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Rapid Report

The presence of *Rickettsia* is associated with increased susceptibility of *Bemisia tabaci* (Homoptera: Aleyrodidae) to insecticides

Svetlana Kontsedalov,[1] Einat Zchori-Fein,[2] Elad Chiel,[2,3] Yuval Gottlieb,[1] Moshe Inbar[3] and Murad Ghanim[1]*

[1]*Department of Entomology, Institute of Plant Protection, the Volcani Centre, Bet Dagan 50250, Israel*
[2]*Department of Entomology, Institute of Plant Protection, Newe-Ya'ar Research Centre, Ramat-Yishai 30095, Israel*
[3]*Department of Evolutionary and Environmental Biology, University of Haifa, Haifa 31905, Israel*

Abstract

BACKGROUND: The presence of certain symbiotic microorganisms may be associated with insecticide resistance in insects. The authors compared the susceptibility of two isofemale lines, *Rickettsia*-plus and *Rickettsia*-free, of the sweet potato whitefly *Bemisia tabaci* (Gennadius) (Homoptera: Aleyrodidae) to major insecticides from different chemical groups, including imidacloprid, acetamiprid, thiamethoxam, pyriproxyfen, spiromesifen and diafenthiuron.

RESULTS: While the *Rickettsia*-plus and *Rickettsia*-free lines showed no differences in their susceptibility to imidacloprid and diafenthiuron, higher susceptibility of the *Rickettsia*-plus line to acetamiprid, thiamethoxam, spiromesifen and especially pyriproxyfen was observed. LC_{90} values indicated that the *Rickettsia*-free line was 15-fold more resistant to pyriproxyfen than the *Rickettsia*-plus line.

CONCLUSION: Findings indicate that the infection status of *B. tabaci* populations by *Rickettsia* is an important consideration that should be taken into account when performing resistance monitoring studies, and may help in understanding the dynamics of *B. tabaci* resistance, symbiont-pest associations in agricultural systems and the biological impact of *Rickettsia* on whitefly biology.

Keywords: insecticide resistance; symbiont; whitefly; *Bemisia tabaci*; *Rickettsia*

1 INTRODUCTION

Symbiotic relationships with bacteria are quite common within the Arthropoda, where the interactions are known to have a substantial influence on the biology of both partners.[1] Such associations can be obligatory or facultative for the host, and, to date, they have been reported to be involved in nutrition, host plant utilization, reproductive manipulation and ability to cope with environmental factors.[1] However, the role of symbiotic bacteria in pesticide resistance is largely unknown.

The sweet potato whitefly *Bemisia tabaci* (Gennadius) (Homoptera: Aleyrodidae) is a cosmopolitan, polyphagous phloem-feeder that inflicts damage in many crops owing to direct feeding and the vectoring of plant viruses.[2,3] *Bemisia tabaci* is a complex of biotypes that vary greatly with respect to characteristics such as host range, fecundity, insecticide resistance, ability to transmit plant viruses and induction of plant disorders.[4,5]

All whitefly species are known to host the obligatory bacterium *Portiera aleyrodidarum* Thao & Baumann, which supplements their unbalanced sap diet. In addition, different *B. tabaci* populations may harbour a diverse array of bacterial tenants, including *Hamiltonella*, *Arsenophonus*, *Cardinium*, *Wolbachia*, *Rickettsia and Fritschea*.[6–9] Although these bacteria are known from other arthropods, virtually nothing is known about their effects on the whitefly host. Interestingly, a correlation has been found between the insect biotype and the bacteria it carries: all B-biotype *B. tabaci* host *Hamiltonella*, but they have not been found to carry either *Wolbachia* or *Arsenophonus*.[10] In contrast, the Q biotype has a frequent association with *Arsenophonus* and *Wolbachia*, and in Israel was never associated with *Hamiltonella*. Although the presence of *Rickettsia* may vary among populations, sites and

* Correspondence to: Murad Ghanim, Department of Entomology, Institute of Plant Protection, the Volcani Centre, Bet Dagan 50250, Israel
E-mail: ghanim@agri.gov.il
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crops, it is the only symbiont that is commonly detected in both biotypes. Therefore, this bacterium was used to test the hypothesis that its presence in *B. tabaci* influences the pest's susceptibility to different pesticides.

