

**University of São Paulo
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**Multipartite interactions of *Aphis* (*Toxoptera*) and their associated
symbionts**

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Thesis presented to obtain the degree of Doctor in
Sciences. Area: Entomology

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DEDICATION

This thesis is dedicated to my loving ones that stood by my side
all over the way.

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EPIGRAPH

I have fought the good fight, I have finished the race, I have kept the faith.

2 Timothy 4:7

RESUMO

Interações multitróficas de *Aphis* (*Toxoptera*) e seus simbioss secundários

A associação insetos - bactérias simbioss resulta em várias implicações bioecológicas para o hospedeiro e se estende em uma rede de interações que inclui outros microrganismos, o ambiente e outros níveis tróficos. A interação bactérias simbioss e pulgões tem sido amplamente estudada, especialmente em espécies polífagas do hemisfério norte, tal como o pulgão modelo *Acyrtosiphon pisum* (Harris). Essas pesquisas indicam que simbioss influenciam vários aspectos ecológicos do hospedeiro, tais como: nutrição, resistência ao estresse térmico e inimigos naturais, capacidade de transmissão de fitovírus, uso de plantas hospedeiras, entre outros. Entretanto, o resultado da interação simbioss-pulgão é contexto dependente, no qual o fenótipo depende da variabilidade intra- e interespecífica, da linhagem do simbioss e de diversos fatores bióticos e abióticos. Assim, este trabalho aborda as interações do pulgão *Aphis* (*Toxoptera*) *citricidus* (Kirkaldy) e seus simbioss, com o objetivo de contribuir com um sistema ecológico diferente. Foi investigada a influência da estratégia alimentar na diversidade da microbiota, comparando-se uma espécie oligófaga, *A. citricidus*, a outra polífaga, *Aphis* (*Toxoptera*) *aurantii* Boyer de Fonscolombe. Foram utilizadas diversas abordagens experimentais (biológica, metagenômica, genômica, transcritômica e proteômica) para atingir os objetivos de *i*) investigar o impacto das plantas hospedeiras na aptidão biológica de *A. citricidus* e *A. aurantii* e sua consequência ao simbioss primário, *ii*) explorar as diferenças no genoma do simbioss primário de *A. citricidus* e *A. aurantii*, *iii*) investigar a influência da planta hospedeira na distribuição de simbioss secundários em *A. citricidus*, *iv*) pesquisar a riqueza e abundância de simbioss secundários nas duas espécies de pulgão e indagar sobre a influência de *Spiroplasma* *v*) na aptidão biológica, *vi*) no transcrito e *vii*) na proteômica de *A. citricidus* em duas plantas hospedeiras (laranja vs. murta). Os resultados indicaram ser laranja o melhor hospedeiro para ambos pulgões, sendo *A. citricidus* mais sensível à planta hospedeira que *A. aurantii*. As duas espécies de pulgão utilizam estratégias distintas para controlar o crescimento de *Buchnera*, assim como a utilização do alimento influenciou diferentemente cada fase do desenvolvimento dos pulgões estudados. A planta hospedeira também influenciou a abundância de simbioss secundários; porém, *Spiroplasma* foi o simbioss secundário mais abundante em ambas espécies. *Spiroplasma* não afetou a biologia de *A. citricidus*, mas causou alterações no transcrito e no proteoma do hospedeiro. A planta hospedeira também exerceu forte regulação na transcrição gênica de *A. citricidus*, mas esse efeito foi dependente da infecção do pulgão por *Spiroplasma*. Análises de transcrito em pulgões infectados indicaram a regulação de transcritos relacionados à resposta imunológica quando em laranja, mas de chaperoninas em murta. A regulação gênica de *A. citricidus* foi fortemente influenciada pela planta hospedeira, mas enquanto insetos livres de *Spiroplasma* apresentaram superexpressão gênica em laranja quando comparada a murta, pulgões infectados com esse simbioss apresentaram padrão de expressão oposto para o mesmo conjunto de transcritos. Análises comparativas do proteoma de pulgões infectados ou não por *Spiroplasma* nas plantas hospedeiras estudadas indicaram diminuição dos mecanismos de defesa em favor de um aumento de proteínas ligadas à nutrição em insetos infectados quando se alimentando em murta.

