Final Scientific Report - US-4304-10 R

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BARD Project Number: US-4304-10 R

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Project Title: *Rickettsia* in the whitefly *Bemisia tabaci*: Phenotypic variants and fitness effects

Investigators

Principal Investigator: Martha S. Hunter

Co-Principal Investigator: Einat Zchori-Fein

Institutions

University of Arizona

ARO

Keywords

Symbiosis, sweetpotato whitefly, insect migration, horizontal transmission, insect symbionts

Abbreviations commonly used in the report, in alphabetical order:

MEAM 1 : "Middle Eastern Minor 1"

Budget: IS: \$ 140,000

US: \$ 150,000

Total: \$ 290,000

Signature
Principal Investigator

Signature
Authorizing Official, Principal Institution

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Publication Summary (numbers)

	Joint IS/US authorship	US Authors only	Israeli Authors only	Total
Refereed (published, in press, accepted) BARD support acknowledged	4	1	1	6
Submitted, in review, in preparation	2	2		4
Invited review papers				
Book chapters				
Books				
Master theses				
Ph.D. theses	2			
Abstracts	9	4	3	16
Not refereed (proceedings, reports, etc.)	1			1

Cooperation Summary (numbers)

	From US to Israel	From Israel to US	Together, elsewhere	Total
Short Visits & Meetings	2	1	3	6

Details of cooperation:

The project was conducted as a part of a long, ongoing collaboration between the two laboratories, and was executed jointly at all stages. Insect samples were shipped from one country to the other throughout the study and various lab members continuously shared protocols and results. The PIs routinely updated each other on the progress made, consulted about results and future experimental designs, and jointly prepared the data for publication. The PIs also met frequently. At the beginning of the project they met at the *Wolbachia* meeting held in June 2010. Dr. Hunter attended the 4th European Whitefly Symposium held in Israel on 2011 and on July 2012 the PIs met again in Poland, where they both attended the International Symbiosis Society meeting. The PIs co-attended the International Whitefly meeting in Crete on 2013, after which Hunter came for a visit to Israel. Toward the end of the project, on 2013, Zchori-Fein visited the Hunter laboratory at the Univ. of Arizona and the two PIs then attended the Entomological Society of America meeting in Austin. Additionally, Hunter took an active part in both the kickoff and annual meetings of an EU ARIMNet project which includes 15 researchers from 7 countries and is headed by Zchori-Fein. This project is largely derived from collaborative projects of Zchori-Fein and Hunter funded by BARD as well as USDA and BSF.

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Abstract

The sweet potato whitefly, *Bemisia tabaci* (Hemiptera: Aleyrodidae) is a major pest of vegetables, field crops, and ornamentals worldwide. This species harbors a diverse assembly of facultative, “secondary” bacterial symbionts, the roles of which are largely unknown. We documented a spectacular sweep of one of these, *Rickettsia*, in the Southwestern United States in the B biotype (=MEAM1) of *B. tabaci*, from 1% to 97% over 6 years, as well as a dramatic fitness benefit associated with it in Arizona but not in Israel. Because it is critical to understand the circumstances in which a symbiont invasion can cause such a large change in pest life history, the following objectives were set: 1) Determine the frequency of *Rickettsia* in *B. tabaci* in cotton across the United States and Israel. 2) Characterize *Rickettsia* and *B. tabaci* genotypes in order to test the hypothesis that genetic variation in either partner is responsible for differences in phenotypes seen in the two countries. 3) Determine the comparative fitness effects of *Rickettsia* phenotypes in *B. tabaci* in Israel and the United States. For Obj. 1, a survey of *B. tabaci* B samples revealed the distribution of *Rickettsia* across the cotton-growing regions of 13 sites from Israel and 22 sites from the USA. Across the USA, *Rickettsia* frequencies were heterogeneous among regions, but were generally at frequencies higher than 75% and close to fixation in some areas, whereas in Israel the infection rates were lower and declining. The distinct outcomes of *Rickettsia* infection in these two countries conform to previously-reported phenotypic differences. Intermediate frequencies in some areas in both countries may indicate a cost to infection in certain environments or that the frequencies are in flux. This suggests underlying geographic differences in the interactions between bacterial symbionts and the pest. Obj. 2, Sequences of several *Rickettsia* genes in both locations, including a hypervariable intergenic spacer gene, suggested that the *Rickettsia* genotype is identical in both countries. Experiments in the US showed that differences in whitefly nuclear genotype had a strong influence on *Rickettsia* phenotype. Obj. 3. Experiments designed to test for possible horizontal transmission of *Rickettsia*, showed that these bacteria are transferred from *B. tabaci* to a plant, moved inside the phloem, and could be acquired by other whiteflies. Plants can serve as a reservoir for horizontal transmission of *Rickettsia*, a mechanism that may explain the occurrence of phylogenetically-similar symbionts among unrelated phytophagous insect species. This plant-mediated transmission route may also exist in other insect-symbiont systems, and since symbionts may play a critical role in the ecology and evolution of their hosts, serve as an immediate and powerful tool for accelerated evolution. However, no such horizontal transmission of *Rickettsia* could be detected in the USA, underlining the difference between the interaction in both countries, or between *B. tabaci* and the banded wing whitefly on cotton in the USA (*Trialeurodes* sp. nr. *abutiloneus*) and the omnivorous bug *Nesidiocoris tenuis*. Additionally, a series of experiments excluded the possibility that *Rickettsia* is frequently transmitted between *B. tabaci* and its parasitoid wasps *Eretmocerus mundus* and *Encarsia pergandiella*. Lastly, ecological studies on *Rickettsia* effects on free flight of whiteflies showed no significant influence of symbiont infection on flight. In contrast, a field study of the effects of *Rickettsia* on whitefly performance on caged cotton in the USA showed strong fitness benefits of infection, and rapid increases in *Rickettsia* frequency in competition population cages. This result confirmed the benefits to whiteflies of *Rickettsia* infection in a field setting.

