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Symbionts and hosts behavioral interactions: a study from the perspective of
host – parasitoid interactions

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RESUMO

Simbiontes e interações comportamentais de hospedeiros: um estudo da perspectiva das interações hospedeiro-parasitoide

A simbiose é um dos principais agentes na evolução e ecologia de organismos. Tais interações são muito íntimas, podendo ser muito diversas e ter grandes impactos na diversidade biológica. Uma das principais associações que ocorrem na natureza é aquela entre insetos e microrganismos. Microrganismos associados a insetos são capazes de alterar uma gama de eventos fisiológicos, comportamentais, ecológicos e evolutivos em seus hospedeiros. Dois simbiontes de insetos muito comuns são *Wolbachia* e *Spiroplasma*. *Wolbachia* é também muito comum a outros artrópodes e nematoides. Para melhor compreender como essas relações podem influenciar o comportamento de insetos, dois sistemas biológicos foram selecionados para investigar como esses simbiontes podem interferir nas interações hospedeiro-parasitoide. O comportamento de duas espécies de parasitoides, *Aphelinus asychis* (Hymenoptera: Aphididae) e *Trichogramma pretiosum* (Hymenoptera: Trichogrammatidae) foi investigado quando explorando *patches* com seus respectivos hospedeiros, *Aphis citricidus* (Hemiptera: Aphididae) e *Anagasta kuehniella* (Lepidoptera: Pyralidae). No primeiro caso foi investigado como a infecção do hospedeiro por *Spiroplasma*, e no segundo caso como a infecção do parasitoide por *Wolbachia*, afetariam a exploração da *patch* pelos parasitoides. O comportamento dos parasitoides ao explorarem suas *patches* foi registrado, assim como os comportamentos de defesa dos pulgões em resposta ao ataque do parasitoide. Os dados obtidos demonstraram que *Spiroplasma* afetaram o comportamento de defesa e a agressividade de *A. citricidus* em resposta aos ataques de *A. asychis*. *Wolbachia* aumentou o tempo de residência e reduziu o sucesso de parasitismo de ovos do hospedeiro por *T. pretiosum*. A compreensão de tais efeitos certamente contribuirá para o melhor entendimento dos efeitos da associação de insetos a simbiontes, fornecendo bases sólidas para a melhor exploração de tais interações para propósitos de controle biológico de pragas.

Palavras-chave: Comportamento de insetos; Inimigos naturais; Parasitismo; Simbiontes

ABSTRACT

Symbionts and hosts behavioral interactions: a study from the perspective of host – parasitoid interactions

Symbiosis is one of the main players in evolution and ecology of organisms. Such intimate interactions may be diverse and have a great impact in biological diversification. One of the main associations that occur in nature is that of insects and microbes. Insect associated microbes are, capable of altering a wide range of physiological, behavioral, ecological and evolutionary events for their hosts. Two very common insect microbial symbionts are *Wolbachia* and *Spiroplasma*. *Wolbachia* is also common to other arthropods and nematodes. To better understand how these relations could influence the behavior of insects, we selected two biological systems to investigate how these symbionts can interfere in the host – parasitoid interactions. We investigated the behavior of two species of parasitoids, *Aphelinus asychis* (Hymenoptera: Aphididae) and *Trichogramma pretiosum* (Hymenoptera: Trichogrammatidae) when exploiting patches with their respective hosts, *Aphis citricidus* (Hemiptera: Aphididae) and *Anagasta kuehniella* (Lepidoptera: Pyralidae). In the first case we looked into how *Spiroplasma* infecting hosts, and in the second case how *Wolbachia* infecting the parasitoid may affect parasitoid patch exploitation. We recorded the wasp's behaviors when exploiting their patches, as well as the aphid defensive behavior in response to parasitoid attack. Our data demonstrate *Spiroplasma* and *Wolbachia* influence the parasitoid patch exploitation decisions. *Spiroplasma* also affected the defense behavior and aggressiveness of *A. citricidus* in response to *A. asychis* attack. *Wolbachia* increased the patch residence time and reduced the successful parasitization of host eggs in *T. pretiosum*. The understanding of such effects will certainly contribute to provide a better knowledge of the outcome of the associations of insects with microbial symbionts, providing ground base for the proper exploitation of such interactions for biological control purposes.