2 MATERIALS AND METHODS

2.1 Insects

Two strains of *B. tabaci* were established by setting isofemale *Rickettsia*-plus and *Rickettsia*-free lines derived from the same population. This was done by allowing 30 females to lay eggs in individual leaf cages. After egg lay, the females were tested for the presence or absence of *Rickettsia* using a genus-specific amplification of an rDNA PCR fragment as previously described.[7,10] Adults that developed from eggs laid by females that tested positive for *Rickettsia* were pooled to establish the *Rickettsia*-plus population. Adults that developed from eggs laid by females that tested negative for *Rickettsia* were pooled, and this population was called *Rickettsia*-free. The B-biotype population used to establish these isofemale lines was obtained from the laboratory culture of Professor Dan Gerling at Tel Aviv University and carries *Portiera*, *Hamiltonella* and *Rickettsia*. All whiteflies were reared on cotton seedlings (*Gossypium hirsutum* L. cv. Acala) under standard laboratory conditions of $26 \pm 2\,^{\circ}C$ and a photoperiod of 14:10 h light:dark.

2.2 Insecticides

Based on their mode of action and usage, six insecticides were chosen (Table 1). The applied concentration of each insecticide was based on LC_{50} values previously determined for the susceptible B-biotype *B. tabaci* strain.[11]

2.3 Bioassays

Because the insecticides differ in their modes of action, application and targeted insect stage, different bioassays were employed. The effect of thiamethoxam, acetamiprid, imidacloprid and diafenthiuron was scored on adults. The assay for thiamethoxam and acetamiprid was performed by dipping cotton seedlings (20–25 cm high, with two true leaves) for 20 s in an aqueous dispersion of the test formulation and then allowing the plant to air dry for 2 h. Adult whiteflies (15–20 per replicate) were then confined on the treated seedlings using clip-on leaf cages for 48 h, after which their mortality was scored. For diafenthiuron, cotton seedlings were treated as described above, and leaf discs were punched out and placed into petri dishes filled with 1.5% agar. Adult whiteflies (15–20 per replicate) were exposed to the treated discs for 72 h and their mortality was scored. For imidacloprid, cotton stems with two true leaves were inserted in plastic vials containing the formulation dispersion for 24 h, and petri dishes with leaf discs were prepared as described above. Adult mortality was determined after 48 h of whitefly exposure to the treated leaf discs. Pyriproxyfen is transovarially active against eggs, and thus egg mortality was scored. After foliar application, adult whiteflies were exposed to treated leaves as described above. They were removed after a 48 h egg-laying period, and the number of hatched eggs was counted after 8 days. Spiromesifen is an ovolarvicidal compound, i.e. it is mostly active against the larvae when eggs are treated. Cotton seedlings infested with 0- to 2-day-old eggs were dipped in the formulation dispersion, and the cumulative larval mortality (expressed as suppression of pupation) was determined 18 days post-application. Deionized water was used as a control for all experiments. Each bioassay was performed with a minimum of five replicates.

2.4 Data analysis

Comparisons of mortality and egg hatch were performed using Student's *t*-test with unequal sample sizes, and percentage data were transformed (angular transformation) before analysis. Probit analyses of the concentration-dependent mortality data were performed using POLO-PC,[12] after correction with Abbott's formula[13] which takes into account the

Table 1. Description and dosage of the tested insecticides

commercial name	producer	active ingredient	applied concentration (ppm)	mode of action	stages most affected
Actara 24SC ($240\,gL^{-1}$ SC)	Syngenta, Switzerland	thiamethoxam	4	Targets nAchRs[1]	All stages
Mospilan 20SP ($200\,g\,kg^{-1}$ SP)	Nippon Soda Co., Japan	acetamiprid	0.25	Targets nAchRs	All stages
Confidor 35SC ($350\,gL^{-1}$ SC)	Bayer CropScience, Germany	imidacloprid	5	Targets nAchRs	All stages
Pegasus 50SC ($500\,gL^{-1}$ SC)	Syngenta, Switzerland	diafenthiuron	25	Inhibits mitochondrial respiration chain	Adults
Tiger 10EC ($100\,gL^{-1}$ EC)	Sumitomo Co., Japan	pyriproxyfen	0.04	Mimics juvenile hormone	Eggs
Oberon 24SC ($240\,gL^{-1}$ SC)	Bayer CropScience, Germany	spiromesifen	3	Inhibits lipid synthesis	Eggs and larvae

[1] nicotinic acetylcholine receptors

mortality in the control experiment run in parallel with the treatment. Control experiments were performed independently for each insecticide tested. Failure of 95% LC to overlap at a particular lethal concentration indicated a significant difference.