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Achievements (three pages maximum, 1.5 spaced, font not smaller than 12)

Significance of the main scientific achievements or innovations

Past experiments in Israel indicated that *Rickettsia* was effectively neutral for its whitefly host. In contrast, results stemming from a study conducted in Arizona suggested a dramatic fitness benefit associated with the presence of *Rickettsia*: doubled fecundity, doubled survival to adulthood and highly female biased sex ratios. Given that only relatively small changes in biology (e.g. in host range, fecundity, insecticide resistance) can exacerbate or reduce the pest status of an insect, this research was initiated to understand the circumstances in which a symbiont invasion can cause such a large change in pest life history.

Whitefly screening in Israel and the USA. We characterized the extent of current *Rickettsia* spread in the USA and Israel samples. A survey of *B. tabaci* B samples revealed the distribution of *Rickettsia* across the cotton-growing regions of ten sites from Israel and twenty-two sites from the USA. Across the USA, *Rickettsia* was generally at very high frequencies, close to fixation, whereas in Israel the infection rates were lower and declining. The distinct outcomes of *Rickettsia* infection in these two countries conform with previously reported phenotypic differences. Intermediate frequencies in some areas in both countries may indicate a cost to infection in certain environments or that the frequencies are in flux. This suggests underlying geographic differences in the interactions between bacterial symbionts and this serious agricultural pest. This study is in revision (Cass et al.).

***Rickettsia* genotyping.** To test the hypothesis that variation in phenotype of R^+ whiteflies in the two countries is due to differences in *Rickettsia* strains, we sequenced representative genes from sites in both countries. We found evidence that *Rickettsia* was highly similar if not identical in both countries; both 16S rDNA and a 984 portion of the citrate synthase gene *gltA* were identical. Furthermore, a much faster evolving intergenic spacer region, *rpmE-tRNA^{fMet}*, used among researchers of human pathogenic *Rickettsia* to type strains within species, was also identical between countries (Cass et al. In revision). We are pursuing this line of research beyond the BARD funding period to compare the genome of *Rickettsia* in *B. tabaci* in Israel with that of *Rickettsia* in *B. tabaci* in the USA, but our working hypotheses is that the same strain of *Rickettsia* is present in both countries.

