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**Impact of pest management practices on the frequency of insecticide resistance alleles in *Bemisia tabaci* (Hemiptera: Aleyrodidae) populations in three countries of West Africa.**

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35 **ABSTRACT**

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37 In West Africa, the use of organophosphates and pyrethroids insecticides to control cotton  
38 pests has led to the evolution of resistance in field populations of *Bemisia tabaci* Gennadius.  
39 Three pest management programs have been commonly recommended: the Conventional  
40 Program (CP) where 6 treatments are applied, the use of *Bt* cotton plants for which only 2  
41 applications of neonicotinoids are required and that has been adopted in many countries, and a  
42 biological program (BP) without any chemical treatment. The present study aims to determine  
43 the influence of these practices on the frequency of mutations that confer resistance to  
44 pyrethroids (mutation L925I in the *para*-type voltage-gated sodium channel gene) and  
45 organophosphates (mutation F331W in the acetylcholinesterase enzyme *ace1*: allele *Ace1<sup>R</sup>*) in  
46 *B. tabaci* populations using *Bt* cotton and CP areas in Pô and Saria (Burkina Faso), CP and  
47 BP areas in Kandi (Benin) and only CP areas in Tové and Infa (Togo). All individuals  
48 sampled belonged to the MED (biotypes MED-Q1) and Africa Silver Leafing (ASL) species.  
49 MED-Q1 was found in sympatry with ASL in Burkina Faso both on CP and *Bt* cotton areas at  
50 variable frequencies. In Togo and Benin, only ASL was found, except in Tové where MED-  
51 Q1 was also detected, but at low frequency. Frequencies of mutations that confer resistance  
52 varied between localities and species but we did not find any strong evidence of a relationship  
53 between the pest management program and these frequencies except for the allele *Ace1<sup>R</sup>* in  
54 Burkina Faso for which the frequencies decrease when chemical applications are reduced.  
55 This study provides valuable information for the development of efficient integrated pest  
56 management programs.

57 **Key words:** Pest management programs, insecticides, *kdr*, *Ace1<sup>R</sup>*, *Bemisia tabaci*.

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## 62 **1. Introduction**

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64 In western Africa, cotton is an economically important crop providing substantial  
65 incomes for farmers. However, the cotton plant is attacked by key pests including the cotton  
66 bollworm, *Helicoverpa armigera* (Lepidoptera: Noctuidae) and the whitefly *Bemisia tabaci*  
67 Gennadius (Hemiptera: Aleyrodidae). Three main spraying programs are recommended to  
68 control these pests in many countries of West Africa. They include the Conventional Cotton  
69 program (CP), the Biological Program (BP) and the transgenic *Bt* cotton program (*Bt* Cotton).  
70 The CP is based on calendar-based applications of insecticides belonging to pyrethroids,  
71 organophosphates and neonicotinoids families separately or as a mixture (Gnankiné et al.,  
72 2013a; Silvie et al., 2013). They are applied with temporal rotations during the whole cotton  
73 season (from May to October). The repeated use of such insecticides have imposed strong  
74 selection pressures on target pests' populations, resulting in the evolution of field resistances  
75 (Houndeté et al., 2010). Over \$60 millions of chemicals were spent for chemical pests control  
76 in Burkina Faso (Greenplate et al., 2006). The BP relies on the use of biopesticides and  
77 natural fertilizers without utilization of any chemical. The *Bt* cotton program uses *Bt* (*Bacillus*  
78 *thuringiensis*) transgenic cotton plants that express two crystal toxins (Cry1Ac and Cry2Ab)  
79 that target some major lepidopteran pests but are harmless to vertebrates and most other  
80 organisms (Mendelsohn et al., 2003; Sanahuja et al., 2011; Pardo-Lopez et al., 2013). In this  
81 program, only neonicotinoids are used at the end of the cotton phenological stages (Héma et  
82 al., 2009). It was initiated in 2008 in West Africa but only in Burkina Faso. In 2016, the  
83 Burkina Faso government suspended the use of *Bt* technology due to the quality of cotton  
84 fiber which is shorter than the fiber of conventional cotton.