Palavras-chave: Fisiologia; Interações multitróficas; Manejo sustentável de pragas; Simbiose

ABSTRACT

Multipartite interactions of *Aphis* (*Toxoptera*) and their associated symbionts

Insect-symbiont interactions have many bioecological consequences to the host. Their relationships expand through a complex network that includes other microorganisms, interactions with the environment and other trophic levels. An extensive literature has been produced on bacterial symbionts and aphids, especially for polyphagous aphids from North America and Europe, such as *Acyrtosiphon pisum* (Harris). They indicated symbionts influence host nutrition, heat tolerance, defense against natural enemies, virus transmission, host plant exploitation, among others. However, the outcome from host-symbiont interactions is context-dependent, with the expressed phenotype depending on intra and interspecific variations, symbiont strain, and biotic/abiotic stimuli. We explored the interaction between aphids and associated symbionts, aiming to contribute to this field by exploring new aphid systems, and focused on *Aphis* (*Toxoptera*) *citricidus* (Kirkaldy) and associated microbiota. We investigated the influence of the feeding habits on symbiont diversity in an oligophagous, *A. citricida*, as compared to a polyphagous species, *Aphis* (*Toxoptera*) *aurantii* Boyer de Fonscolombe. We employed several approaches (biological, metagenomics, genomic, and proteomic) to investigate *i*) the impact of host plants on fitness traits and primary symbiont abundance in the oligophagous and polyphagous species, *ii*) differences in the draft genome of the primary aphid symbiont between *A. citricidus* and *A. aurantii*, *iii*) the influence of host plant in secondary symbiont distribution in *A. citricidus*, *iv*) secondary symbiont richness and abundance in both aphids, and investigate the effects of *Spiroplasma* infections *v*) in the fitness traits, *vi*) transcriptome and *ii*) proteome of *A. citricidus* when reared on two host plants (sweet orange and orange jasmine). Our data indicated that sweet orange is a better host plant than orange jasmine for both aphids, and that *A. citricidus* was more negatively affected by lower-quality host than *A. aurantii*. *A. citricidus* and *A. aurantii* had different strategies regarding *Buchnera* growth and the use of food in different stages of development. We observed differences in the draft genome of *Buchnera* associated to *A. citricidus* and *A. aurantii*. Host plant affected secondary symbiont abundance, but *Spiroplasma* was the most abundant symbiont in both aphids. *Spiroplasma* had neutral effects on *A. citricidus* biology, but affected host transcriptome and proteome. The host plant affected gene expression of *A. citricidus*, but the effect was dependent on *Spiroplasma* infection. Transcriptome analysis indicated *Spiroplasma* down-regulated aphid immune response genes on sweet orange, while regulating an entire different set of genes on orange jasmine, mainly chaperonins. Gene transcription of *A. citricidus* was strongly influenced by the host plant. But while a large number of transcripts were up-regulated in uninfected aphids in sweet orange as compared to orange jasmine, the same set of genes had an opposite pattern of expression in *Spiroplasma*-infected aphids. Comparative proteomic analysis of *Spiroplasma*-infected and uninfected aphids on sweet orange and orange jasmine demonstrated regulation of a larger number proteins on orange jasmine than on sweet orange. *Spiroplasma* down-regulated the immune response of aphids and up-regulated proteins related to nutritional processes when developing on a low-quality host plant, orange jasmine, while no such trend was observed on sweet orange.

Keywords: Physiology; Multitrophic interactions; Sustainable pest management; Symbiosis

1 INTRODUCTION

Plants, herbivorous insects, and associated microorganisms participate of complex multitrophic interactions in which insect-plant relationships are influenced by microbial associates of either plants or insects (SUGIO et al., 2015). This thesis will not cover plant microbial diversity, but instead address how insect-associated bacterial endosymbionts are influenced by host plant use, and how a particular symbiont affects host fitness traits, host transcriptome and host proteome on different host plants.

Aphids are model insects used to study insect-symbiont interactions, and their relationships with host plants and natural enemies. The association between aphids and the obligate symbiont *Buchnera aphidicola* is largely known, and there is a robust body of information for *Acyrtosiphum pisum* (Harris), including its host races and secondary symbionts (MORAN; TELANG, 1998; SABATER et al., 2001; SIMON et al., 2003; TSUCHIDA et al., 2004; LAUGHTON; FAN; GERARDO, 2014).