Keywords: Insect behavior; Natural enemies; Parasitism; Symbionts

1. INTRODUCTION

Symbiosis is a common process in nature and it is distributed in many hosts ranging from aquatic to terrestrial habitats, playing an important part in the evolution and ecology of organisms (Oliver et al. 2003; Wernegreen 2004). Symbiosis is so diverse and involves the living together of a range of organisms that the outcome of such associations can be extremely diverse (Nikoh et al. 2014; Lewis and Lizé 2015). The age of the association is one of the major factors affecting the type of relationship the associated organisms will establish. Longer the history of association more likely is the reduction of pathogenesis and high is the development of coadaptive processes due to the coevolutionary history (Hentschel et al. 2000; Steinert et al. 2000).

Symbiosis has a great impact in biological diversification (Zabalou et al. 2004). In the case of insects, microorganisms that established mutualistic associations can be fundamental in providing essential nutrients to insect hosts (Hansen and Moran 2011; McCutcheon and Moran 2012), recycling nitrogen (Fox-Dobbs et al 2010), assisting with food digestion (Engel and Moran 2013) and in supporting host reproduction (Dedeine et al. 2001). These associations most involve endosymbionts or endocytobionts, but there are cases in which the coevolutionary history of the association led to the establishment of external mutualistic associations. One of such example is the ant – fungus association, in which fungus-growing ants rely on the cultivation and use of a mutualistic fungus as food resource (Weber 1966). From the many associations organisms present in the animal kingdom, the ones that are maternally transmitted, like bacteria in insects, are often obligate, reaching such an intimate relationship that one could not survive without the other (Douglas 1998; Ferrari and Vavre 2011).

Facultative symbionts in the other hand are not required for the completion of their hosts life cycle but usually establish beneficial symbiotic interactions (Oliver et al. 2010). Although not always required for their hosts survival, the facultative symbionts may contribute positively to several host fitness traits, such as resistance against natural enemies and xenobiotics, protection against heat stress, and expansion of food sources (Chen and Purcell 1997; Oliver et al. 2003; Ferrari et al. 2007; Kontsedalov et al. 2008; Burke et al. 2010; Xie et al. 2010; Simon et al. 2011). As shown by Oliver et al. (2003), the facultative symbiont *Serratia symbiotica* improved aphid resistance against its natural enemy, causing high mortality of the developing parasitoid larvae.

Other researchers have even found evidence that the presence of facultative symbionts could increase their host fitness, sometimes doubling their offspring (Leonardo and Muir 2003). Moreover, non-parasitic microbial symbionts can also alter the behavior of their hosts in order to optimize their reproductive fitness or to protect them against pathogens (Brownlie and Johnson 2009).

Facultative symbionts can be acquired from the environment or be horizontally transmitted, but vertical transmission is not uncommon even if not leading to infection fixation (Werren et al. 2008). The most spread and common group of non-obligate symbiont associated with arthropods are the sex-determinant bacteria, particularly. *Wolbachia* is the most common representative microorganism associated with the reproductive system of arthropods, influencing progenie sex determination or even host reproductive fitness *Wolbachia* (Ma et al. 2013; Newton et al. 2016). Other known bacteria that also establish such type of relationship with their arthropod hosts are *Arsenophonus*, *Cardinium*, *Rickettsia* and *Spiroplasma* (Enigl and Schausberger 2007; Duron et al. 2008; Shropshire and Bordenstein 2016; Zhang et al. 2016).

The discoveries in the last decades of the importance of the microbiota in phenotype definition (Sonnenburg et al. 2005; Lyte et al. 2016) and the increased understanding of the role of obligate and non-obligate bacteria in insects, made clear the associated microbiota acts as manipulators of their hosts. In manipulating the host, the microbiome acts as one of the determinants of evolutionary, physiological and behavioral processes driving adaptation, diversification and speciation (Forsythe and Kunze 2013; Rohrscheib and Brownlie 2013; Rohrscheib et al. 2015).

Manipulators of behavioral responses of insect hosts are seen among symbiotic bacteria (Lewis and Lize 2015), fungi (Roy et al. 2006) and virus (Burand et al. 2005), but in many of these cases microorganisms established a pathogenic relationship with their host insects (Evans 1982; van Houte; Ros; van Oers 2013). The entomopathogenic fungus *Ophiocordyceps unilateralis* induces the infected host ant *Camponotus leonardi* (Hymenoptera: Formicidae) to wander towards the north/northwest direction as infected ants die around noon in a position that enhances fungus transmission and spread of infection to other workers of the colony (Holldobler and Wilson 1990; Hughes, et al. 2011). Pathogenic viruses are also reported to affect the behavior of infected hosts, inducing the precocious onset of the courthip behavior in *Gryllus texensis* (Orthoptera: Gryllidae) male crickets to assure female infection during mating (Knell and Webberley 2004; Adamo et al. 2014).

