

**University of São Paulo
“Luiz de Queiroz” College of Agriculture**

**Phenotypic diversity in the biological and behavioral responses of isolines of
Myzus persicae (Sulzer, 1776) (Hemiptera: Aphididae) to *Diaeretiella rapae*
(M’Intosh, 1855) (Hymenoptera: Braconidae)**

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Dissertation presented to obtain the degree of Master in
Science. Area: Entomology

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Teaching licensed and Bachelor in Biological Sciences

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“Those who pass by us, do not go alone, and do not leave us alone; they leave a bit of themselves, and
take a little of us.”

(Antoine de Saint-Exupéry)

RESUMO

Diversidade fenotípica nas respostas biológicas e comportamentais de isolinhagens de *Myzus persicae* (Sulzer, 1776) (Hemiptera: Aphididae) a *Diaeretiella rapae* (M'Intosh, 1855) (Hymenoptera: Braconidae)

Interações ecológicas são onipresentes e ocorrem entre todos os organismos, macro ou microrganismos. Na relação inseto-inseto, parasitoides são os principais entomofagos e são explorados em programas de controle biológico de diferentes insetos de importância agrícola. A compreensão da interação hospedeiro-parasitoide é essencial para o desenvolvimento de pesquisas aplicadas para a implantação de programas bem-sucedidos de controle biológico em campo. A relação estabelecida entre hospedeiro e parasitoide é resultado de processos coevolutivos, nos quais fenótipos de parasitoides com habilidades de ataque foram selecionados em resposta às adaptações de defesa do hospedeiro. Em contraste, mecanismos de defesa desenvolvidos para driblar estratégias de ataque de parasitoides, envolvendo adaptações comportamentais e fisiológicas foram selecionadas na população hospedeira. A evolução de mecanismos de defesa desenvolvidos pelo hospedeiro pode resultar em custos biológicos, refletidos na fecundidade ou tamanho do inseto, influenciando, assim, a aptidão biológica do hospedeiro. Com o objetivo de identificar a diversidade fenotípica nas respostas biológicas e comportamentais apresentadas por hospedeiros ao ataque por inimigos naturais, investigamos a interação hospedeiro *Myzus persicae* - parasitoide *Diaeretiella rapae* usando isolinhagens de *M. persicae* com diferentes respostas ao ataque do parasitoide. O sucesso do parasitismo observado de *M. persicae* por *D. rapae* variou entre 43% e 76% nas 14 isolinhagens testadas, das quais foram selecionadas três isolinhagens do primeiro (maior parasitismo) e do quarto (menor parasitismo) quartis para avaliação de parâmetros biológicos e comportamentais. Os parâmetros biológicos avaliados demonstraram diferenças significativas entre isolinhagens de *M. persicae* com diferentes respostas ao parasitismo, mas sem a associação uniforme de custo à capacidade de resposta ao parasitismo, para as diferentes isolinhagens estudadas. A associação de *M. persicae* com o simbiote secundário normalmente relatados a pulgões, *Rickettsia*, sugerem que esses organismos podem não afetar diretamente a capacidade do pulgão em responder ao parasitoide *D. rapae*, mas linhagens infectadas apresentaram maior capacidade reprodutiva. *Rickettsia* também demonstrou influenciar o comportamento de defesa das isolinhagens testadas, com as fêmeas de pulgões infectadas apresentando maior frequência de agitação do corpo do que linhagens não-infectadas. Infecção do hospedeiro por *Rickettsia* também interferiu no comportamento de seleção hospedeira dos parasitoides, que atacaram com maior frequência as isolinhagens livres do simbiote secundário, bem como pulgões do grupo de menor parasitismo. A presença de comportamento de defesa, representado por movimentos do corpo mais intensos no grupo de menor parasitismo, pode justificar o maior número de ataques necessários para parasitá-los, enquanto que o menor número de ataques pelo parasitoide em pulgões infectados por *Rickettsia*, mesmo que eles tenham apresentado comportamento de defesa semelhante ao do grupo de menor parasitismo, sugere que esse simbiote pode ter induzido outras alterações nos pulgões que interferiram no processo de seleção hospedeira do parasitoide, indicando a necessidade de estudos fisiológicos para entender os fatores envolvidos no parasitismo observado nas isolinhagens selecionadas de *M. persicae*.