B. aphidicola is an obligate intracellular bacterium sharing a long history of association with aphids, with differences among strains closely representing the phylogeny of their hosts (NOVAKOVA et al., 2013). This bacterium is harbored in specific cells of the host called bacteriocytes (BUCHNER, 1965), and is maternally transmitted to the progeny (WILKINSON; FUKATSU; ISHIKAWA, 2003). *B. aphidicola* is known to supplement the aphid diet by supplying the host mainly with essential amino acids that are not provided by the host diet, but also vitamins and sterols (DOUGLAS, 2009). In return, the host aphid provides *B. aphidicola* an optimum niche for survival (HANSEN; MORAN, 2014).

The long history of association of this symbiont with host aphids led to the reduction of the *B. aphidicola* genome, as symbionts lose several of their genes in the process of symbiogenesis (WERNEGREN, 2002). *B. aphidicola* lost critical genes for DNA replication and repair, and genes coding for most enzymes in the biosynthetic pathways for non-essential amino acids (MORAN; DEGNAN, 2006). However, whole genome sequencing of *B. aphidicola* from multiple aphid species demonstrated interspecific variation in the ability to synthesize amino acids (JIANG et al., 2013). Interspecific variation was observed due to the presence of specific mutations in *B. aphidicola* genome that drove host plant adaptation, leading to the development of host-adapted races in *Schizaphis graminum* (Rondani) (VOGEL; MORAN, 2011), and also resulted from the intensity with which *Buchnera* genome reduction occurred in particular host species (KOGA; TSUCHIDA; FUKATSU, 2003; PEREZ-BROCAL et al., 2006). Genome reduction in *B. aphidicola* harbored by *Cinara* sp. was so intense that this

symbiont can no longer provide the host aphid with all of the required nutritional needs, and the host has to rely on an additional secondary symbiont to aid *Buchnera* to fulfill its nutritional role (KOGA; TSUCHIDA; FUKATSU, 2003; SAKURAI et al., 2005; PEREZ-BROCAL et al., 2006).

Several secondary symbionts are known to be associated with aphids. *Hamiltonella defensa*, *Regiella insecticola*, *Serratia symbiotica*, *Rickettsiella viridis*, *Rickettsia*, *Spiroplasma*, X-type, *Arsenophonus* and *Wolbachia* are among the most common aphid-associated secondary symbionts. Although these symbionts are not vital for host survival and/or reproduction, they may influence host fitness traits by altering the host response to a number of abiotic and biotic conditions (OLIVER et al., 2010; SU; ZHOU; ZHANG, 2013; WAGNER et al., 2015).

Several aphid-associated secondary symbionts are considered to aid the aphid defensive response to natural enemies (SU; ZHOU; ZHANG, 2013; OLIVER; SMITH; RUSSELL, 2014). Therefore, increased survival to parasitism was linked to aphids harboring *H. defensa* and *S. symbiotica* (OLIVER et al., 2003), although pea aphid resistance to parasitoids in *H. defensa*-associated aphids was dependent on the symbiont strain and on the presence of the infective bacteriophage APSE (OLIVER et al., 2009). But protection against fungal pathogens was observed in aphids carrying *R. insecticola*, *R. viridis*, *Rickettsia*, *Spiroplasma* and the X-type symbiont (FERRARI et al., 2001; LUKASIK et al., 2013; TSUCHIDA et al., 2014; HEYWORTH; FERRARI, 2015). Additionally, *R. viridis* was reported to indirectly protect the host aphid against predatory insects (TSUCHIDA et al., 2014). *Wolbachia* are known to protect *Drosophila* and mosquitoes against viruses; nonetheless, no protective phenotype was recognized in aphids so far (TEIXEIRA; FERREIRA; ASHBURNER, 2008; MOREIRA et al., 2009; AUGUSTINOS et al., 2011).

Furthermore, secondary symbionts can also lead to other phenotypic responses of aphid to abiotic and/or biotic stressors. *S. symbiotica* and *R. insecticola* were associated with aphid tolerance to heat stress (MONTLLOR; MAXMEN; PURCELL, 2002; RUSSELL; MORAN, 2006), *R. insecticola* and *Arsenophonus* with host plant utilization by aphids (LEONARDO; MUIRU, 2003; FERRARI et al., 2006), and *Spiroplasma* with sex determination in sexual generations and induced fitness costs, as it reduced aphid fecundity and longevity (FUKATSU et al., 2001; SIMON et al., 2011).