3 RESULTS

A comparison using Student's *t*-test with unequal sample sizes between the two *B. tabaci* populations revealed a clear trend of higher insecticide susceptibility to five out of the six tested compounds in the presence of *Rickettsia* (Fig. 1). Non-significant trends in adult mortality of the *Rickettsia*-plus and *Rickettsia*-free lines were found for imidacloprid (81 and 65% respectively; $t = 1.37$, $P = 0.2$) and diafenthiuron (30 and 33% respectively; $t = 0.04$, $P = 0.96$). Mortality rates of the *Rickettsia*-plus strain were significantly higher compared with the *Rickettsia*-free line when treated with acetamiprid (95 versus 79% adult mortality respectively; $t = 2.86$, $P = 0.021$), thiamethoxam (87 versus 63% adult mortality respectively; $t = 2.45$, $P = 0.025$) and spiromesifen (68 versus 47% larval mortality respectively; $t = 1.9$, $P = 0.046$) (Fig. 1).

The most striking difference was observed when the two *B. tabaci* strains were treated with pyriproxyfen: while 43% egg mortality was observed in the *Rickettsia*-free strain, the *Rickettsia*-plus strain exhibited 90% mortality ($t = 9.33$, $P = 0.00024$) (Fig. 1). A log-response pyriproxyfen concentration curve was built (on a probit scale) for the two strains. All four concentrations tested resulted in a significantly higher susceptibility of the *Rickettsia*-plus strain relative to the *Rickettsia*-free one (Table 2 and Fig. 2).

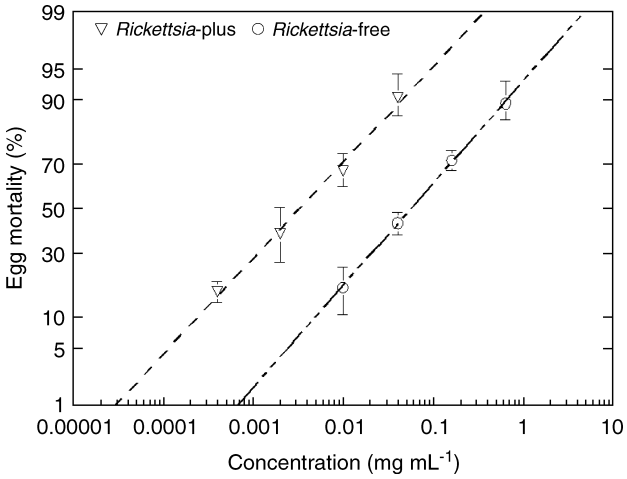

Figure 2. Log concentration–response curves (probit scale) of the effect of pyriproxyfen on *Rickettsia*-plus and *Rickettsia*-free strains. Effect is on egg hatch in the two *Bemisia tabaci* strains. Bars represent mean standard errors of mortality in each concentration tested.

4 DISCUSSION

The assumption tested in this study was that the presence of *Rickettsia* alters the response of *B. tabaci* to pesticide application. It was found that, in the presence of *Rickettsia*, the whitefly's susceptibility to five out of the six insecticides tested was increased, in spite of their variable mode of action and target stages (Table 1).

It has been suggested that symbiotic microorganisms are associated with insecticide resistance in insects. For example, the cigarette beetle *Lasioderna serricorne* (F.) (Coleoptera: Anobiidae) contains a symbiotic gut yeast *Symbiotaphrina kochii* Jurzitza ex Gams & v. Arx,