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Whitefly alternative genetic backgrounds. Given the high degree of genetic similarity between *Rickettsia* in whiteflies in the USA and Israel, we tested the hypothesis that differences in whitefly genetic background could influence *Rickettsia* expression. We cannot compare living whiteflies from the USA and IS for biosecurity reasons, so instead we established a second whitefly genetic background for *Rickettsia* in the USA, collected from Maricopa, AZ. As we did previously, we collected whiteflies, separately pooled uninfected individuals and infected individuals, and then conducted a series of crosses and backcrosses between the two lines (for 6 generations) to homogenize the nuclear background between them. The result was two whitefly inbred backgrounds: “Mac1” and “Mac2,” each with separate R^+ and R^- lines that shared >98% nuclear whitefly genes. Our comparisons of performance of these two backgrounds showed an interaction between background and *Rickettsia*-infection; while *Rickettsia* appeared to increase fitness in both backgrounds, *Rickettsia* had smaller effects in the Mac2 line. We then introgressed the two backgrounds in order to create new R^+ lines that had the cytotype of Mac1 and the nuclear genotype of Mac2 or vice versa. Performance experiments with the six lines (the four original and the two new lines) enabled us to distinguish between cytotype effects and nuclear effects on the symbiont phenotype. Our results strongly supported the role of whitefly nuclear genes in influencing the *Rickettsia* phenotype, but did not show a role for the whitefly cytotype (mitochondrial genes or genes from the other symbionts). Differences in whitefly genotype or genetic population structure could explain the differences between *Rickettsia* effects in Israel relative to the USA. This work is in preparation (Hunter et al. In prep).

Within-species horizontal transmission of *Rickettsia*. The fact that *Rickettsia*, best known as vertebrate pathogens vectored by blood-feeding arthropods, is also found in phytophagous insects presupposes a means of horizontal transmission, but no mechanism for this transmission has been described. Here we found that *Rickettsia* were transferred from *B. tabaci* to a plant, moved inside the phloem, and could be acquired by other whiteflies. In one experiment, *Rickettsia* was transferred from the whitefly host to leaves of cotton, basil and black nightshade, where the bacteria were restricted to the phloem cells of the plant. In another experiment, *Rickettsia*-free adult whiteflies, physically segregated, but sharing a cotton leaf with *Rickettsia*-plus individuals, acquired the *Rickettsia* at a high rate. Plants can serve as

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a reservoir for horizontal transmission of *Rickettsia*, a mechanism which may explain the occurrence of phylogenetically similar symbionts among unrelated phytophagous insect species. This plant-mediated transmission route may also exist in other insect-symbiont systems, and since symbionts may play a critical role in the ecology and evolution of their hosts, serve as an immediate and powerful tool for accelerated evolution. This work has been published (Caspi-Fluger et al. 2011).

Between-species horizontal transmission of Rickettsia. In USA cotton fields, *B. tabaci* harbors the secondary symbionts *Rickettsia* and *Hamiltonella*, and co-occurs with *Trialeurodes* sp. nr. *abutiloneus* whiteflies. To determine whether symbionts may be transferred from one whitefly genus to the other, the microbial diversity of these whiteflies on cotton across the USA was analyzed. *Trialeurodes* sp. nr. *abutiloneus* bore *Portiera*, *Pseudomonas*, *Serratia*, *Arsenophonus* and *Wolbachia*, but no *Rickettsia* or *Hamiltonella* were detected. These results provide no evidence for horizontal transmission of symbionts between these two whitefly genera. More work is necessary to understand the interactions of ecological and physiological barriers that determine the complex patterns of interspecific transmission we see in phylogenetic reconstructions of symbionts and their arthropod hosts. This work has been published (Cass et al. 2014).

Agricultural and/or economic impacts of the research findings.

This research illustrates how the biology of an insect pest can be strongly influenced by an intracellular symbiont. *Rickettsia*, in the USA sweetpotato whitefly, *B. tabaci*, confers strong fitness benefits that make it superior to uninfected individuals and potentially a worse pest. That the relationship is different in Israel, where our work showed that *Rickettsia* is actually declining in frequency in the field, illustrates an important and overlooked aspect of intracellular symbionts, that host background and genetic diversity may mediate the effects of symbiont infection. Furthermore, these studies showed that horizontal transmission of a common symbiont via the plant can be an important route for symbiont infection of insects. In general, the interaction between symbionts of herbivores and plant responses is likely to be an important area in the future with significant agricultural implications.