There are also a few cases of non-pathogenic symbionts that influence the host behavior. Viral particles infecting *Leptopilina boulardi* (Hymenoptera: Figitidae) alter the wasp parasitization behavior inducing a higher tendency to host superparasitization, increasing the chances of horizontal transmission within the parasitized host larva *Drosophila* (Varaldi et al. 2006). Reproductive parasites usually manipulate their hosts' reproduction by increasing the production or survival of female hosts at some cost to the males (Montenegro et al 2006). *Wolbachia*-infected males and females of *Drosophila melanogaster* (Diptera: Drosophilidae) had higher locomotor activity and foraging behavior than uninfected adults probably due to an increase in their metabolic rates (Evans et al. 2009; Caragata et al. 2011). *Wolbachia* was also shown to reduce *D. melanogaster* male aggressiveness by lowering males octopamine levels (Rohrscheib et al. 2015). Additionally, *Wolbachia* was demonstrated to influence *Trichogramma brassicae* (Hymenoptera: Trichogrammatidae) oviposition behavior by affecting the capacity of females to discriminate previously parasitized hosts. In this case, infected females were less effective in host evaluation, leading female to select low quality hosts more frequently (Farahani et al. 2015). The manipulation of the reproductive fitness or traits of the host are very important for determination of the efficiency of symbiont infections. Nonetheless, such manipulations can directly affect host fitness and influence symbiont ability to persist in natural populations (Montenegro et al., 2005b).

Spiroplasma is another important symbiont present in nearly 5% of insects (Duron et al. 2008). *Spiroplasma* is better known as a male-killing symbiont (Harumoto et al. 2014), but that can also stimulate the host immune response capacity (Herren and Lemaitre 2011) and protect the host against natural enemies (Jaenike et al. 2010; Xie et al. 2010).

Thus, microbial manipulators can play important roles in the evolution and ecology of their hosts, and the understanding of these effects may have important applied implications (Lewis and Lizé 2015). Parasitoids are the most common biological control agents in applied biological control of a great number of insect pests, constituting one of the most important strategies in integrated pest management programs (Waage and Hassell 1982; Fernández-Arhex and Corley 2003; Yazdani and Keller 2016). As parasitoids usually have a very short lifespan, they maximize the deposition of their eggs early in their adult life to avoid dying before females are able to lay their full complement of eggs (Rosenheim 1999; Wajnberg et al. 2006; 2016).

Thus, female parasitoids evolved foraging strategies to avoid time limitation by optimization of patch use (Outreman et al. 2005; Wajnberg 2006). A patch is defined as a spatial subunit of the foraging area where host aggregations are available (Hassell and Southwood 1978; Wajnberg 2006). Hosts occurring in discrete and individual patches in the environment lead parasitoids to optimize the time they invest in host exploitation, actively contributing to the costs of reproduction (Charnov and Skinner 1984; Godfray 1994; Rosenheim 1999). A series of theoretical models was proposed to explain the decisions parasitoids make to allocate time for patch exploitation and to maximize the individual capacity of exploitation (Outreman et al. 2005). To enhance egg production, parasitoids need to balance their investments in longevity and host searching in an attempt to lay their full egg complement (Rosenheim et al. 2008).

Female parasitoids may locate patches ranging in quality within their foraging area. Differences in patch quality require females to decide the best time allocation to each particular patch in the process of host selection and exploitation. There are a number of factors that can affect the female's decision to allocate time to exploit a patch. The identification of such factors and the understanding of how they influence female's decision to select and exploit a particular patch are not only of relevance for comprehending the behavioral ecology of parasitoids (Outreman et al. 2001, 2005; Desneux et al. 2004; Tentelier et al. 2005; Wajnberg 2006), but also for predicting the efficacy of parasitoids selected for use as biocontrol agents (Waage 1990; Wajnberg et al. 2016).