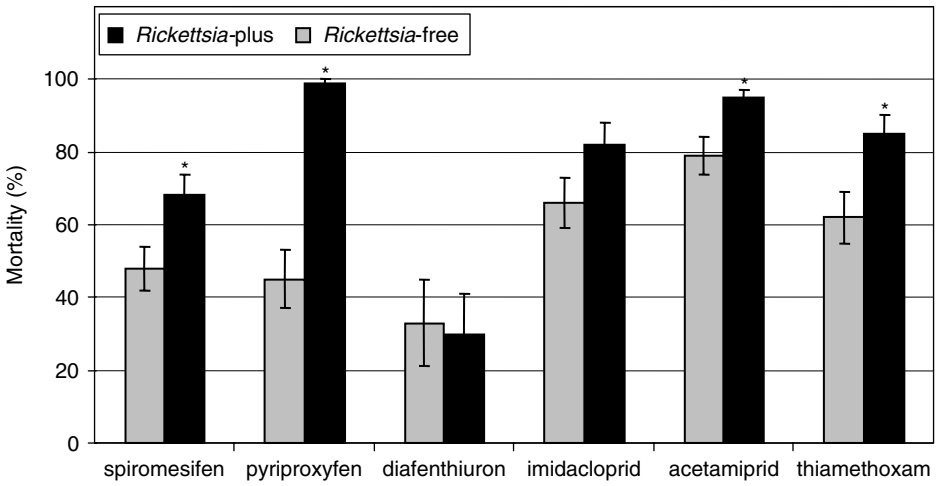

Figure 1. Mortality rates caused by application of various insecticides (Table 1) of *Bemisia tabaci* B biotype from *Rickettsia*-plus and *Rickettsia*-free lines. Asterisk indicates a statistically significant difference (see text).

Table 2. Susceptibility of *Rickettsia*-plus and *Rickettsia*-free strains to pyriproxyfen (LC Ratio = LC *Rickettsia*-free/LC *Rickettsia*-plus)

Strain	*n*	Slope±SEM	LC_{50} (F.L.)	LC_{90} (F.L.)	Ratio LC_{50}	Ratio LC_{90}
Rickettsia-plus	761	1.31 ± 0.14	0.004 (0.000–0.010)	0.04 (0.02–0.23)	1	1
Rickettsia-free	871	1.18 ± 0.10	0.05 (0.02–0.10)	0.6 (0.3–3.4)	13	15

which is involved in the detoxification of natural and synthetic poisons.[14] Similarly, the ability of the apple maggot fly *Rhagoletis pomonella* Walsh (Diptera: Tephritidae) to degrade and detoxify the phytotoxin phloridzin depends on the presence of the bacterial gut symbiont *Enterobacter agglomerans* (Beijerinck) Ewing & Fife.[15] Furthermore, a positive correlation has been found between the presence of insecticide resistance genes and *Wolbachia* density in the mosquito *Culex pipiens* L. (Diptera: Culicidae).[16] Although the presence of *Wolbachia* does not directly affect the insect's susceptibility, the density of the symbiont increases the cost of resistance.[17] These authors hypothesized that, in the presence of resistant genes, the mosquitoes suffer a physiological cost that, in turn, reduces their ability to control *Wolbachia* densities. The consequent increase in *Wolbachia* infection levels has deleterious effects on the host, thus increasing the cost of insecticide resistance.

The number of *Rickettsia* in *B. tabaci* nymphs and adults varies greatly among individuals and may reach very high densities.[7] This high bacterial load may partially explain the significant fitness disadvantage associated with the presence of *Rickettsia*. The increased mortality exhibited by the population carrying the *Rickettsia* in response to pesticide applications may restrict the proliferation of infected individuals and thereby contribute to the fact that the presence of the symbiont is not fixed in field populations.[10] Another possibility is that *Rickettsia* possesses an as yet undiscovered fitness disadvantage for its whitefly host. This disadvantage might weaken the whitefly and make it vulnerable to environmental stresses, including pesticide pressure.

In spite of the insecticide resistance burden, most *B. tabaci* in the field carry *Rickettsia*.[10] Either frequent horizontal transmission of the symbiont or a fitness advantage that has yet to be discovered in this system may be helpful in maintaining the *Rickettsia* in different populations of the pest.

The two *B. tabaci* lines compared in this study were established by setting isofemale lines with individuals originating from one population that had been kept in the laboratory for many years; thus, both lines are B biotype that harbour *Portiera* and *Hamiltonella*, and they differ only in the presence or absence of *Rickettsia*. Crosses between the two lines showed that they successfully mate and produce fertile offspring (Chiel E, unpublished data). These facts, together with the very straight line obtained on the concentration–response curves to pyriproxyfen for the two lines (Fig. 2), suggest that they differ only in the presence or absence of *Rickettsia*, and that the presence of *Rickettsia* is responsible for the differences observed.

More experiments on lines with and without *Rickettsia* are required in order to understand the mechanisms by which the symbiont affects the biology of *B. tabaci* in general, and insecticide resistance in particular.

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