbidity by 50% and global mortality by 20–30% in The Gambia, Ghana, and Kenya.[2–4] The insecticides of choice for bed net impregnation are pyrethroids because of their high efficacy, rapid rate of knockdown, strong mosquito excito-repellent properties, and low mammalian toxicity. The World Health Organization (WHO) recommends the large-scale use of ITNs to control malaria transmission because they offer a good cost-efficiency ratio based on active community involvement. Analysis of pyrethroid resistance in *Anopheles gambiae* s.l. is complicated by the presence of several members of the species complex throughout much of its range, and the occurrence of different chromosomal forms within *An. gambiae* s.s. Resistance to pyrethroids in *An. gambiae* s.l. has been reported in west Africa.[5–7] Field and laboratory studies in Côte d'Ivoire in 1999 showed that *An. gambiae* s.l. had developed cross-resistance to many pyrethroids.[8] Despite this, pyrethroid impregnated ITNs still achieve good control of resistant populations.[9] As in several other insect species, a knockdown resistance (kdr)–based mechanism caused by a single point mutation in the parasodium channel gene is the main mechanism of resistance to pyrethroids in *An. gambiae* s.l.[10–12] This kdr mutation was present only in the S molecular form of *An. gambiae* s.s. in the tropical savanna area. Recently, a different kdr mutation was found that conferred pyrethroid resistance in association with a monooxygenase-based mechanism in *An. gambiae* s.s. from eastern Africa.[13]

The present survey was carried out in Burkina Faso in 1999 and 2000. It was designed to evaluate resistance to pyrethroids in *An. gambiae* s.l. at different sites and correlate resistance with the use of insecticides in these areas.

MATERIALS AND METHODS

Study area. The study was carried out in four localities in Burkina Faso chosen because of their different patterns of insecticide use (Figure 1). The localities were 1) a rice field cultivation area with two sampling sites, village VK5, located in the center of the rice fields and village VK7, located on the outskirts of the rice fields; 2) a cotton-growing area (Léna); 3) an urban area (Bobo-Dioulasso) with two sampling sites, Dsso ba, located in the center of the city and Kuinima, located on the outskirts of the city; and 4) a control area, Batié, a site with very limited use of insecticides. All of these study sites were located in the same climatic area (annual rainfall = 1,000 mm).

Mosquito strains and bioassays. Mosquitoes were collected as larvae during the rainy season and brought back to the laboratory for emergence of adults. A susceptible strain of *An. gambiae* s.s. from Kisumu (Kenya) was provided by the Laboratoire de Lutte Contre les Insectes Nuisibles, WHO Collaborating Center for Vector Control (Montpellier, France) and used as a reference strain.

Adult susceptibility assays were carried out using 1% permethrin (cis:trans = 25:75)- and 0.05% deltamethrin-impregnated filter papers as recommended by the WHO. Resistance to 4% DDT was checked in the same populations for an initial prediction of cross-resistance patterns and underlying resistance mechanisms. Filter paper impregnated according to WHO specifications was provided by the Institut Pierre Richet de Bouaké, WHO Collaborating Center for Vector Control (Bouaké, Côte d'Ivoire). The WHO test kits for adult mosquitoes were used.[14] Tests were carried out in the laboratory of the Center Muraz in Bobo-Dioulasso, Burkina Faso. All tests were done on 2–5-day-old, non-blood-fed, female mosquitoes. In addition to mortality after a 24-hour recovery period, insecticide knockdown effects were recorded after 10-, 20-, 30-, 40-, and 60-minute exposures. Fifty and ninety-five percent knockdown times (KDT_{50} and KDT_{95}) were es-