Microbial associations are quite spread in insects and several insect-associated microorganisms are reported to affect the host insect behavioral decisions. In this dissertation we focused in evaluating two host-parasitoid systems to investigate the impact microbial associations would have on host-parasitoid interactions, particularly in parasitoid patch exploitation. In one of the systems used, the aphid *Aphis citricidus* (Hemiptera: Aphididae) – the wasp *Aphelinus asychis* (Hymenoptera: Aphelinidae), we used sister isolines of *Spiroplasma*-infected and uninfected aphids *A. citricidus* to investigate the role of host infection in patch time use by the parasitic wasp. In the second system, the host *Anagasta kuehniella* (Lepidoptera: Pyralidae) – the wasp *Trichogramma pretiosum* (Hymenoptera: Trichogrammatidae), we investigated how *Wolbachia* could affect patch exploitation by using sister isolines of *Wolbachia*-infected and uninfected *T. pretiosum*.

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2. *SPIROPLASMA*-INFECTING APHIDS INTERFERES WITH THE *Aphis citricidus* (HEMIPTERA: APHIDIDAE) – *Aphelinus asychis* (HYMENOPTERA: APHELINIDAE) INTERACTIONS

Abstract

There are a wide range of studies focusing on the benefits of secondary associations of insects and microorganisms at the molecular, physiological and behavioral levels. Secondary symbionts have been demonstrated to influence several insect host responses to a number of stressors, including the third trophic level. Insects associated with many secondary symbionts were shown to better survive to the attacks of their natural enemies. Understanding the effects associations with secondary symbionts may have on the output of host-parasitoid interactions are important in the implementation of biological control strategies in the field. Thus, we investigated the effects of *Aphis citricidus* infection with *Spiroplasma* in the patch-time exploitation by the aphid parasitoid *Aphelinus asychis*. We also investigated if *Spiroplasma* infection could affect the host defensive behavior. Investigations were done by recording the host defensive behaviors and the parasitoid patch use in a patch comprised of five-host aphids under controlled conditions ($25\pm 2^\circ\text{C}$; $70\pm 10\%$ RH; 14:10 h). We did not detect overall differences in the exploitation of *Spiroplasma* infected and uninfected hosts by *A. asychis*. The only factors leading to an effect on the patch leaving decisions of *A. asychis* were the time spent in grooming by the parasitic wasp and the host agitated behavior. The aphid's agitated behavior was positively influenced by *Spiroplasma* infection. *Spiroplasma* also influenced the aphid kicking and whipping behaviors. Kicking was reduced and antennal whipping was increased by *Spiroplasma* infection in response to parasitoid attack. *Spiroplasma*-infected hosts were less accepted than uninfected aphids for egg laying by *A. asychis*, demonstrating *Spiroplasma* directly affects *A. asychis* parasitization efficiency by reducing host acceptance and indirectly by enhancing host defensive behaviors that limits parasitoid successful attacks.

Keywords: Host selection; Integrated pest management; Natural enemies; Parasitoid efficacy; Symbiosis

2.1. Introduction

A wide range of studies are focused on the diverse interactions insects have with non-pathogenic microorganisms, with some of which dedicated to understand and explain how these microorganisms interact with their hosts at the molecular, physiological and behavioral levels (Goodacre and Martin 2012). Bacteria that are obligately associated with insects are often involved with host-nutrition supplementation, while the non-obligate, secondary symbionts have been reported to affect a variety of host traits, influencing the host phenotype and the host interactions with other trophic levels (Oliver et al. 2003; Vásquez et al. 2012; Guidolin et al. 2018).

Non-obligate symbionts can affect host fitness traits by conferring resistance against natural enemies and xenobiotics, protection against heat stress, and the host diet breadth (Leonardo and Muir 2003; Ferrari et al. 2004; Oliver et al. 2003; Kontsedalov et al. 2008; Burke et al. 2010; Xie et al. 2010), and even increase their response to selection pressures (Oliver et al. 2003). There are symbionts able to induce behavioral changes in the host and improve the host's reproductive capacity (Brownlie and Johnson 2009) while others are capable to confer protection to hosts against entomophagous or entomopathogens (Oliver et al. 2003).

