Biotype status and resistance to neonicotinoids and carbosulfan in Bemisia tabaci (Hemiptera: Aleyrodidae) in Burkina Faso, West Africa

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Abstract

Bemisia tabaci Gennadius is a one of the major pests of cotton crops worldwide. In Burkina Faso, data on resistance to neonicotinoids and carbamate insecticides related to species/biotypes remain very scarce. To evaluate the resistance status of *B. tabaci* in Burkina Faso, four insecticides were tested using the leaf dip method on ten field populations collected from cotton. The status of biotypes was also determined. Two biotypes, Q and ASL were recorded. A significant resistance to neonicotinoids and carbosulfan was showed in most of the populations tested. The highest resistance ratios (RR) were recorded in populations from locations exhibiting only Q1 biotype. On the contrary, the populations with a mix of Q1 and ASL biotypes appeared to be more susceptible to insecticides. Resistance to neonicotinoids may be related not only to the biotype status but also to the environmental factors and agricultural practices. The exclusive use of neonicotinoids against whiteflies on cotton in Burkina Faso is expected to continue to select this resistant Q biotype and might be threaten the very short-term control of whitefly populations thereby increasing the risk of outbreaks in different host plants and begomovirus transmission.

Key words: *Bemisia tabaci*, insecticide resistance, neonicotinoid, carbamate, biotype.

1. Introduction

The sweet potato whitefly *Bemisia tabaci* (Gennadius) is one of the most serious pests of many agricultural and ornamental crops in all tropical and subtropical regions. *B. tabaci* is considered as a complex of morphologically indistinguishable species which vary greatly with respect to host range, fecundity, insecticide resistance, ability to transmit plant viruses, and induction of plant disorders (Brown et al. 1995; Beitia et al. 1997; Devine et al. 2004; Perring 2001; Simon et al. 2003). Although, De Barro et al. (2011) suggested that many biotypes of *B. tabaci* are cryptic species rather than races of *B. tabaci*, the taxonomy and systematics remain controversial. To date, 28 putative cryptic species within the *B. tabaci* complex were identified (McKenzie et al. 2009; Dinsdale et al. 2010; Shu-Sheng et al., 2012). Among the 28 putative species, the Middle East–Asia Minor 1 (MEAM1), known commonly as biotypes B and B2 and the Mediterranean (MED), known as biotypes Q, J, L and Subsaharan Africa Silverleaf biotypes species (ASL), are recognized as predominant in many areas (Dinsdale et al. 2010). In Q biotype, three groups were recently designated as Q1 (Western Mediterranean populations), Q2 (Middle Eastern populations) (Chu et al. 2008), and Q3, restricted to populations from Burkina Faso (Gueguen et al. 2010). Hereafter, we have retained the commonly used term biotype here to link this study with previous literature.

Life-history traits such as resource exploitation, and resistance to insecticides may affect the distribution and frequency between populations of the *B. tabaci* species complex (Pascual and Callejas 2004; Horowitz et al. 2005; Crowder et al. 2010). In agricultural areas, human activities including cultivation practices, the use of cultivated plants and pesticide treatments, create intense selection pressure on populations and may have a major influence on population demographics and on patterns of species distribution (Reitz and Trumble 2002; Crowder et al. 2010).

In West Africa, population outbreaks were reported in 1998 in cotton fields in Burkina Faso, Mali and Côte-d'Ivoire inducing severe crop damages (Otoidobiga et al. 2002). The resulting loss severely impacted economic activity of these countries since cotton production and distribution is one of the main financial resources, especially in Burkina Faso. In addition, in this country, monitoring of population dynamics on cotton revealed high levels of *B. tabaci* at the boll opening stage (Gnankiné et al. 2007). At this stage, *B. tabaci* causes damages indirectly through honeydew excretion leading to the sticky cotton. Consequently, the recommended pest management strategy at this stage is two foliar neonicotinoid treatments against whiteflies in combination with a pyrethroid against bollworms (Gnankiné et al. 2007). Farmers generally spray a mixture of acetamiprid and cypermethrin. This leads to select resistance particularly observed in Burkina Faso (Otoidobiga et al. 2002; Gnankiné et al. 2002; Houndété et al. 2010a). Recently, Houndété et al. (2010a) showed resistance of *B. tabaci* to pyrethroids, such as deltamethrin and bifenthrin, to organophosphates (OPs), such as dimethoate and chlorpyriphos ethyl, and to neonicotinoids, such as acetamiprid and thiamethoxam. Unfortunately, that study was not related to *B. tabaci* populations identity collected in Burkina Faso in connection with agricultural practices. In the meantime, the presence of Q1 living in sympatry with the local biotype, Sub-saharan Africa Silverleafing (ASL), has been observed on cotton and vegetables crops (Gnankiné et al. 2012; Gueguen et al. 2010). Q1 was shown to be dominant in Burkina Faso but was not detected in Benin and Togo where ASL was the only biotype (Gnankiné et al. 2012). In these ASL populations, pyrethroid and OP resistance was highlighted (Houndeté et al. 2010a). In contrast, no neonicotinoid resistance was detected in populations from Benin.