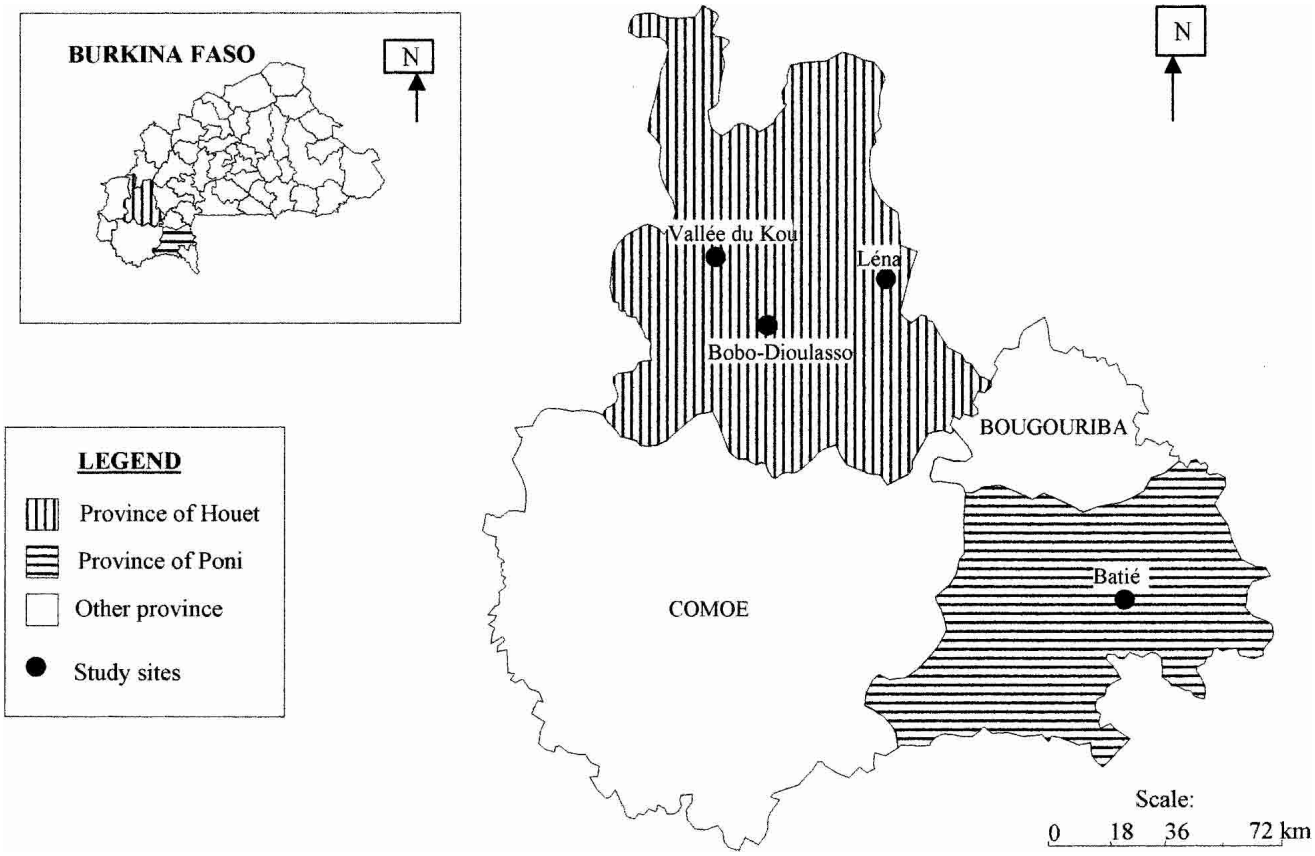

FIGURE 1. Study areas in Burkina Faso.

timated using a log time-probit model.[15] Samples were defined as resistant if they showed less than 90% mortality with 4% DDT and less than 95% mortality with 1% permethrin and 0.05% deltamethrin.[6]

Mosquito species, molecular forms, and the presence of the kdr mutation. Mosquitoes used in bioassays were identified to the species level using the polymerase chain reaction technique described by Scott and others[16] and analyzed for the prevalence of kdr mutations.[11] *Anopheles gambiae* s.s. mosquitoes were identified as being the M or S form by polymerase chain reaction–restriction fragment length polymorphism.[17]

RESULTS

Mortality. Mortality in control groups was consistently less than 5%. Thus, no correction of test sample data for observed control mortality was required. All WHO recommended discriminating dosages of insecticides caused 100% mortality in the susceptible Kisumu strain.

Mosquitoes collected in cotton-growing and urban areas during the 1999 rainy season were resistant to permethrin and DDT, but susceptible to deltamethrin (Figure 2). In the control area, complete susceptibility to the three insecticides was observed. The rice field area showed variable results. In July, mosquitoes collected in VK5, a village located in the center of the rice fields, were susceptible to permethrin and deltamethrin, but resistant to DDT. Four months later, when the rice was fully grown, mosquitoes collected from VK7, a village located on the outskirts of rice fields, were resistant to permethrin and DDT. When compared with the control area, significantly lower mortality with permethrin and DDT ($P < 0.05$) was obtained with mosquitoes from all test areas except from VK5, the rice field area. The numbers of mosquitoes tested from the different field sites ranged from 75 to more than 100.