Host plant use is the most debatable phenotype in aphid-symbiont interactions. Regardless the unquestionable contribution and importance of *Buchnera* to support aphids in exploiting a nutritionally limited food resource as the phloem (DOUGLAS, 2009), secondary

symbionts place host plant use by aphids into an interesting ecological perspective, since secondary symbionts allow the use of additional plant species and promote dietary specialization by increasing performance on one species while decreasing on another (HANSEN; MORAN, 2014).

Host race adaptation driven by secondary symbionts has been reported in aphids (LEONARDO, 2004; WAGNER et al., 2015). In the aphid *A. pisum* for example, the secondary symbiont *R. insecticola* was associated with a race adapted to white clover (*Trifolium repens*), while *H. defensa* to a race adapted to alfalfa (*Medicago sativa*) (LEONARDO; MUIRU, 2003). But this issue remains controversial due to variable intra and interspecific results (LEONARDO, 2004; TSUCHIDA; KOGA; FUKATSU, 2004; FERRARI; VIA; GODFRAY, 2008 ; PECCOUD et al., 2015). Another issue affecting the proper evaluation of the role of secondary symbionts in aphid host-race adaptation is the biased screening for secondary symbionts on aphids and the limited biogeographic coverage, limiting the proposal of a hypothetical pattern of association with secondary symbionts and host plant specialization (ZYTYSKA; WEISSER, 2016).

Host plants are also reported to limit the spread of secondary symbionts, as secondary symbiont - host plant interactions are also dependent on plant nutritional quality and plant species diversity in a patch. *S. symbiotica* was demonstrated to be affected by plants exposed to low levels of nitrogen (WILKINSON; KOGA; FUKATSU, 2007), while plant diversity in a patch favored aphid symbiont diversity (HENRY et al., 2015).

The range of host plants exploited depends on the aphid feeding strategy and on the co-evolutionary history with its host plants. Monophagous aphids are specialized in host plant use, and are able to tolerate or even sequester host plant defensive compounds. Polyphagous aphids, on the other hand, are commonly spread geographically and are reported to survive periods of environmental instability, even though they are less prone to tolerate secondary defensive compounds from the host plant (ALI; AGRAWAL, 2012). The association of secondary symbiont distribution with aphids-feeding habits has been recently discussed. *S. symbiotica* was commonly associated with aphids with a narrow host-plant range (HENRY et al., 2015). However, few studies that screened for secondary symbionts included oligophagous aphids, and none of them discussed symbiont distribution and feeding habits (HENRY et al., 2015; ZYTYSKA; WEISSER, 2016).

Additionally, the increasing use of next generation sequencing technologies to elucidate insect-symbiont interactions is shedding some light on key questions. The use of metagenomics based on the 16S rRNA gene revealed a more precise symbiont diversity, as it allowed the

detection of low density or even new symbionts (RUSSELL et al., 2013; BANSAL; MIAN; MICHEL, 2014). Furthermore, transcriptome analyses allowed for investigations on the molecular mechanisms by which phenotypes induced by symbionts act on host plants and vice-versa (LIU et al., 2012; BAUER et al., 2014; UPADHYAY et al., 2015). Moreover, proteomic approaches investigated insect proteins that could influence host phenotype, such as proteins of the aphid saliva that could be involved in virus transmission to plants or act as effector proteins (CILIA et al., 2011; ELZINGA; DE VOS; JANDER, 2014; PINHEIRO et al., 2014).