Spiroplasma is better known as a male-killer in a range of host associations (Ebbert 1991; Montenegro et al. 2000; 2005). Male-killing is due the production of high levels of a protein that contains ankyrin repeats and a deubiquitinase domain, which is designated Spaid. Male mortality was suggested to result from the effects of Spaid on the dosage compensation machinery on the male X-chromosome (Harumoto and Lemaitre 2018). Nevertheless, *Spiroplasma* can establish associations with insect hosts playing a range of roles (Jaenike et al. 2010; Xie et al. 2010; Herren and Lemaitre 2011; Guidolin et al. 2018). In some of these associations, *Spiroplasma* infection can result in increased protection of the host against bacterial pathogens (Herren and Lemaitre 2011) and parasitoids (Jaenike et al. 2010; Xie et al. 2010). *Spiroplasma* is not commonly reported associated to aphids, but this secondary symbiont prevailed in field collected Brazilian populations of *Aphis citricidus* (Hemiptera: Aphididae) and *Aphis aurantii* (Hemiptera: Aphididae) (Guidolin and Cônsoli 2018). *Spiroplasma* infections of *A. citricidus* were also shown to affect the aphid proteome. Moreover, the aphid proteome was differently affected depending on the host plant quality, altering the abundance of proteins involved in protein-protein interactions, cell functioning and energy metabolism (Guidolin et al. 2018). Metabolomics analysis also indicated *Spiroplasma* infections affected a large variety of metabolites of *A. citricidus*, including metabolites involved in host selection by aphid's parasitoids (Duarte 2017).

Thus, based on the fact secondary symbionts can alter host phenotypic traits, affect the interactions of the host insect with other trophic levels and that *Spiroplasma* did alter the proteome and metabolome of the host aphid *A. citricidus*, we hypothesize *Spiroplasma* infection affects *A. citricidus* interactions with the parasitoid *Aphelinus asychis* (Hymenoptera: Aphelinidae), and tested two predictions to prove our hypothesis: 1) *Spiroplasma*-infected aphids will display more efficient defensive

behavior against parasitoid attack, and 2) patch time allocation of female parasitoids will be affected when exploiting *Spiroplasma*-infected aphids.

2.2. Conclusions

- *Spiroplasma* infections affect the defensive behavior of *Aphis citricidus*;
- *Spiroplasma*-infected aphids kick and whip females of *Aphelinus asychis* with their antennae more often than uninfected aphids;
- The oviposition decisions of female *A. asychis* are negatively impacted in patches of *Spiroplasma*-infected *A. citricidus*.

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3. *Wolbachia* INFECTION INTERFERES WITH THE PATCH EXPLOITATION DECISIONS OF THE EGG PARASITOID *Trichogramma pretiosum* (HYMENOPTERA: TRICHOGRAMMATIDAE)

Abstract

Several factors influence the host selection process and the behavioral ecology of parasitic wasps. The assessment and understanding of the factors that result in successful host selection and optimal patch time allocation by parasitic wasps are required for the implementation of successful applied biological control and promotion of conservation biological control. Insect microbial symbionts are diverse and are reported to influence several aspects of the physiology and behavior of their hosts, including *Wolbachia*, the most common non-obligate symbiont associated with arthropods. We investigated the effects of *Wolbachia* infection on patch time allocation and host selection behavior of *Trichogramma pretiosum* (Hymenoptera: Trichogrammatidae) using infected and cured sister lines, when exploiting patches with host eggs of *Anagasta kuehniella* (Lepidoptera: Pyralidae). A new sequence type (ST-493) of *Wolbachia* was identified infecting *T. pretiosum* using the multi-locus sequencing typing approach. We recorded the behaviors of infected and cured wasps when exploiting the patch. We found infected females remain longer in the patch when compared to cured females. We also detected mated females remained longer than virgin females regardless of their infection status. The patch leaving decisions of *T. pretiosum* females increased with rate of contact with previously parasitized eggs regardless the mating and infection status. In conclusion, the ST-493 of *Wolbachia* affects the patch leaving decisions and the efficiency of parasitization of *T. pretiosum*, influencing the field efficiency of this parasitoid if used as a biological control agent.

Keywords: Natural enemies; Parasitism; Quality control; Risk assessment; Symbiosis

3.1. Introduction

There is a wide range of symbiont bacteria directly related to their hosts attributes, which can alter the hosts physiology, phenotype expression and behavior. Some bacterial symbionts are key to their hosts as they provide essential nutrients to complement the host's nutritional requirements, while other can contribute to host defense and host utilization, for example (Steinhaus 1960; Dillon and Dillon 2004).

