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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ABSTRACT

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

Key words: Pest management programs**,** insecticides, *kdr*, Ace1^R, *Bemisia tabaci.*

1. Introduction

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

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

complex of cryptic species whose taxonomy is still not entirely resolved. Based on the current consensus, *B. tabaci* is mostly represented by the MED species in western Africa, even though AnSL species can also be found (Gnankiné et al., 2013b; Gnankiné et al., 2013c). Within the MED species, different biotypes are encountered including MED-Q1, MED-Q3 and ASL. Actually, recent analyses suggest that MED-Q1 and ASL do not hybridize in the field and that ASL is thus a different species (Mouton et al., 2015). Interestingly, these species/biotypes differ in terms of host plants range and insecticide resistance traits. Generally, MED-Q1 is predominant in cotton areas and sometimes found in sympatry with ASL on vegetables crops (Gnankiné et al., 2013b). It is also associated with higher levels of resistance to some insecticides as pyrethroids, organophosphates and neonicotinoids (Gnankiné et al., 2013a). In *B. tabaci*, two mutations in the *para*-type voltage-gated sodium channel gene, L925I and T929V, and one mutation in the acetylcholinesterase enzyme *ace1* (F331W: allele Ace1^R) confer resistance to pyrethroids and organophosphates, respectively (Roditakis et al., 2006; Alon et al., 2008; Tsagkarakou et al., 2009). Recent studies showed that the Ace1^R was found in both MED-Q1 and ASL but, while this resistant allele was almost fixed in MED-Q1 (0.99), its frequency was 0.59 in ASL (Mouton et al., 2015). In addition, while the L925I mutation in the sodium channel gene is almost fixed in MED-Q1 populations, it is rarely detected in ASL. The T929V was never found in *B. tabaci* populations from West Africa (Mouton et al., 2015). The objectives of the present study were to perform a first analysis of the impact of the agricultural practices on the *B. tabaci* biotypes/species composition and diversity, and the frequencies of alleles that confer resistance to pyrethroids and organophosphates. Sampling was performed in three countries of Western Africa: Burkina Faso, Benin and Togo.

2. Materials and methods

2.1 Management of cotton pests

Three pest control programs are recommended in West African countries:

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

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

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

2.2 *B. tabaci* sampling

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

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

2.3 Molecular analysis

DNA extraction

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

Identification of *B. tabaci*

Species/biotypes were identified using the Polymerase Chain Reaction-Random Fragment Length Polymorphism (PCR-RFLP) diagnostic assay based on the mitochondrial cytochrome oxidase 1 gene sequence (*mtCO1*) described in Henri et al. (2013). This technique allows discriminating the species/biotypes present in West Africa (Gnankiné et al., 2013b).

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

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

The frequencies of *kdr and ace-1*^{R} mutations were calculated according to the formula

$p = n♂(R) + 2n♀(RR) + n♀(RS) / n♂ + 2n♀$ where RR was the number of homozygotes, RS the number of heterozygotes and n the size of specimens analysed.

2.4 Statistical analyses

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

3. Results

3.1 Geographic distribution of biotypes

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

3.2 Frequency of the L925I mutation

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

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

3.3 Frequency of the Ace-1^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.

4. Discussion

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

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

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

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

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

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

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

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

Acknowledgments

The authors are grateful to the following institute for the samples collection and the technical assistance from Inera and University of Lomé.

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