85 One of major threat to cotton plant remains the whitefly *B. tabaci*. It causes damages directly  
86 through phloem feeding and indirectly through the transmission of plant viruses. *B. tabaci* is a

87 complex of cryptic species whose taxonomy is still not entirely resolved. Based on the current  
88 consensus, *B. tabaci* is mostly represented by the MED species in western Africa, even  
89 though AnSL species can also be found (Gnankiné et al., 2013b; Gnankiné et al., 2013c).  
90 Within the MED species, different biotypes are encountered including MED-Q1, MED-Q3  
91 and ASL. Actually, recent analyses suggest that MED-Q1 and ASL do not hybridize in the  
92 field and that ASL is thus a different species (Mouton et al., 2015). Interestingly, these  
93 species/biotypes differ in terms of host plants range and insecticide resistance traits.  
94 Generally, MED-Q1 is predominant in cotton areas and sometimes found in sympatry with  
95 ASL on vegetables crops (Gnankiné et al., 2013b). It is also associated with higher levels of  
96 resistance to some insecticides as pyrethroids, organophosphates and neonicotinoids  
97 (Gnankiné et al., 2013a). In *B. tabaci*, two mutations in the *para*-type voltage-gated sodium  
98 channel gene, L925I and T929V, and one mutation in the acetylcholinesterase enzyme *aceI*  
99 (F331W: allele *AceI<sup>R</sup>*) confer resistance to pyrethroids and organophosphates, respectively  
100 (Roditakis et al., 2006; Alon et al., 2008; Tsagkarakou et al., 2009). Recent studies showed  
101 that the *AceI<sup>R</sup>* was found in both MED-Q1 and ASL but, while this resistant allele was almost  
102 fixed in MED-Q1 (0.99), its frequency was 0.59 in ASL (Mouton et al., 2015). In addition,  
103 while the L925I mutation in the sodium channel gene is almost fixed in MED-Q1 populations,  
104 it is rarely detected in ASL. The T929V was never found in *B. tabaci* populations from West  
105 Africa (Mouton et al., 2015). The objectives of the present study were to perform a first  
106 analysis of the impact of the agricultural practices on the *B. tabaci* biotypes/species  
107 composition and diversity, and the frequencies of alleles that confer resistance to pyrethroids  
108 and organophosphates. Sampling was performed in three countries of Western Africa:  
109 Burkina Faso, Benin and Togo.

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## 113 **2. Materials and methods**

### 114 2.1 Management of cotton pests

115 Three pest control programs are recommended in West African countries:

116 (i) The Conventional Program (CP) is based on two to four treatments with pyrethroids (PY)  
117 plus organophosphates (OP) and 2 other treatments with neonicotinoids (see table 1 for  
118 details).

119 (ii) The Biological control Program (BP) does not use chemicals for plant protection. Farmers  
120 worked under the supervision of technicians from the Beninese Organization for Organic  
121 Farming Promotion (OBEPAP) who participated in the implementation and the survey of  
122 good agricultural practices on organic cotton.

123 (iii) The transgenic cotton program (*Bt* Cotton). In this program, pesticides belonging to OP  
124 and PYR are not used. Only neonicotinoids are used at boll opening stage to control sucking  
125 pests. In this case, farmers worked under the supervision of technicians from societies of  
126 textile fibers in Burkina Faso.

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### 128 2.2 *B. tabaci* sampling

129 Sampling was performed in october and november between 2009 and 2015 in three countries  
130 of Western Africa: Burkina Faso, Benin and Togo (Figure 1, Table 2). In Burkina Faso,  
131 whiteflies were collected randomly in two localities, Pô in 2013 and Saria in 2015, from *Bt*  
132 cotton and CP fields. In Pô, *Bt* cotton represented 90% of the sampled fields while in Saria it  
133 represented 15%. In Benin, sampling was done at Kandi in 2009 in CP and BP areas (BP  
134 represent around 10% of areas). In Togo, collection was done randomly in two sites in 2009,  
135 Infa and Tové, where only CP is used. The collected adult whiteflies were stored in ethanol

136 95%. The origin of the samples (location) and the number of individuals are summarized in  
137 Table 2.

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### 139 2.3 Molecular analysis

#### 140 DNA extraction

141 For each individual, total DNA was extracted in 25 µl of an extraction buffer containing  
142 50 mM KCl, 10 mM Tris-base pH 8, 0.45% Nonidet P-40, 0.45% Tween 20 and 50 mg/ml  
143 proteinase K. After 3 h at 65°C, samples were incubated at 100°C for 15 min. Pure water  
144 (35 µl) was then added to the extract.

#### 145 Identification of *B. tabaci*

146 Species/biotypes were identified using the Polymerase Chain Reaction-Random Fragment  
147 Length Polymorphism (PCR-RFLP) diagnostic assay based on the mitochondrial cytochrome  
148 oxidase 1 gene sequence (*mtCOI*) described in Henri et al. (2013). This technique allows  
149 discriminating the species/biotypes present in West Africa (Gnankiné et al., 2013b).