Resistance levels were again tested in the cotton-growing, urban, and rice field areas in 2000 to assess the temporal variation of the resistance in mosquitoes. In the cotton-growing area, mosquitoes were susceptible to permethrin in the dry season (January and June). As the rainy season began, selection for resistance to permethrin was observed and mosquitoes became more resistant from July to September (Figure 3). In VK5, mosquito susceptibility remained unchanged throughout 2000. In the dry season, mosquitoes were already resistant to permethrin in the urban area and resistance increased during the rainy season. The KDT_{50} of the mosquitoes tested, when compared with that of the Kisumu strain, was slightly increased even with deltamethrin, which achieved a high mortality in all areas tested (Table 1). This knockdown time to permethrin and DDT was significantly increased ($P < 0.05$) in VK7, Léna, and Dsso ba compared with the susceptible strain.

Mosquito species, molecular forms, and presence of the kdr mutation. Two hundred eighty mosquitoes were identified to species and molecular forms and analyzed for the kdr mutation. *Anopheles arabiensis* composed only a small percentage of the total *An. gambiae* s.l. population throughout the year,

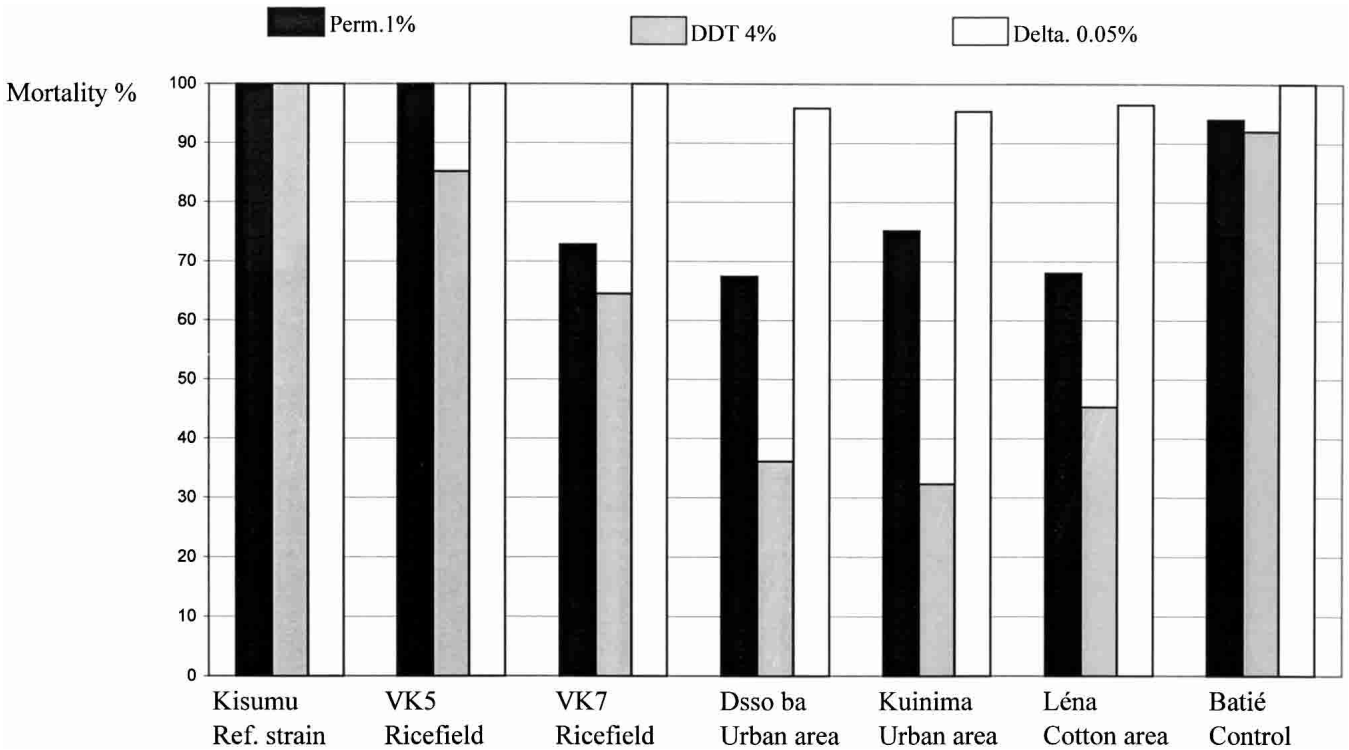

FIGURE 2. Mortality of *Anopheles gambiae* s.l. in Burkina Faso after a one-hour exposure to insecticide-impregnated paper in World health organization tubes. Perm. = permethrin; Delta. = deltamethhrin; Ref. = reference.

except in Léna during the dry season (Table 2). During the rainy season, most *An. gambiae* s.s. were of the S form, with the M form being found only in the rice field area. During the dry season, the frequencies of the M forms of both *An. gambiae* s.s. and *An. arabiensis* increased, especially in the cotton-growing area, where the S form of *An. gambiae* s.s. was almost completely eliminated. This occurred at a significantly higher frequency in the cotton-growing and urban areas than in

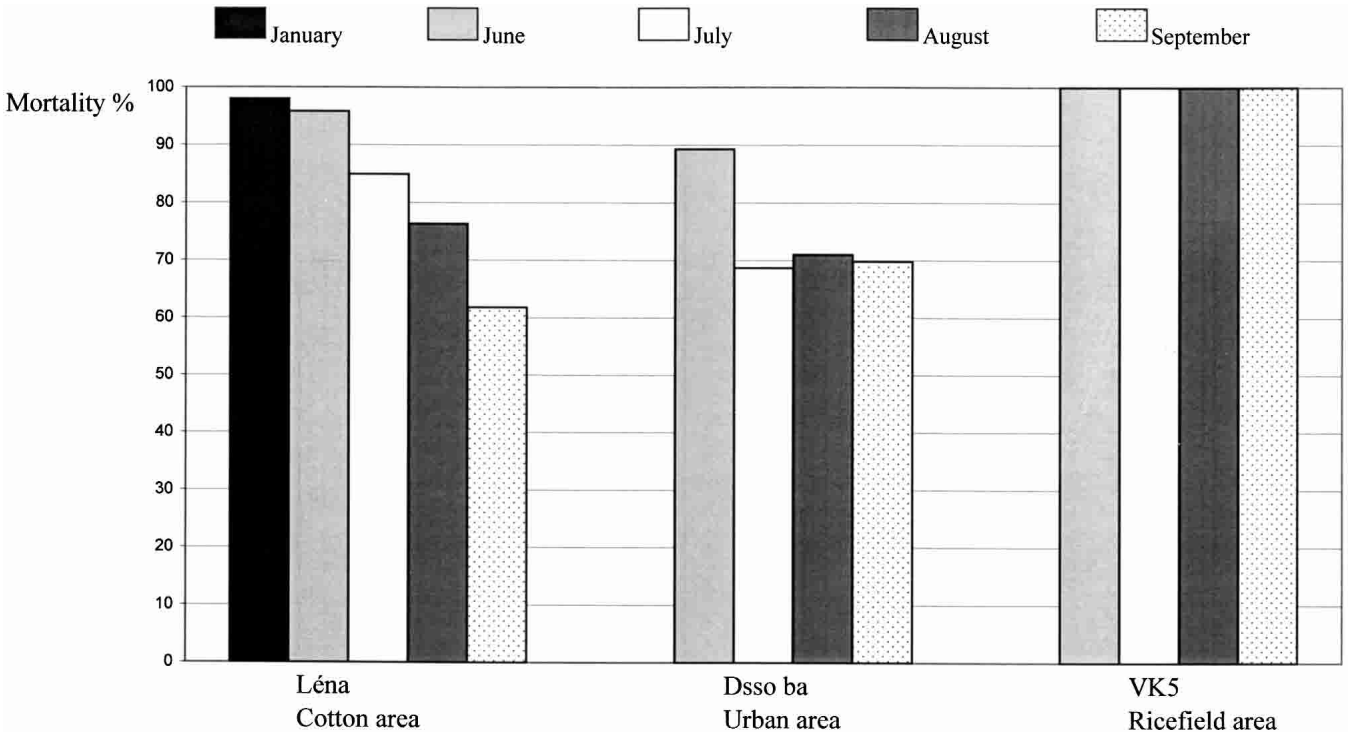

FIGURE 3. Mortality levels of *Anopheles gambiae* s.l. after exposure to 1% permethrin in three areas of Burkina Faso from January to September 2000.