We investigated host-aphid-plant relationship of an oligophagous aphid inhabiting tropical areas of South America, an area still underrepresented in the growing body of information that accumulates in this field of research. *Aphis (Toxoptera) citricidus* (Kirkaldy) (Hemiptera, Aphididae) is an oligophagous aphid with a diet range restricted to *Citrus* species and a few closely-related genera (HALBERT; BROWN, 1996). This aphid vectors the *citrus tristeza virus* (CTV), a disease that caused severe damage to global citriculture until resistant varieties became available (MORENO et al., 2008). CTV is mainly transmitted by *A. citricidus*, although *Aphis (Toxoptera) aurantii* Boyer de Fonscolombe can also vector this disease, but with low efficiency. *A. aurantii* can co-occur with *A. citricidus* in *Citrus* trees, and in such conditions it is usually misidentified as *A. citricidus*, since they are morphologically similar. Besides, *A. aurantii* differs from *A. citricidus* in many ecological aspects, particular the diet breadth. While *A. citricidus* is reported almost exclusively on citrus (HALBERT; BROWN, 2011), *A. aurantii* hosts includes coffee, tea, cacao, avocado, macadamia, litchi, mango, piper and fig as host plants, and it has been considered an important pest in many of them (CARVER, 1978; CORTEZ-MADRIGAL et al., 2003; SEVIM; CELEBI; SEVIM, 2012; WAHEED et al., 2014). There are few studies on the biology of *A. citricidus* and *A. aurantii* (TSAI, 1998; TSAI; WANG, 1999; WANG; TSAI, 2001), but data are quite variable from one study to another, which has been conferred to the variation in the genetic background of aphids and host plants tested (TANG et al., 1999). Nothing is known on secondary symbiont diversity in these species.

Thus, we provide new data on aphid-symbiont associations for aphids inhabiting tropical areas in the New World. We employed several approaches to investigate the association of secondary symbionts with aphids and their role in host plant use, aphid/primary symbiont gene expression and aphid proteomics. We also investigated the genome of the primary symbiont - *Buchnera* - of two closely related aphid species with different feeding habits – oligophagy vs. polyphagy – to search for differences in their symbionts to explain their range of host plants they use.

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2 NUTRITIONAL ECOLOGY OF OLIGOPHAGOUS AND POLYPHAGOUS APHIDS AFFECTED BY HOST SUITABILITY AND ITS EFFECTS ON THE OBLIGATE SYMBIONT GROWTH

Abstract

Nutrition is an important aspect of insect physiology and ecology, and insects may develop different levels of diet specialization leading herbivores to explore from a narrow to a broad range of hosts. Polyphagous insects feed on a broad range of host plants belonging to different botanic families, while oligophagous insects feed on a restrict number of plants, usually within the same family. As a consequence, diet limitation will lead to nutritional specialization in oligophagous. Nutritional specialization may become even more severe in cases in which the diet can pose nutritional quality limitations. Hemipterans are examples of insects that feed on nutritionally poor diets. In such cases, diet use was made possible through the establishment of associations with nutritional symbionts that supply essential nutrients as diet supplements. Here we investigate the effects of host plant suitability on the biology of the oligophagous aphid *Aphis (Toxoptera) citricidus* (Kirkaldy) and the polyphagous *Aphis (Toxoptera) aurantii* Boyer de Fonscolombe, and on their obligate symbiont *B. aphidicola*. The effects of host plant quality on fitness of oligophagous and polyphagous aphids were assessed by investigating their development when restricted to host plants with different nutritional value (sweet orange and orange jasmine) and when alternating host plant quality between immature and adult stages. Sweet orange was the most adequate host for both aphid species, but the poor nutritional quality of orange jasmine was much more severe on the fitness of the oligophagous aphid than the polyphagous aphid. The growth pattern of *Buchnera* differed between aphids and host plants. *Buchnera* increases in density as *A. citricidus* develops on sweet orange, but *Buchnera* density will decrease in aphids feeding on orange jasmine. Yet, *Buchnera* will increase in density in *A. aurantii* regardless the host plant in use, although following a different growth trend. Finally, *Buchnera* density fluctuation reflects the contribution of food source in each developmental stage of both aphids and we argue on the dynamic of the interaction among aphids, *Buchnera* and host plant suitability considering the oligophagous - polyphagous dichotomy.