#### 150 Identification of susceptible and resistant alleles of the sodium channel and the 151 acetylcholinesterase *ace1* genes

152 Resistant and susceptible alleles in the *para*-type voltage-gated sodium channel and *ace1*  
153 genes were identified using the diagnostic assays developed by Tsagkarakou et al. (2009).  
154 Briefly, *ace1*-susceptible (F331) and -resistant (W331) alleles, as well as susceptible (L925)  
155 and resistant (I925) *para*-type voltage-gated sodium channel alleles were detected using PCR-  
156 RFLP (Tsagkarakou et al. 2009). Some PCR products were sequenced for each susceptible  
157 and resistant allele and each country. We never found the T929V mutation in the sequences.

158 The frequencies of *kdr* and *ace-1<sup>R</sup>* mutations were calculated according to the formula

159  $p = \frac{n_{\sigma^{\uparrow}}(R) + 2n_{\ominus}(RR) + n_{\ominus}(RS)}{n_{\sigma^{\uparrow}} + 2n_{\ominus}}$  where RR was the number of homozygotes, RS the

160 number of heterozygotes and n the size of specimens analysed.

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## 162 2.4 Statistical analyses

163 Statistical analysis were performed using the R statistical software ([http://www.R-](http://www.R-project.org)  
164 [project.org](http://www.R-project.org)). The effects of the pest management practices on the proportions of *B. tabaci*  
165 composition and the frequencies of resistance alleles were tested by using Fisher's exact tests.

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## 168 3. Results

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### 170 3.1 Geographic distribution of biotypes

171 All the 170 *B. tabaci* individuals collected in 5 localities in Burkina Faso, Benin and Togo  
172 belonged to MED-Q1 or ASL (Table 2). In Togo and Benin, only ASL was found (except one  
173 MED-Q1 individual in Tové), while in Burkina Faso, MED-Q1 and ASL were found in  
174 sympatry at variable frequencies: depending on the locality, it was either ASL or MED-Q1  
175 that predominated. In Pô, the frequency of ASL reached more than 80% whatever the control  
176 strategy (CP or Cotton *Bt*). In Saria, MED-Q1 was more common than ASL, but their relative  
177 frequencies depended on the management program: on CP areas, 93 % of individuals  
178 belonged to MED-Q1 while only 57% of whiteflies were MED-Q1 on *Bt* Cotton fields (Fisher  
179 exact test,  $p < 0.005$ ).

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### 182 3.2 Frequency of the L925I mutation

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185 For the *para*-type voltage-gated sodium channel gene, we studied the frequency of the L925I  
186 mutation that correspond to the allele called r1 by Alon et al. (2008). We found high  
187 variations depending on the country (Fisher exact test,  $p < 0.0005$ ). Indeed, in Burkina Faso, r1



188 was fixed for both MED-Q1 and ASL in the two localities (Table 3). In Benin, this allele has  
189 not been found (Table 4). In Togo, its frequency varied between 0.5 and 0.75 (Table 5).

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### 3.3 Frequency of the Ace- $I^R$ allele

For the acetylcholinesterase gene, we studied the presence of the F331W mutation (R allele). Globally, the frequency of this allele was more homogeneous among countries than for the L925I mutation and ranged between 0.71 and 1 (Fisher exact test,  $p=0.08$ ). In Togo, this frequency did not differ between the two localities, Infa and Tové (Table 5; Fisher exact test,  $p=0.1$ ). In Benin, where sampling was performed in only one locality, the frequency of R varied between 0.7 and 0.9 but did not significantly differ between the fields that were treated either with BP or CP program (Table 4; Fisher exact test,  $p=0.49$ ). In Burkina Faso, the frequency of the resistant allele changed in the two localities according to the treatment: it was lower in *Bt* Cotton areas than in CP fields (Table 3; Fisher exact test,  $p=0.003$  for Pô and  $p<0.0005$  for Saria). In the two localities, R was fixed in MED-Q1 in the CP fields while it had a frequency of 0.5 and 0.875 in Pô and Saria respectively in *Bt* areas (Fisher exact test,  $p<0.0005$ ). For ASL, differences were not so important and not statistically significant (Fisher exact test,  $p>0.05$ ) with a range of frequencies between 0.91 and 1 for CP fields and 0.71 - 0.83 for *Bt* cotton fields.

#### 222 4. Discussion

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225 In this paper, we present the distribution of *B. tabaci* biotypes/species and the frequencies of  
226 the mutations in the para sodium channel gene (*kdr*) and in the Acetylcholinesterase gene  
227 (*ace-1<sup>R</sup>*) associated with resistance to Pyrethroids and Organophosphates respectively of the  
228 pest *B. tabaci* in 3 countries of Western Africa in connection with the pest management  
229 programs.

