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

Olivier Gnankiné^a, Laurence Mouton^b, Aly Savadogo^c, Thibaud Martin^d, Antoine Sanon^a,
Roch K. Dabire^e, Fabrice Vavre^b, Frédéric Fleury^b

^a *Laboratoire d'Entomologie Appliquée, Université de Ouagadougou, Burkina Faso.*

^b *Laboratoire de Biométrie et Biologie Evolutive, UMR 5558, Université de Lyon, CNRS, Villeurbanne, France*

^c *Laboratoire de Microbiologie, CRSBAN/UFR-SVT, Université de Ouagadougou, Burkina Faso*

^d *UR Hortsys, CIRAD, Montpellier, France*

^e *IRSS/Centre Muraz, BP 390 Bobo-Dioulasso, Burkina Faso*

* Corresponding author: Email : olivier.gnankine@univ-ouaga.bf

1 **Abstract**

2

3 *Bemisia tabaci* Gennadius is a one of the major pests of cotton crops worldwide. In Burkina

4 Faso, data on resistance to neonicotinoids and carbamate insecticides related to

5 species/biotypes remain very scarce. To evaluate the resistance status of *B. tabaci* in Burkina

6 Faso, four insecticides were tested using the leaf dip method on ten field populations collected

7 from cotton. The status of biotypes was also determined. Two biotypes, Q and ASL were

8 recorded. A significant resistance to neonicotinoids and carbosulfan was showed in most of

9 the populations tested. The highest resistance ratios (RR) were recorded in populations from

10 locations exhibiting only Q1 biotype. On the contrary, the populations with a mix of Q1 and

11 ASL biotypes appeared to be more susceptible to insecticides. Resistance to neonicotinoids

12 may be related not only to the biotype status but also to the environmental factors and

13 agricultural practices. The exclusive use of neonicotinoids against whiteflies on cotton in

14 Burkina Faso is expected to continue to select this resistant Q biotype and **might be threaten**

15 the very short-term control of whitefly populations thereby increasing the risk of outbreaks in

16 different host plants and begomovirus transmission.

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

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1 **1. Introduction**

2 The sweet potato whitefly *Bemisia tabaci* (Gennadius) is one of the most serious pests
3 of many agricultural and ornamental crops in all tropical and subtropical regions. *B. tabaci* is
4 considered as a complex of morphologically indistinguishable species which vary greatly with
5 respect to host range, fecundity, insecticide resistance, ability to transmit plant viruses, and
6 induction of plant disorders (Brown et al. 1995; Beitia et al. 1997; Devine et al. 2004; Perring
7 2001; Simon et al. 2003). Although, De Barro et al. (2011) suggested that many biotypes of *B.*
8 *tabaci* are cryptic species rather than races of *B. tabaci*, the taxonomy and systematics remain
9 controversial. To date, 28 putative cryptic species within the *B. tabaci* complex were
10 identified (McKenzie et al. 2009; Dinsdale et al. 2010; Shu-Sheng et al., 2012). Among the 28
11 putative species, the Middle East–Asia Minor 1 (MEAM1), known commonly as biotypes B
12 and B2 and the Mediterranean (MED), known as biotypes Q, J, L and Subsaharan Africa
13 Silverleaf biotypes species (ASL), are recognized as predominant in many areas (Dinsdale et
14 al. 2010). In Q biotype, three groups were recently designated as Q1 (Western Mediterranean
15 populations), Q2 (Middle Eastern populations) (Chu et al. 2008), and Q3, restricted to
16 populations from Burkina Faso (Gueguen et al. 2010). Hereafter, we have retained the
17 commonly used term biotype here to link this study with previous literature.