Keywords: *Buchnera aphidicola*; Comparative biology; Host plant; Nutrition

3 DRAFT GENOME OF *Buchnera aphidicola* FROM TWO CLOSELY RELATED APHIDS WITH DIFFERENT HOST RANGES

Abstract

Buchnera aphidicola is the obligate symbiont of aphids. This bacterium provides essential amino acids and vitamins to the host, while host provides a safe intracellular environment. One of the consequences of the specialization to the intracellular mode of life and the establishment of a long co-evolutionary history with aphid hosts is the drastic reduction in the genome of *Buchnera*. Gene losses among different lineages of *Buchnera* were unequal, but all *Buchnera* strains sequenced so far lack genes related to DNA repair and replication and members of pathways for non-essential amino acids. However, there are also cases in which losses of specific sets of genes in *Buchnera* were related to the aphid nutrition ecology. We produced *de novo* assemblies of draft genomes for *Buchnera* strains associated with close-related aphid species, *Aphis* (*Toxoptera*) *citricidus* (Kirkaldy) and *Aphis* (*Toxoptera*) *aurantii* Boyer de Fonscolombe, which differ in the range of host plants they explore, in order to investigate if differences in the genome of the obligate symbiont would reflect the oligophagy/polyphagy strategies of their host aphids. Draft genomes of *Buchnera* associated with *A. citricidus* (oligophagous) and *A. aurantii* (polyphagous) were assembled following paired-end sequencing (250 bp-pairs) on an Illumina MiSeq platform. *Buchnera* associated with *A. citricidus* (*BAC* strain) and *A. aurantii* (*BAa*) were quite similar, with 570 predicted coding sequences (CDS) in common. Only two CDS and one tRNA were observed to differ between the *BAC* and *BAa* strains: a riboflavin kinase, a DNA helicase and a tRNA-Leu. The riboflavin kinase is a pseudogene in *BAa*, while DNA helicase and a duplication in the tRNA-Leu were only observed in *BAC*. Indeed, the *BAC* strain is the only *Buchnera* strain so far to carry a DNA helicase. Duplications in tRNA-Leu were also observed in the APS strain of *Buchnera*. We compared both draft genomes with those available for other *Buchnera* strains and discuss their differences based on the importance of these gene functions to the host nutritional ecology, considering the oligophagous-polyphagous dichotomy.

Keywords: Comparative genome; Genomics; Host nutrition ecology; Obligatory symbiont

4 SYMBIONT DIVERSITY OF *Aphis (Toxoptera) citricidus* (HEMIPTERA: APHIDIDAE) AS INFLUENCED BY HOST PLANTS

Abstract

Aphids are well known for their association with endosymbiont bacteria. Almost all aphids harbor *Buchnera aphidicola* as an obligate symbiont and several other bacteria as facultative symbionts. Associations of facultative symbionts and aphids are quite variable in terms of diversity and prevalence across aphid species. Facultative symbionts can have a major impact on aphid bioecological traits. A number of factors shape the outcome of the facultative symbiont-aphid association, including aphid clone, bacterial genotype, geography, and host plant association. The effects of host plant on aphid-facultative symbiont associations are the least understood. We performed deep sequencing of the bacterial community associated with field populations of the oligophagous aphid *Aphis (Toxoptera) citricidus* (Kirkaldy) collected from different host plants. We demonstrate that *i)* *A. citricidus* has low symbiont diversity, *ii)* symbiont diversity is affected by host plant, and *iii)* host plants affect the relative abundance of the obligate symbiont *Buchnera* and an unknown genus of Enterobacteriaceae.

Keywords: Bacterial community; Food source; Illumina; Metagenomics; Symbiont

5 FACULTATIVE SYMBIONT DIVERSITY AT THE SAME ECOLOGICAL NICHE IN TWO CLOSELY-RELATED APHIDS WITH DIFFERENT HOST RANGES

Abstract

Richness and abundance of facultative symbionts vary strongly with aphid species and genotype, symbiont strain, host plant, biogeography and a number of abiotic factors. Despite indications that aphids in the same ecological niche show similar levels of facultative symbiont richness, existing reports do not consider the potential role of host plants on aphid microbial community. Little is known about how oligophagy and polyphagy may be influenced by secondary symbiont distribution, mainly because studies on secondary symbiont diversity are biased towards polyphagous aphids from the Northern Hemisphere. Here, we demonstrate the richness and abundance of the most common aphid-associated facultative symbionts in two tropical aphid species, the oligophagous *Aphis (Toxoptera) citricidus* (Kirkaldy) (Hemiptera: Aphididae) and the polyphagous *Aphis (Toxoptera) aurantii* Boyer de Fonscolombe (Hemiptera: Aphididae). *A. citricidus* is restricted to *Citrus* sp. host plants and closely-related genera, whereas *A. aurantii* successfully exploits a wide variety of host plants from different families. Both were collected in the same ecological niche and our data basically indicated the same richness of secondary symbionts, but the abundance at which secondary symbionts occurred was very distinct between the two species. *Spiroplasma* was the most abundant facultative symbiont associated with *A. citricidus* and *A. aurantii* in the ecological niche studied. Single and multiple secondary symbiont infections were observed, but diversity of multiple infections was particularly high in *A. citricidus*. We discuss our findings and suggest hypotheses to explain causes and consequences of the differences in secondary symbiont diversity observed between these two aphid species.