To prevent outbreaks and to establish sustainable control of this pest, the susceptibility to pesticide used must be surveyed in connection with identification of *B. tabaci* populations, human activities and environmental factors. In Spain, the Q biotype exhibited a greater degree

of pesticide resistance than the B biotype, which had better fecundity and competitive abilities (Pascual and Callejas 2004). In Israel, Q excluded B biotype when insecticides were used whereas B rapidly evolved resistance to insecticides in the United States (Horowitz et al. 2005; Khasdan et al. 2005).

The present study aimed to estimate the proportion of different biotypes in populations sampled from cotton fields not yet investigated and evaluate their susceptibility to three neonicotinoids and one carbamate insecticide. Among them, acetamiprid is the only insecticide usually sprayed on cotton fields in Burkina Faso.

2. Materials and Methods

2.1 Whitefly populations

In 2007, at least 10,000 adults of *B. tabaci* were collected from cotton fields close to the villages of Sidéradougou, Houndé, Diébougou, Boromo, Solenzo, Datomo, Diabo, Diapaga, Pô and Bittou (Figure 1)**.** The environmental factors and agricultural practices varied considerably in the different zones of the country (Table 1). In each area, after neonicotinoid treatments, *B. tabaci* adults were collected at random from 50 cotton plants using a mouth aspirator, then confined in a wooden rearing cage (50 by 35 by 35 cm) containing cotton seedlings, and returned to the laboratory within 2-5 h. Whiteflies of both sexes and different ages were tested the same day or the following day. At least, thirty (30) individuals of *B. tabaci* were collected from fields and conserved in alcohol (80 %) for molecular analysis in laboratory.

2.2 Insecticides

The following formulated insecticides were used for the bioassays: imidacloprid (Confidor 200 SL), provided by Bayer AG (Leverkusen, Germany), acetamiprid (Mospilan 200 SL) provided by Arysta Life Science (Noguères, France), thiamethoxam (Actara 240 SC) from Syngenta Crop Protection AG, (Basel, Switzerland) and carbosulfan (Marshal 25 EC)

provided by Syngenta Agro AG (Dielsdorf, Switzerland). Among the insecticides tested, only acetamiprid at 12 g active ingredient/ha is used by farmers for cotton protection in Burkina Faso during the last treatments in combination with cypermethrin at 36 g a.i./ha (Gnankine et al. 2007). The other insecticides may be used at any time and any dosages for protecting vegetables in growing areas.

2.3 Determination of B. tabaci biotypes or genetic groups

Genomic DNA was extracted from each individual adult of *B. tabaci* in 26 µl of Nonidet P-40 extraction buffer (Delatte et al. 2005) and stored at - 20 °C. Biotypes were identified using a PCR-RFLP based diagnostic assay. Briefly, in this method, a fragment of the mitochondrial marker CO1 (Cytochrome Oxidase 1 gene sequences, *mtCOI*) gene is amplified by PCR (Frohlich et al. 1999) using universal COI primers C1-J-2195 (5'-TTGATTTTTTGGTCATCCAGAAGT-3)' and TL2-N-3014 (5'-TCCAATGCAC TAATCTGCCATATTA-3') (Khasdan et al. 2005). The PCRs were composed of 25 µl Platinum PCR SuperMix 0.5 µl forward primer (10 pmol), 0.5 µl reverse primer (10 pmol) and 2 µl DNA template. The PCR products are then digested by the restriction endonucleases XapI (Fermentas) and/or BfmI (Fermentas), which generates clear polymorphism between biotypes B, MS, Q and Q1, Q2 or Q3 genetic groups. The PCR products were incubated with 10 U/µL XapI (Fermentas) at 37 °C for 3 h before loading onto agarose gel (Henri et al. 2012 submitted).

2.4 Bioassay

A leaf dip bioassay method was performed based on previous studies (Rowland et al. 1991; Cahill et al. 1995). For each insecticide, discs (35 mm in diameter) of cotton leaves were immersed for 10 s in seven aqueous solutions of various concentrations of insecticide, or in distilled water (controls). Leaf discs were air dried for 30 min. Discs were then positioned individually on an agar-coated (7 $g.l^{-1}$) in a vial (45 mm diameter). Adults of *B. tabaci* (30

individuals of mixed sex) were removed from cotton leaves with a mouth aspirator, chilled and transferred into small plastic vials containing the treated leaf discs. Each vial was then sealed with a transparent ventilated lid. When adults recovered from chilling, vials were stored upside down and maintained at 25 °C (± 2), 60% r.h. (± 5) and a 12:12 h light: dark photoperiod. An insect was considered alive if any sign of movement was observed. Mortality was recorded 24 h later. Three replicates were carried out for each concentration of insecticide and untreated controls. Mortality in the control was always <10% and data from all bioassays were corrected for control mortality using Abbott's formula (Abott 1925).