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

1 In West Africa, population outbreaks were reported in 1998 in cotton fields in Burkina
2 Faso, Mali and Côte-d'Ivoire inducing severe crop damages (Otoïdobia et al. 2002). The
3 resulting loss severely impacted economic activity of these countries since cotton production
4 and distribution is one of the main financial resources, especially in Burkina Faso. In addition,
5 in this country, monitoring of population dynamics on cotton revealed high levels of *B. tabaci*
6 at the boll opening stage (Gnankiné et al. 2007). At this stage, *B. tabaci* causes damages
7 indirectly through honeydew excretion leading to the sticky cotton. Consequently, the
8 recommended pest management strategy at this stage is two foliar neonicotinoid treatments
9 against whiteflies in combination with a pyrethroid against bollworms (Gnankiné et al. 2007).
10 Farmers generally spray a mixture of acetamiprid and cypermethrin. This leads to select
11 resistance particularly observed in Burkina Faso (Otoïdobia et al. 2002; Gnankiné et al.
12 2002; Houndété et al. 2010a). Recently, Houndété et al. (2010a) showed resistance of *B.*
13 *tabaci* to pyrethroids, such as deltamethrin and bifenthrin, to organophosphates (OPs), such as
14 dimethoate and chlorpyrifos ethyl, and to neonicotinoids, such as acetamiprid and
15 thiamethoxam. Unfortunately, that study was not related to *B. tabaci* populations identity
16 collected in Burkina Faso in connection with agricultural practices. In the meantime, the
17 presence of Q1 living in sympatry with the local biotype, Sub-saharan Africa Silverleafing
18 (ASL), has been observed on cotton and vegetables crops (Gnankiné et al. 2012; Gueguen et
19 al. 2010). Q1 was shown to be dominant in Burkina Faso but was not detected in Benin and
20 Togo where ASL was the only biotype (Gnankiné et al. 2012). In these ASL populations,
21 pyrethroid and OP resistance was highlighted (Houndété et al. 2010a). In contrast, no
22 neonicotinoid resistance was detected in populations from Benin.

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

1 of pesticide resistance than the B biotype, which had better fecundity and competitive abilities
2 (Pascual and Callejas 2004). In Israel, Q excluded B biotype when insecticides were used
3 whereas B rapidly evolved resistance to insecticides in the United States (Horowitz et al.
4 2005; Khasdan et al. 2005).

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

9 **2. Materials and Methods**

10 ***2.1 Whitefly populations***

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

21 ***2.2 Insecticides***

22 The following formulated insecticides were used for the bioassays: imidacloprid
23 (Confidor 200 SL), provided by Bayer AG (Leverkusen, Germany), acetamiprid (Mospilan
24 200 SL) provided by Arysta Life Science (Noguères, France), thiamethoxam (Actara 240 SC)
25 from Syngenta Crop Protection AG, (Basel, Switzerland) and carbosulfan (Marshal 25 EC)

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

6 **2.3 Determination of *B. tabaci* biotypes or genetic groups**

7 Genomic DNA was extracted from each individual adult of *B. tabaci* in 26 µl of
8 Nonidet P-40 extraction buffer (Delatte et al. 2005) and stored at - 20 °C. Biotypes were
9 identified using a PCR-RFLP based diagnostic assay. Briefly, in this method, a fragment of
10 the mitochondrial marker COI (Cytochrome Oxidase 1 gene sequences, *mtCOI*) gene is
11 amplified by PCR (Frohlich et al. 1999) using universal COI primers C1-J-2195 (5'-
12 TTGATTTTTTGGTCATCCAGAAGT-3)' and TL2-N-3014 (5'-TCCAATGCAC
13 TAATCTGCCATATTA-3') (Khasdan et al. 2005). The PCRs were composed of 25 µl
14 Platinum PCR SuperMix 0.5 µl forward primer (10 pmol), 0.5 µl reverse primer (10 pmol)
15 and 2 µl DNA template. The PCR products are then digested by the restriction endonucleases
16 XapI (Fermentas) and/or BfmI (Fermentas), which generates clear polymorphism between
17 biotypes B, MS, Q and Q1, Q2 or Q3 genetic groups. The PCR products were incubated with
18 10 U/µL XapI (Fermentas) at 37 °C for 3 h before loading onto agarose gel (Henri et al. 2012
19 submitted).