TABLE 1
Knockdown times (KDTs) in *Anopheles gambiae* s.l. in Burkina Faso after exposure to fixed insecticide dosages in a tarsal contact assay*

Insecticide	Area	Locality	KDT_{50} (min)	KDT_{95} (min)	Status
1 %					
Permethrin	†	–	10.4	15.2	S
		VK5	16.9	31.3	S
	Rice field	VK7	22.9	94	R
		Dsso ba	86.8	382.1	R
	Urban	Kuinima	57	382.1	R
	Cotton-growing	Léna	39.3	295.4	R
	Control	Batié	12.3	36	S
4%					
DDT	†	–	30.3	49.9	S
		VK5	33	93.9	R
	Rice field	VK7	36.2	163.5	R
		Dsso ba	992.1	Impossible	R
	Urban	Kuinima	123.3	Impossible	R
	Cotton-growing	Léna	105.9	Impossible	R
	Control	Batié	24.8	124.4	S
0.05%					
Deltamethrin	†	–	9.6	13.4	S
		VK5	14.3	27.8	S
	Rice field	VK7	21.8	37.4	S
		Dsso ba	29.4	53.8	S
	Urban	Kuinima	30.6	58.7	S
	Cotton-growing	Léna	22.4	46.7	S
	Control	Batié	17.8	34.2	S

* S = susceptible; R = resistant. Mortality was significantly lower than in the Kisumu strain ($P < 0.05$).
†

Batié. The kdr mutation was present in all areas tested except in the rice field village (VK5). The M forms of *An. gambiae* s.s. and *An. arabiensis* did not have the kdr mutation.

DISCUSSION

In this study, resistance to permethrin and DDT in *An. gambiae* s.l. was found in the cotton-growing and urban areas of Burkina Faso. We also observed higher mosquito mortality with the WHO discriminating dosage of deltamethrin. Extensive use of insecticides for in the cotton-growing area may explain the high level of resistance observed in this region.[7] Cotton farmers in Burkina Faso are obliged to use increased amounts of insecticides to avoid losses that can amount to half of their yield.[18] In the urban area of Bobo-Dioulasso, resistance to both permethrin and DDT can be explained by the extensive domestic use of insecticides as bomb sprays or coils. A sociologic knowledge, attitude, and practice (KAP) survey in this city showed that more than 95% of the households use coils frequently to protect themselves against mosquito bites (Ouédraogo JB and others, unpublished data). This finding is consistent with a study carried out in 1993 in Bouaké, Côte d'Ivoire in which Elissa and others[5] reported permethrin resistance in *An. gambiae* s.l. that was attributed to massive use of pyrethroids in households (coils, aerosols). In the rice-growing area, resistance differed in mosquitoes from the two study villages. During the rainy season, when mosquitoes were collected in VK5, we observed low resistance to DDT but not to permethrin. At the end of the rainy season, when the rice was fully grown, which prevented larval breeding in

TABLE 2
Frequency of knockdown resistance (kdr) mutations, species identification, and molecular forms*

		Anopheles gambiae s.s.			S form			M form			*An. arabiensis*		
Season	Area	%	N	F (kdr)	%	N	F (kdr)	%	N	F (kdr)	%	N	F (kdr)
Rainy	VK5 Rice field	98.2	55	0	0	–	–	100	55	0	1.8	1	0
	VK7 Rice field	96.7	30	44.2	46.4	13	88.5	53.6	15	0	3.3	1	0
	Dsso ba Urban	100	61	95.6	100	61	95.6	–	0	–	–	0	–
	Léna Cotton-growing	96.2	51	89.6	100	51	89.6	–	0	–	3.8	2	0
	Batié Control	94.2	33	18.1	100	33	18.1	–	0	–	5.8	2	0
Dry	Dsso ba Urban	91.7	22	83.3	85.7	18	97.2	14.3	3	0	8.3	2	0
	Léna Cotton-growing	75	15	20	28.6	4	75	71.4	10	0	25	5	0

* F values are frequences of the kdr mutation.

rice fields, mosquitoes in VK7, a village at the periphery of the rice fields, were resistant to both permethrin and DDT. Mosquitoes collected in VK5 were mainly of the M form, while those collected in VK7 were of mixed forms (M and S). The resistant mosquitoes from VK7 were only of the S form and might have migrated from the areas of high resistance into the rice fields. Insecticides are used less in the rice fields than in cotton-growing areas and the selection pressure is therefore lower in this area.