Keywords: Host plant; Microbial community; Oligophagy; Polyphagy; Symbiont richness

6 NO FITNESS COSTS ASSOCIATED WITH INFECTIONS OF *Aphis (Toxoptera) citricidus* BY THE SECONDARY SYMBIONT *Spiroplasma* ON TWO HOST PLANTS

Abstract

Aphids are associated with many bacterial symbionts, with the obligate symbiont *Buchnera aphidicola* supplementing the host diet with essential amino acids and vitamins, while secondary symbionts inducing different host phenotypes. Aphids can harbor several secondary symbionts that alter important aphid-related ecological traits, such as defense against natural enemies, heat tolerance and host plant utilization. One of these secondary symbionts, *Spiroplasma*, is well known in *Drosophila* as a sex modulator and by interacting with the host immune system. However, little is known on the effects of *Spiroplasma* on aphids, such as its influence on the host immune defense against fungi and on host plant utilization. Aphid infections by *Spiroplasma* are low and few aphid species were reported to be infected with this secondary symbiont. Thus, we investigated the association of *Spiroplasma* with the tropical aphid *Aphis (Toxoptera) citricidus* (Kirkaldy) through comparative biology experiments on two host plants with different nutritional value to the aphid. We demonstrate *Spiroplasma* induced no significant fitness costs to *A. citricidus* on either host plant, as no changes in the fitness traits we assessed were observed. *Spiroplasma* infection only induced subtle changes on host longevity and fecundity. Therefore, we concluded *Spiroplasma* established a neutral interaction with *A. citricidus* under the selection pressure we tested, and argue on stress conditions that could better demonstrate the role of *Spiroplasma* in *A. citricidus* bioecology and associated costs involved.

Keywords: Aphid-symbionts interactions; Comparative biology; Host plant suitability; Nutritional ecology

7 TRANSCRIPTOME ANALYSIS OF *Spiroplasma*-INFECTED *Aphis* (*Toxoptera*) *citricidus* ON TWO HOST PLANTS

Abstract

Aphids are associated with several bacterial symbionts. The obligate symbiont *Buchnera aphidicola* is known for supplying the host with essential amino acids and vitamins, while the host provides a safe environment for *Buchnera* growth. This relationship is ancient and led to the establishment of a complementary metabolism between host and *Buchnera*. However, aphids may be associated with a variety of secondary symbionts that alter host ecology and interacts with host immune system. Secondary symbionts may affect host plant use and promote dietary specialization, leading to the establishment of host-adapted races. Moreover, host plant, aphids, and primary and secondary symbionts establish an interesting multipartite interaction. *Spiroplasma* is the least studied secondary symbiont due to low occurrence on aphids, but we observed high abundance of this symbiont on *Aphis* (*Toxoptera*) *citricidus* (Kirkaldy). Biology of *A. citricidus* infected with *Spiroplasma* showed a neutral relationship. However, identification of host-induced phenotypes by secondary symbionts may appear only under selection pressure. Therefore, we used transcriptome analysis to investigate the influence of *Spiroplasma* on *A. citricidus* at the molecular level. We also explored the effects of host suitability on the interaction *A. citricidus*-*Spiroplasma*. We found that *Spiroplasma* affected *A. citricidus* transcriptome, and the intensity of the effects on gene expression depends on host plant quality. *Spiroplasma* infections induced up-regulation of transcripts related to immune defense in aphids feeding on sweet orange, while on orange jasmine most of the up-regulated transcripts were linked to nutrition (digestive enzymes and detoxification pathways). Thus, we argue that *Spiroplasma* affects the transcriptome of *A. citricidus*, and regulation of gene expression differs in response to the suitability of the host plant to the host aphid.