20 **2.4 Bioassay**

21 A leaf dip bioassay method was performed based on previous studies (Rowland et al.
22 1991; Cahill et al. 1995). For each insecticide, discs (35 mm in diameter) of cotton leaves
23 were immersed for 10 s in seven aqueous solutions of various concentrations of insecticide, or
24 in distilled water (controls). Leaf discs were air dried for 30 min. Discs were then positioned
25 individually on an agar-coated (7 g.l⁻¹) in a vial (45 mm diameter). Adults of *B. tabaci* (30

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

9 ***2.5 Statistical analyses***

10 ***2.5.1 Impact of geographical location on the distribution of biotypes***

11 The data collected were subjected to Fisher's exact test with simulated p-values based on
12 2000 replicates using R statistical software (<http://www.R-project.org>).

13 ***2.5.2 Doses response bioassays***

14 All bioassay replicates were combined for analysis. LC₅₀ values were calculated by global
15 optimisation by simulated annealing (GOSA), available at <http://bio-log.biz>. This software
16 tests the linearity of dose-mortality responses and provides the slope, lethal concentrations
17 (LC₅₀) and 95% confidence limits (CL). A population is considered to be significantly ($P <$
18 0.05) more (or less) resistant than another population when there is no overlap of the 95%
19 confidence limits of the LC₅₀. The resistance ratios (RR) were calculated following the report
20 of various LC₅₀ on the LC₅₀ of the most susceptible field population.

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1 **3. Results**

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3 **3.1 Biotype or genetic groups**

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

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11 **3.2 Resistance to insecticides**

12 Populations with only Q1 appeared to be more resistant to acetamiprid, thiamethoxam and
13 carbosulfan (Table 2). Among the ten *B. tabaci* populations tested, those living in sympatry
14 (ASL and Q1) from Boromo, Houndé and Pô were in most cases more susceptible to the three
15 neonicotinoids (Figure 1; Table 2). On the contrary, the highest neonicotinoid resistant
16 populations were all from pure Q1 such as the populations from Diabo, Solenzo and Bittou
17 highly resistant to acetamiprid, imidachloprid and thiamethoxam respectively. In the absence
18 of reference strain, the *B. tabaci* population from Houndé was used as reference to calculate
19 the resistance ratio (RR) for neonicotinoids. **Q1 populations from Diapaga, Boromo and**
20 **Bittou exhibited some variation in their response towards acetamiprid compared to**
21 **Houndé (RR₅₀ 2 to 9)**, while highest resistance (RR₅₀ 23 to 50) was observed in the
22 populations from Sidéradougu (Q1), Po (Q1 and ASL), and Diabo (Q1). **Q1 and ASL**
23 **populations from Boromo and Po exhibited some variation in their response towards**
24 **imidacloprid compared to Houndé (RR₅₀ 1 to 2)**, while highest resistance (RR₅₀ 5 to 14)
25 was observed in the Q1 populations from Datomo, Diebougou, and Solenzo. **Q1 and ASL**
26 **populations from Boromo and Po exhibited some variation in their response towards to**

1 **thiamethoxam compared to Houndé (RR₅₀ 2 to 4)**, while highest resistance (RR₅₀ 13 to 33)
2 was observed in the Q1 populations from Diabo, Solenzo and Bittou.
3 As for neonicotinoids, the three *B. tabaci* populations from Boromo, Houndé and Pô, where
4 ASL and Q1 lived in sympatry, were among the most susceptible to carbosulfan (Figure 1;
5 Table 2). In the absence of reference strain, the *B. tabaci* population from Boromo was used
6 as reference to calculate the resistance ratio (RR) for carbosulfan. The highest carbosulfan
7 resistant populations were observed in pure Q1 populations such as Datomo and
8 Sideradougou (RR₅₀ 7 to 14). **Q1 populations from Houndé, Diebougou and Po exhibited**
9 **some variation in their response towards carbosulfan compared to Boromo (RR₅₀ 2 to**
10 **5).**