Results from the survey conducted in 2000 showed that resistance in mosquitoes varied with the season. In the dry season, mosquitoes were susceptible to all three insecticides tested in the cotton-growing area. During this season, insecticides are not used. During the rainy season, use of insecticides to protect cotton plants increases, which exerts selective pressure on the mosquito population and results in an increase in resistance. The kdr mutation, which is probably the main mechanism of pyrethroid resistance in this area, was found only in the S form of *An. gambiae* s.s.[12,13] The absence of the kdr mutation in the M form mosquitoes is at least partly due to the M form being genetically isolated from the S form by a strong barrier to gene flow. In tropical areas, the molecular study of natural populations support the stability of genetic differentiation and the existence of effective isolation barriers, presumably acting at the premating level.[17,19] However, in southern Benin, the kdr mutation was recently detected in the M population.[20,21] The low genetic diversity in the sodium channel introns of the resistant M and S mosquitoes suggests that a genetic sweep has occurred through an introgression from the S form.[21] On the basis of this resistance survey in 2000, resistance is absent in *An. arabiensis* or the M form of *An. gambiae* s.s. in our study area. The frequencies of these species increase during the dry season. There is no intensive use of insecticides during that period. Mosquitoes that were collected from VK5 were resistant to DDT, but not to permethrin. The kdr mutation was not detected in mosquitoes from this site, suggesting that a glutathion-S-transferases (GST)-based metabolic mechanism of resistance may be present. The knockdown time is an important parameter in detecting early resistance in mosquitoes since the KDT_{95} to DDT in the control area consistently increased, although mosquitoes were susceptible to this insecticide.

Resistance to insecticides in insect vectors constitutes a great problem in disease control. In a previous study, we demonstrated that resistance to pyrethroids occurred primarily in the cotton-growing area of Burkina Faso.[7] This study has shown the variability of resistance to pyrethroids throughout the year, which is related to the use of insecticide in this country. We now plan to look for pesticide residues in mosquitoes-breeding sites to confirm the actual involvement of agriculture in the selection of resistance in mosquitoes. Any strategy of resistance management needs to consider the different selection pressures and allow accurate resistance monitoring. The use of molecular and biochemical techniques can provide a better understanding of gene flow between members of the *An. gambiae* sibling species complex. We will continue to monitor these field populations for movement of resistance genes between taxa as an indicator of gene flow.

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Authors' addresses: Abdoulaye Diabate, Thierry Baldet, and T. Robert Guiguemde, Laboratoire d'Entomologie et de Parasitologie, Centre Muraz, BP 153, Bobo-Dioulasso, Burkina Faso, Telephone: 226-97-01-02, Fax: 226-97-04-57, E-mail: a_diabate@hotmail.com. Fabrice Chandre, Institut Pierre Richet Bouaké, BP 1500, Bouaké 01, Côte d'Ivoire, Telephone: 225-81-32-14, Fax: 225-63-27-38. Martin Akogbeto, Centre de Recherches Entomologiques de Cotonou, 06 BP 2604, Cotonou, Bénin, Telephone: 229-33-08-25, Fax: 229-33-08-25. Jean Marc Hougard, Cécile Brengues, and Frédéric Darriet, Laboratoire de Lutte Contre les Insectes Nuisibles (Institut de Recherche pour le Développement/Montpellier), 911 Avenue Agropolis, BP 5045, 34032 Montpellier Cedex 1, France, Telephone: 33-4-67-04-32-23, Fax: 33-4-67-54-20-44. Pierre Guillet, World Health Organization, 1211 Geneva 27, Switzerland, Fax: 41-22-791-4869. Janet Hemingway and Graham J. Small, School of Biosciences, Cardiff University, Main College, Cardiff, United Kingdom, Telephone: 44-1222-874-621, Fax: 44-1222-874-305.

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