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Details of cooperation

This project would not have been possible as a sole-PI effort. One of the central findings of the work is that the interaction of *Rickettsia* and whiteflies is fundamentally different in the USA and Israel. This finding was only possible with close collaboration of the US and IS teams. Interaction and feedback between the team members informed the work done in each laboratory, and made both teams more productive. In many instances, data and specimens were shared for additional analysis in the other team's laboratories, and several joint publications resulted from the collaboration (see **List of Publications**, below).

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List of publications

Published research papers

Caspi-Fluger, A., Inbar, M., Mozes-Daube, N., Mouton, L., **Hunter M.S.** and **E. Zchori-Fein**. 2011. *Rickettsia* 'in' and 'out': two different localization patterns of a bacterial symbiont in the same insect species. *PLoS One* 6: e21096.doi:10.1371/journal.pone.0021096.

Caspi-Fluger, A., Mozes-Daube, N., Inbar, M., Katzir, N., Portnoy, V., Belausov, E., **Hunter M.S.** and **Zchori-Fein E.** 2012. Horizontal transmission of the insect symbiont *Rickettsia* is plant-mediated. *Proc. Roy. Soc. Lon., B.* **279**: 1791-1796.

Zchori-Fein, E., E. Chiel, S. Steinberg, J. Klapwijk, K. Bolckmans and D. Gerling. 2012. Contagious sterility in the parasitoid wasp *Eretmocerus mundus* (Hymenoptera: Aphelinidae). *Biocontrol Sci. Technol.* 22: 61-66.

Cass, B.N., N. Mozes-Daube, L. Iasur-Kruh, E.C. Bondy, S.E. Kelly, **M.S. Hunter** and **E. Zchori-Fein**. **2014** Bacterial endosymbionts in field-collected samples of *Trialeurodes* sp. nr. *abutiloneus* (Hemiptera: Aleyrodidae). *Res. Microbiol.* 165: 77-81.

Chiel, E., Kelly, S.E., Harris, A.M., Gebiola, M., Li, X., **Zchori-Fein, E.** and **M.S. Hunter**. 2014 Characteristics, phenotype and transmission of *Wolbachia* in the sweet potato whitefly, *Bemisia tabaci* (Homoptera: Aleyrodidae), and its parasitoid *Eretmocerus* sp. nr. *emiratus* (Hymenoptera: Aphelinidae). *Environ. Entomol.* 43: 353-362.

Asiimwe, P., Kelly, S.E. and **Hunter, M.S.** 2014. Symbiont infection affects whitefly dynamics in the field. *Basic Appl. Ecol.* In press.

Submitted and in preparation

Cass, B.N., Yallouz, R., Bondy, E.C., Mozes-Daube, N., Horowitz, A.R., Kelly, S.E., **Zchori-Fein E.** and **Hunter, M.S.** Dynamics of the endosymbiont *Rickettsia* in an insect pest. *Microb. Ecol.* In revision.

Hendry, T., **Hunter, M.S.** and Baltrus, D. The facultative symbiont *Rickettsia* protects an invasive whitefly against entomopathogenic *Pseudomonas syringae* strains. *Appl. Environ. Microbiol.* In revision.

Cass, B.N., Bondy, E.C., Kelly, S.E., Fung S.K. and **M.S. Hunter**. Conditional fitness effects of the *Rickettsia* bacterial symbiont in an insect pest. In preparation for *Oecologia*.

Hunter, M.S., Asiimwe, P., Himler, A.G., Kelly, S.E. Cass, B.N. and **Zchori-Fein, E.** Host genetic background influences the phenotype of a mutualist facultative endosymbiont. In preparation for *J.Evol. Biol.*

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Meeting abstracts

Caspi-Fluger, A., N. Mozes-Daube, L. Mouton, M. Inbar, N. Katzir, V. Portnoy, E. Belausov, **M. S. Hunter** and **E. Zchori-Fein**. 2011. Horizontal transmission of the insect symbiont *Rickettsia* is plant-mediated. 4th European Whitefly Symposium, September 2011, Rehovot, Israel.

Hunter, M.S., A.G. Himler, T. Adachi-Hagimori, E. Chiel, A. Kozuch, S. E. Kelly and **E. Zchori-Fein**. 2011. Breakneck spread of the symbiont *Rickettsia* in *Bemisia* B biotype is fueled by female bias and fitness benefits. 4th European Whitefly Symposium, September 2011, Rehovot, Israel.