Keywords: Gene expression; Host plant suitability; Immune defense; Nutrition; RNA-Seq

8 PROTEOMICS OF THE INTERACTION *Aphis (Toxoptera) citricidus*- *Spiroplasma* ON TWO HOST PLANTS

Abstract

Bacterial symbionts are broadly distributed among insects, influencing insect biology and ecology to different degrees. Aphids are commonly associated with primary and secondary symbionts. *Buchnera aphidicola* is the primary symbiont that provides essential amino acids the host is unable to produce or acquire from the host plant. Aphids also carry a number of secondary symbionts that can influence aphid physiology and fitness attributes, including changes in host metabolites and immune defense. *Spiroplasma* is one of such secondary symbionts associated with aphids, but very little is known about such association and its effects on the aphid trophic interactions. *Spiroplasma* is not usually reported as a common aphid secondary symbiont, but a high level of infection has been demonstrated in one population of *Aphis (Toxoptera) citricidus*. We used sister isolines of *Spiroplasma*-infected (Ac-BS) and *Spiroplasma*-free (Ac-B) aphids reared on sweet orange and orange jasmine as host plants to demonstrate the effects of *Spiroplasma* infection on the proteomics of *A. citricidus*. *Spiroplasma* infection affected the aphid proteome in both host plants. A higher number of proteins were found to be affected in aphids feeding on orange jasmine, indicating the host plant quality influence the outcome of the aphid-*Spiroplasma* interactions. In both host plants, the majority of proteins affected by *Spiroplasma* were heat shock proteins, proteins linked to cell function and structure, and energy metabolism. However, *Spiroplasma* also induced changes in proteins involved in antimicrobial activity, carbohydrate processing and metabolism, amino acid synthesis and metabolism in aphids feeding on orange jasmine. We also provide a discussion on how the host proteome is differentially affected by *Spiroplasma* infection when the host is exploiting different host plants.

Keywords: Aphid-symbiont interaction; Host plant use; Mass spectrometry; Metabolism alterations

9 FINAL CONCLUSIONS

We were able to demonstrate the effects of host plant suitability (optimal vs. suboptimal host plants) on the development and reproduction of the aphid *Aphis (Toxoptera) citricidus* (Kirkaldy) and on the growth pattern of the aphid-associated primary symbiont, *Buchnera aphidicola*. We also demonstrated that aphids reproductive strategies and regulation of the growth pattern of the primary symbiont differ between the oligophagous aphid *A. citricidus* and the closely-related polyphagous *Aphis (Toxoptera) aurantii* Boyer de Fonscolombe. Our investigation on the genomic composition of the primary symbiont associated with these oligophagous and polyphagous aphid species indicated differences in their strategies to explore optimal and suboptimal hosts were not sustained by differences in the genomic composition of their primary symbionts.

We also demonstrated that host plants can affect the diversity of secondary symbionts associated with *A. citricidus*, even if restricted to *Citrus* and *Citrus*-related genera, and that the effects on the aphid associated microbiota followed the phylogeny of the host plants and their suitability to *A. citricidus*. Our investigations also allowed us to explore the diversity of secondary symbionts associated with *A. citricidus* and *A. aurantii* and demonstrate they share the same secondary symbiont richness, but not the secondary symbiont abundance when living in the same ecological niche. Nevertheless, *Spiroplasma* was the most common secondary symbiont infecting both aphid species. We then demonstrated associations with *Spiroplasma* induced no fitness costs to *A. citricidus*, but *Spiroplasma* did affect the proteome of aphid host. The effects of *Spiroplasma* infection on aphid proteome were influenced by the quality of the host plant. Transcriptomic analysis between *Spiroplasma*-infected and *Spiroplasma*-free *A. citricidus* reared on optimal and suboptimal hosts indicated *Spiroplasma* interferes with gene expression of *A. citricidus* and its associated primary symbiont, *B. aphidicola*. Once again, the effects of *Spiroplasma* on gene expression profile of infected aphids were influenced by the quality of the host plant (optimal or suboptimal) the aphid was developing on.

In conclusion, we demonstrated that the association of aphid-primary symbiont is influenced by secondary symbionts and that the nutritional quality of the aphid host plant interferes in the multipartite interactions aphids establish with their associated symbionts. We found no clear role of symbiotic associations on the feeding strategies of *A. citricidus* (oligophagy) and *A. aurantii* (polyphagy).