2.5 Statistical analyses

2.5.1 Impact of geographical location on the distribution of biotypes

The data collected were subjected to Fisher's exact test with simulated p-values based on 2000 replicates using R statistical software (http://www.R-project.org).

2.5.2 Doses response bioassays

All bioassay replicates were combined for analysis. LC_{50} values were calculated by global optimisation by simulated annealing (GOSA), available at http://bio-log.biz. This software tests the linearity of dose-mortality responses and provides the slope, lethal concentrations (LC_{50}) and 95% confidence limits (CL). A population is considered to be significantly ($P < 0.05$) more (or less) resistant than another population when there is no overlap of the 95% confidence limits of the LC_{50}. The resistance ratios (RR) were calculated following the report of various LC_{50} on the LC_{50} of the most susceptible field population.

3. Results

3.1 Biotype or genetic groups

In our study, all *B. tabaci* from populations collected belonged to the Africa/Middle East/Asia Minor group (biotypes B, Q, ASL, Ms) (Figures 1; 2). A significant relationship was found between the geographical range and the biotype distribution (Fisher's Exact Test, P=0.0005). Q1 biotype was largely predominant according to various areas. It has been observed in Sidéradougou, Diébougou, Solenzo, Datomo, Diabo, Diapaga, and Bittou. The Q1 biotype was found in sympatry with the ASL biotype in populations from Boromo, Houndé, and Pô.

3.2 Resistance to insecticides

Populations with only Q1 appeared to be more resistant to acetamiprid, thiamethoxam and carbosulfan (Table 2). Among the ten *B. tabaci* populations tested, those living in sympatry (ASL and Q1) from Boromo, Houndé and Pô were in most cases more susceptible to the three neonicotinoids (Figure 1; Table 2). On the contrary, the highest neonicotinoid resistant populations were all from pure Q1 such as the populations from Diabo, Solenzo and Bittou highly resistant to acetamiprid, imidachloprid and thiamethoxam respectively. In the absence of reference strain, the *B. tabaci* population from Houndé was used as reference to calculate the resistance ratio (RR) for neonicotinoids. **Q1 populations from Diapaga, Boromo and Bittou exhibited some variation in their response towards acetamiprid compared to Houndé (**$\mathbf{RR_{50}}$ **2 to 9)**, while highest resistance (RR_{50} 23 to 50) was observed in the populations from Sidéradougou (Q1), Po (Q1 and ASL), and Diabo (Q1). **Q1 and ASL populations from Boromo and Po exhibited some variation in their response towards imidacloprid compared to Houndé (**$\mathbf{RR_{50}}$ **1 to 2),** while highest resistance (RR_{50} 5 to 14) was observed in the Q1 populations from Datomo, Diebougou, and Solenzo. **Q1 and ASL populations from Boromo and Po exhibited some variation in their response towards to**

thiamethoxam compared to Houndé (RR_{50} 2 to 4), while highest resistance (RR_{50} 13 to 33) was observed in the Q1 populations from Diabo, Solenzo and Bittou.

As for neonicotinoids, the three *B. tabaci* populations from Boromo, Houndé and Pô, where ASL and Q1 lived in sympatry, were among the most susceptible to carbosulfan (Figure 1; Table 2). In the absence of reference strain, the *B. tabaci* population from Boromo was used as reference to calculate the resistance ratio (RR) for carbosulfan. The highest carbosulfan resistant populations were observed in pure Q1 populations such as Datomo and Sideradougou (RR_{50} 7 to 14). **Q1 populations from Houndé, Diebougou and Po exhibited some variation in their response towards carbosulfan compared to Boromo (RR_{50} 2 to 5).**

3. Discussion

According to the determination of biotypes, the populations of *B. tabaci* tested belonged to the Africa/Middle East/Asia Minor group (biotypes B, Q, ASL, Ms). Q and Africa-SL biotypes were identified in MED species (Boykin et al. 2007; De Barro et al. 2011). In Q biotype, three groups were recently designated as Q1, Q2 (Chu et al. 2008) and Q3 (Gueguen et al. 2010). Gnankiné et al. (2012) showed that the Q1 was predominant in cotton fields and is probably progressively displacing the African ASL biotype. Q1 originating from the Mediterranean region is generally considered to be an invasive biotype like B. It is the real *B. tabaci* (Tay et al. 2012), and has now begun its own global invasion spreading from its Mediterranean home range to at least 10 different countries (De Barro et al. 2011; Dalton 2006).