11 **3. Discussion**

12 According to the determination of biotypes, the populations of *B. tabaci* tested belonged
13 to the Africa/Middle East/Asia Minor group (biotypes B, Q, ASL, Ms). Q and Africa-SL
14 biotypes were identified in MED species (Boykin et al. 2007; De Barro et al. 2011). In Q
15 biotype, three groups were recently designated as Q1, Q2 (Chu et al. 2008) and Q3 (Gueguen
16 et al. 2010). Gnankiné et al. (2012) showed that the Q1 was predominant in cotton fields and
17 is probably progressively displacing the African ASL biotype. Q1 originating from the
18 Mediterranean region is generally considered to be an invasive biotype like B. It is the real *B.*
19 *tabaci* (Tay et al. 2012), and has now begun its own global invasion spreading from its
20 Mediterranean home range to at least 10 different countries (De Barro et al. 2011; Dalton
21 2006).

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

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

12 Our results suggest that the neonicotinoid resistance status of *B. tabaci* is linked to the
13 biotype, especially to Q1. Indeed, the highest resistance factors to acetamiprid and
14 thiamethoxam were recorded in the Q1 populations. One noteworthy finding is that the
15 correlation between the neonicotinoid resistance and the biotype was not clearly
16 demonstrated. However the most susceptible *B. tabaci* populations were collected in Boromo
17 and Houndé. These populations were composed by Q1 and ASL individual with quite the
18 same prevalence. In Benin, previous studies have already identified neonicotinoid susceptible
19 populations as belonging to the ASL biotype compared with heterogeneous populations from
20 Burkina Faso that were resistant (Gueguen et al. 2010; Houndété et al. 2010b; Gnankiné et al.
21 2012). The susceptibility of *B. tabaci* population from Diapaga compared to other areas with
22 pure Q1 biotype could be due to the low insecticide pressure coupled with the absence of
23 vegetable crops around. This result suggests that individuals from Q1 biotype should be
24 always susceptible to neonicotinoid. That did not exclude resistance to other insecticides.
25 Recently, Mouton et al. (unpublished data) have demonstrated the presence of *kdr* and *Ace1-R*

1 genes in this population. In Israel, Horowitz et al. (2003) showed that the Q-type was
2 predominant in areas where resistance to pyriproxyfen evolved rapidly. Cases of resistances
3 of *B. tabaci* Q populations to pyrethroids were demonstrated in Greece by Roditakis et al.
4 (2009). Moreover, in southern Spain, the Q biotype was linked with high and cross-resistance
5 to neonicotinoids (Nauen et al. 2002; Guirao et al. 1997). After a few years, Q had almost
6 displaced the B, especially in southern Spain (Simón et al. 1999) probably because of the
7 increased use of neonicotinoids against whiteflies. Our results confirmed also the cross
8 resistance between neonicotinoids as Sideradougou population for example was highly
9 resistant to both acetamiprid (23.8-fold) and thiametoxam (24.7-fold). The extreme resistance
10 to acetamiprid in Diabo Q1 populations (50-fold) is probably due to the extensive use of this
11 insecticide. As far as imidachloprid is concerned, Solenzo appears to be the only populations
12 displaying highest resistance (13.8-fold). Karunker et al. (2008) showed that cytochrome
13 P450 CYP6CM1 was implied in the metabolic resistance of neonicotinoids and appears to be
14 specific for imidachloprid (Roditakis et al. 2011). **It has been demonstrated that**
15 **overexpression of CYP6CM1 is associated with high levels of imidacloprid resistance in**
16 ***B. tabaci* (Karunker et al. 2008).**

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

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

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