Cass, B.N., and **M.S. Hunter**. 2011 Distribution of the bacterial symbiont *Rickettsia* in USA populations of the sweetpotato whitefly, *Bemisia tabaci*. The Entomological Society of America Annual Meeting, Reno, NV, November 2011.

Zchori-Fein, E. 2012. The diversity of whitefly symbionts. COST FA0701 meeting on metagenomics of whitefly symbionts, Valencia, Spain.

Hunter, M.S. A. G. Himler, B. N. Cass, S. E. Kelly, E. Chiel, and **E. Zchori-Fein**. 2012. *Rickettsia* infection is dynamic and influences fitness and ecology of the global invasive pest, the sweetpotato whitefly, *Bemisia tabaci*. 7th International Symbiosis Society Congress. August 2012, Krakow, Poland.

Cass, B.N., Yallouz, R., Bondy, E.C., Mozes-Daube, N., Horowitz, A.R., Kelly, S.E., **Zchori-Fein E.** and **Hunter, M.S.** *Rickettsia* dynamics in populations of the sweetpotato whitefly, *Bemisia tabaci*, in Israel and the USA. 7th International *Wolbachia* conference, June 2012, St. Pierre d'Oleron, France.

Himler, A.G., Bergen, J., Zhong, L., Cass, B.N., Macias, S., Gomez, B., Byrne, D. and **Hunter, M.S.** 2012. Effects of the endosymbiont *Rickettsia* on whitefly flight. 7th International *Wolbachia* conference, June, 2012, St. Pierre d'Oleron, France.

Hunter, M.S., Himler, A.G., Kelly, S.E., Bergen, J.E., Rodriguez, B.A., Zhong, L. Byrne, D.B. 2012. Tracking dynamic *Rickettsia*-whitefly interactions: how a rapidly spreading symbiont influences host biology and plant-insect interactions. Entomological Society of America Annual Meeting, November, 2012, Knoxville, TN.

Zchori-Fein, E. 2012. Moving around - Horizontal transmission of the insect symbiont *Rickettsia* among trophic levels. The 21st International Congress of Zoology, Haifa, Israel.

M.S. Hunter, A. G. Himler, B. N. Cass, S. E. Kelly, E. Chiel, and **E. Zchori-Fein**. 2012. *Rickettsia* infection is dynamic and influences fitness and ecology of the

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global invasive pest, the sweetpotato whitefly, *Bemisia tabaci*. 7th International Symbiosis Society Congress. August 2012, Krakow, Poland.

Hunter, M.S. Cass, B.N., Asiimwe, P., Kelly, S.E., **Zchori-Fein, E. 2013.** *Rickettsia* infection influences the fitness and ecology of *Bemisia tabaci*. **Keynote Speaker**, First International Whitefly Symposium, Crete, Greece.

Zchori-Fein, E. 2013. Current status of whitefly symbionts research. First International Whitefly Symposium, Kolymbari, Crete.

Hunter, M.S. 2013. The effects of an intracellular symbiont on plant-whitefly interactions. The Entomological Society of America Annual Meeting, Austin, TX, USA.

Hunter, M.S., Himler, A.G., Asiimwe, P., Kelly, S.E., Cass, B.N., Mozes-Daube, N. **Zchori-Fein, E. 2014.** Fitness benefits of a facultative symbiont are influenced by host nuclear genotype. 8th International *Wolbachia* Conference, Innsbruck, Austria.

Hunter, M.S., Himler, A.G., Asiimwe, P., Kelly, S.E., Nabity, P., Cass, B.N. 2014. Exploring the mechanism of fitness benefits of a facultative symbiont of whiteflies. Plenary speaker, Hemipteran-Plant Interactions Symposium, Riverside, CA.

Hunter, M.S., Himler, A.G., Nabity, P., Cass, B. N., Asiimwe P. 2014 (November, upcoming). The intracellular symbiont *Rickettsia* influences whitefly biology in multiple ways. Entomological Society of America Annual Meeting, Portland, OR.

Non refereed

Caspi-Fluger, A., M. Inbar, N. Mozes-Daube, **M.S. Hunter** and **E. Zchori-Fein. 2013.** The symbiotic bacterium *Rickettsia* in the sweet potato whitefly *Bemisia tabaci*, and its transmission routes among hosts. Yevul Si 80:14-19. (in Hebrew)