230 Our results confirmed the diversity of *B. tabaci* biotypes on cotton in these countries. As  
231 previously described by Gueguen et al. (2010) and Gnankiné et al. (2013b), MED-Q1 and  
232 ASL were detected in Burkina Faso, frequently in the same areas. Despite this sympatry,  
233 population genetics analyses on microsatellite markers suggested that MED-Q1 and ASL do  
234 not hybridize in the field (Mouton et al. 2015). It was also showed that they do not share  
235 insecticide resistance alleles. ASL previously classified in MED-species might thus be  
236 considered now as putative species (Mouton et al. 2015).

237 In the current study, the L925I mutation was fixed in MED-Q1 and ASL individuals in  
238 Burkina Faso. Regarding ASL, this is in sharp contrast with the situation observed in 2009  
239 and 2010, as this mutation only reached 0.02 at that time in the western part of Burkina Faso  
240 (Mouton et al. 2015). This is also in contrast with the situation found in Benin, where this  
241 resistant allele was not found, whatever the control program. Finally, the situation is  
242 intermediate in Togo where the frequencies varied between 0.5-0.75 in CP or BP programs.  
243 The fixation of this L925I mutation might be explained by a high selection pressure due to the  
244 repetitive pyrethroids treatments applied in western Africa particularly on cotton and  
245 vegetables (Gnankiné et al., 2007; Ahouangninou et al., 2011; Gnankiné et al. 2013b).

246 The management of the resistance mutations in the *para*-type voltage-gated sodium channel  
247 gene, L925I but also T929V, seems to be difficult as they prove to be persistent even without

248 selection in laboratory, suggesting a low fitness cost (Roditakis et al., 2006). This low fitness  
249 cost was confirmed by Alon et al. (2006) where no departures from Hardy–Weinberg  
250 equilibrium were observed when the frequency of resistant genotypes was investigated in *B.*  
251 *tabaci* populations reared without any insecticide selection for many years. Accordingly, we  
252 could not detect any impact of the control program on the frequency of these resistance  
253 mutations.

254 For the F331W mutation (*ace1* gene), the frequencies of resistant alleles were very variable.  
255 According to Mouton et al. (2015), the frequencies of the F331W mutation in the *ace1* gene in  
256 individuals from Burkina Faso were 0.98 and 0.59 for MED-Q1 and ASL, respectively. Our  
257 data indicated that the frequency of resistance may be lower in individuals sampled in fields  
258 using *Bt* cotton strategy than in the fields using CP. This effect was more pronounced in  
259 MED-Q1 than ASL, and also more pronounced in Pô than in Saria. The high frequencies  
260 observed in Saria could be due to the number of applications done for the pest control. Indeed,  
261 near Saria, many farmers cultivate vegetables that are systematically treated with pyrethroids  
262 and Organophosphates. On the contrary, in Pô, no vegetables were cultivated and the  
263 insecticide pressure was low. Moreover, a drastic lack of resistant homozygous in the *Bt*  
264 cotton fields is consistent with the hypothesis of a high fitness cost associated with the F331W  
265 mutation in this species. As 90% of the zone was using *Bt* in Pô, while it was only 15% in  
266 Saria, the refuge zone without treatment is thus much more important in Pô, which could  
267 allow a more rapid counter-selection of resistance alleles without treatment. On the other  
268 hand, we could not detect any effect of the control program in Benin (CP vs. BP). This may  
269 be explained by the fact that only 10% of the surface were cultivated using BP program. In  
270 Togo, we did not detect any significant difference in the mutation frequencies in the two areas  
271 but they were under the same insecticide pressure.

272 Other mechanisms of insecticide resistance, like metabolic resistance may be associated to  
273 high frequencies of the two mutations in individuals insects conferring a multiple resistance.  
274 Indeed, detoxifying enzymes such as esterases, glutathione S-transferases, and cytochrome  
275 P450-dependent monooxygenases are involved in resistance to numerous insecticide classes  
276 (Alon et al., 2008; Rauch and Nauen, 2003; Ma W et al., 2010).

277 In conclusion, our results suggest that different control programs may alter the frequency of  
278 resistance alleles. However, the counter-selection of resistance alleles may depend on the one  
279 hand on the fitness cost associated with resistance, **and the other hand** to the relative surface  
280 of untreated areas. This clearly advocates for a better integration of control measures against  
281 *B. tabaci* across the different host crops.

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