Our results confirmed the resistance of *B. tabaci* populations from Burkina Faso to neonicotinoids (Houndété et al. 2010a) and showed the resistance to one carbamate. The resistance ratios (RR) showed a variation between the populations from different localities and genetic groups. It varied from 4 to 50 for the neonicotinoids and from 2 to 14 for the

carbamate. The resistance ratios for the neonicotinoids were much higher than those obtained three years ago by Houndété et al (2010a) in populations collected in Soumousso and Tiara, Burkina Faso (RR = 3 to 8). The increase of the neonicotinoid resistance ratio might be explained by the use of acetamiprid in the two last insecticide sprays on cotton recommended by the ginning companies providing insecticides, at credit, to cotton farmers. However, a part of these insecticides is deflected to the black economy. That is the reason why most of insecticides used for protecting vegetable comes from cotton (Ahouangninou et al. 2012). On the contrary, the low resistance level of *B. tabaci* to carbosulfan may be explained by the no-use of this insecticide for cotton protection. Up to now, to control whiteflies, farmers use acetamiprid that was progressively introduced in 2000 as a substitute for OPs that become inefficient due to acetylcholinesterase resistance (Houndété et al. 2010b).

Our results suggest that the neonicotinoid resistance status of *B. tabaci* is linked to the biotype, especially to Q1. Indeed, the highest resistance factors to acetamiprid and thiamethoxam were recorded in the Q1 populations. One noteworthy finding is that the correlation between the neonicotinoid resistance and the biotype was not clearly demonstrated. However the most susceptible *B. tabaci* populations were collected in Boromo and Houndé. These populations were composed by Q1 and ASL individual with quite the same prevalence. In Benin, previous studies have already identified neonicotinoid susceptible populations as belonging to the ASL biotype compared with heterogeneous populations from Burkina Faso that were resistant (Gueguen et al. 2010; Houndété et al. 2010b; Gnankiné et al. 2012). The susceptibility of *B. tabaci* population from Diapaga compared to other areas with pure Q1 biotype could be due to the low insecticide pressure coupled with the absence of vegetable crops around. This result suggests that individuals from Q1 biotype should be always susceptible to neonicotinoid. That did not exclude resistance to other insecticides. Recently, Mouton et al. (unpublished data) have demonstrated the presence of *kdr* and *Ace1-R*

genes in this population. In Israel, Horowitz et al. (2003) showed that the Q-type was predominant in areas where resistance to pyriproxyfen evolved rapidly. Cases of resistances of *B. tabaci* Q populations to pyrethroids were demonstrated in Greece by Roditakis et al. (2009). Moreover, in southern Spain, the Q biotype was linked with high and cross-resistance to neonicotinoids (Nauen et al. 2002; Guirao et al. 1997). After a few years, Q had almost displaced the B, especially in southern Spain (Simón et al. 1999) probably because of the increased use of neonicotinoids against whiteflies. Our results confirmed also the cross resistance between neonicotinoids as Sideradougou population for example was highly resistant to both acetamiprid (23.8-fold) and thiametoxam (24.7-fold). The extreme resistance to acetamiprid in Diabo Q1 populations (50-fold) is probably due to the extensive use of this insecticide. As far as imidachloprid is concerned, Solenzo appears to be the only populations displaying highest resistance (13.8-fold). Karunker et al. (2008) showed that cytochrome P450 CYP6CM1 was implied in the metabolic resistance of neonicotinoids and appears to be specific for imidachloprid (Roditakis et al. 2011). **It has been demonstrated that overexpression of CYP6CM1 is associated with high levels of imidacloprid resistance in *B. tabaci* (Karunker et al. 2008).**

The current status of *B. tabaci* resistance to neonicotinoid insecticides from West Africa requires urgent attention if neonicotinoids really selected the invasive Q biotype. These results show again the negative effect of using continuously the same molecule, or the same family of insecticides, year after year until inefficiency. Moreover, our results suggested the selection of a new multiresistant phenotype may threaten the production of cotton or vegetable in the future. Indeed, the spread of a potentially more prolific vector for begomovirus virus will surely lead to a crisis in the production of Solanaceae and especially tomatoes by small-scale farmers in West and Central Africa (Hanafi 2000).

The use of genetically modified *Bt* cotton in Burkina Faso since 2008 could be one way to avoid the selection of *B. tabaci* resistant populations by reducing insecticide use, which naturally enhances the impact of natural enemies. The suppression of insecticide treatments against whiteflies that are systematically applied before harvest could have a significant impact on sticky cotton. But this problem can easily be solved by harvesting cotton not too late after boll opening or by controlling the degree of stickiness before the ginning process.

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