Wolbachia is by far the most common and widely distributed symbiont in arthropods and nematodes. *Wolbachia* is better known by affecting sex host determination using several different processes, but a number of different interactions can also be established (Werren et al. 2008). There associations in which *Wolbachia* establishes highly pathogenic interactions with their hosts (Min and Benzer 1997; Woolfit et al. 2013), while in others they can be fundamental in providing nutrients to the host (Brownlie et al. 2009) or in inducing oogenesis in others (Dedeine et al. 2001;

Dedeine et al. 2005). In associations in which *Wolbachia* infection has low adaptive costs, infection by this bacterium enhances the host immune system and contributes to the host immune defense against pathogenic infections (Teixeira et al. 2008).

Several insect associated symbionts are also known to affect the host behavior even when leading of pathogenic interactions (Dion et al. 2011; Ferrari and Vavre 2011). *Wolbachia* is also one of such symbionts, and can induce high levels of octopamine synthesis in their hosts, resulting in increased host aggressiveness (Rohrscheib et al. 2015). *Wolbachia* has also been demonstrated to affect the host selection behavior of natural enemies, reducing the capacity of female parasitic wasps to discriminate parasitized from health hosts (Farahani et al. 2015).

There are also *Wolbachia* strains that adds high fitness costs to their associate hosts negatively affecting fitness traits that are of particular interest when hosts are important as biocontrol agents in applied or conservative biological control (Mochiah et al. 2002). Biological Control is one of the most important strategies available for sustainable pest control (Jonsson et al. 2014). Implementation of biological control strategies require the understanding of natural enemies' behavior and ecology and their interactions with the hosts for their successful exploitation (Wajnberg et al. 2015).

Egg parasitoids of the genus *Trichogramma* (Hymenoptera: Trichogrammatidae) are the most common and important parasitoids used as biocontrol agents worldwide (Cônsoli et al. 2010). *Trichogramma pretiosum* is polyphagous and widely distributed in this genus, and it has been mass reared and released in millions of hectares for the control of pest species in a number of agroecosystems, including Brazil (Cônsoli et al. 2010; Parra and Zucchi 2004; Parra 2014). *Trichogramma* is also a common host to *Wolbachia*, with several species been associated with this bacterium (Pintureaeu et al. 2002; Werren et al. 2008; Almeida and Stouthamer 2017). Thelytokous parthenogenesis is the most common phenotype induced by *Wolbachia* infections in *Trichogramma* (Werren et al. 2008), and the fact that *Wolbachia*-infected *Trichogramma* would only produce females as progenies has been argued to favor the mass production and use of this natural enemy in applied biological control programs (Stouthamer et al. 1990; 1999; Stouthamer 1993;). But little is known on the effects of *Wolbachia* infections on behavioral and fitness traits that would interfere with the optimal strategies for host use and successful parasitization (Farahani et al. 2015).

There are a number of factors capable of altering the host selection and behavior ecology of parasitoids. Over recent years, the role of the host microbiome in host phenotype expression and fitness traits attributes has been discovered and increased concern to understand how associates microbes, particularly bacteria, can interfere in host biology, physiology and ecology has fostered investigations on a wide range of topics, from their role in speciation processes, host selection, host adaptation, among others (Brucker and Bordenstein 2012; Lewis and Lizé 2015; Shropshire and Bordenstein 2016).

In order to better understand the effects of *Wolbachia* in infected wasps target to use as biocontrol agents, we tested the hypothesis that *Wolbachia* affects patch utilization and host parasitization of *T. pretiosum* by analyzing the patch leaving decisions and the successful host parasitization of *Wolbachia*-infected and *Wolbachia*-cured sister isolines when exploiting patches with eggs of *Anagasta kuehniella* (Lepidoptera: Pyralidae). We expect our data will contribute to the understanding of *Wolbachia* – *Trichogramma* associations, and the possible use of *Wolbachia*-infected strains in applied biological control programs.

3.2. Conclusions

- *Trichogramma pretiosum* is infected by the new ST-493 of *Wolbachia*;
- ST-493 of *Wolbachia* affects the patch leaving decisions and the patch residence time of *Trichogramma pretiosum*;
- ST-493 of *Wolbachia* decreases the rate of host encounter per unit time of *Trichogramma pretiosum*;
- ST-493 of *Wolbachia* decreases the number of eggs successfully parasitized by *Trichogramma pretiosum*;
- ST-493 of *Wolbachia* decreases the number of rejected hosts and increases the rate of rejection of eggs per unit time by *Trichogramma pretiosum*;
- Virgin females remain short in the patch as compared to mated females regardless of their infection status.

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