Palavras-chave: Interação hospedeiro-parasitoide; Variação fenotípica; Resposta ao parasitismo; Custos adaptativos

ABSTRACT

Phenotypic diversity in the biological and behavioral responses of isolines of *Myzus persicae* (Sulzer, 1776) (Hemiptera: Aphididae) to *Diaeretiella rapae* (M'Intosh, 1855) (Hymenoptera: Braconidae)

Ecological interactions are ubiquitous and occur between all macro or microorganisms. In insect-insect interactions, parasitoids are the main group of entomophagous that are exploited in biological control programs for many agricultural insect pests. Understanding the host-parasitoid interactions is essential for the development of applied research for the implementation of successful biological control programs in the field. The relationship established between host and parasitoid is the result of coevolutionary processes, in which phenotypes of parasitoids with attacking abilities were selected in response to the host's defense adaptations. In contrast, defense mechanisms developed to circumvent parasitoid attack strategies, involving behavioral and physiological adaptations were selected in the host population. The evolution of defense mechanisms developed by the host can result in biological costs, reflected, for example, in the low fertility or small size of the insect, influencing the biological aptitude of the host. To identify the phenotypic diversity in the biological and behavioral responses presented by hosts to the attack of natural enemies, we investigated the interaction between the host *Myzus persicae* and the parasitoid *Diaeretiella rapae*, using isolates of *M. persicae* with different responses to the attack of the parasitoid. The success of the observed parasitism of *M. persicae* by *D. rapae* ranged from 43% to 76% among 14 tested isolines. Three isolines with parasitism rate in the first (high parasitism) and fourth (low parasitism) quartiles were selected for the evaluation of biological and behavioral parameters. The biological parameters evaluated showed significant differences between lineages of *M. persicae* with different responses to parasitism, but without a uniform association of cost with the aphid capacity to respond to parasitism. The association of *M. persicae* with the secondary symbiont *Rickettsia* demonstrates this symbiont does not interfere directly with the aphid's ability to respond to the parasitoid *D. rapae*, but it had a positive effect in the fecundity of infected isolines. *Rickettsia* infection also influenced the defense behavior of the tested aphid isolines, with infected aphid females showing a higher frequency of body wiggling than the uninfected females. Host infection with *Rickettsia* also interfered in the host selection behavior of *D. rapae*. Parasitoid females attacked more frequently *Rickettsia*-free isolines. Aphids from selected isolines with low parasitism by *D. rapae* were also more attacked than the aphids from isolines with high parasitism. The defensive behavior displayed by wiggling the body more intensively in the group aphids with low parasitism can justify the required larger number of attacks for their successful parasitization. In *Rickettsia*-infected aphids we observed a low number of attacks, although aphids also wiggled their bodies more intensively than uninfected aphids. In this case, we argue that *Rickettsia* induces other physiological changes in the host that it affects the host selection behavior of *D. rapae*, suggesting the need of further physiological studies for a better understanding of the factors involved in the observed parasitization of the selected isolines of *M. persicae*.

Keywords: Host-parasitoid interaction; Phenotypic variation; Response to parasitism; Adaptive costs.

1. INTRODUCTION

Aphids are phytophagous, sap-sucking hemipterans considered to have a great importance for agriculture worldwide due to the damage they cause to several crops, such as cereals, vegetables, and fruits, resulting in great economic losses (Dedryver et al., 2010; Katis et al., 2007). Aphids cause direct damage by sucking the sap of host plants and by secreting toxic saliva, leading to leaf malformation and to host nutritional deficiencies (Blackman & Eastop, 2021; Dedryver et al., 2010; Ellis et al., 1996). Aphids can also cause indirect damage by reducing the host photosynthetic capacity due to the growth of fungi on the honeydew accumulated over the surface of leaves (Blackman & Eastop, 2021; Ellis et al., 1996). But the major indirect damage aphids cause to host plants is due to the vectoring of more than one hundred types of plant viruses, such as the Bean Leafroll Virus (BLRV), Beet Yellow Net Virus (BYNV), Pea Enation Mosaic Virus (PEMV), Barley yellow dwarf virus (BYDV) and the Potato Leafroll Virus (PLRV), which delay host plant development by interfering with the growth, flowering, and fruiting of the host plant (Blackman & Eastop, 2021; Dixon, 1981; Jarosová et al., 2016; Ortiz et al., 2005). In tropical countries, most aphid species, such as *Myzus persicae*, reproduce exclusively by ameiotic (apomictic) parthenogenesis, producing genetically identical progenies. This reproductive strategy seems to be advantageous to aphids when we consider the number of descendants generated, since they produce larger offspring by reproducing asexually than sexually. But asexual reproduction in aphids is largely disadvantageous to host plants, once this mode of reproduction leads to a rapid accumulation of individuals, which can cause more intense and irreversible damage to host plants (Blackman & Eastop, 2021; Guerrero et al., 2013; Oliver et al., 2006; Sunnucks et al., 1996).

Aphid infestations of agricultural crops often require the implementation of pest control measures, and the use of insecticides is usually the most commonly taken (Foster et al., 2002; Ikbāl & Pavela, 2019). However, the side-effects on non-target organisms and the environmental risks associated with the intense and excessive use of insecticides as a single strategy for pest control is often discussed (Costa, 2018; Debach & Rosen, 1991; Foster et al., 2002; Huffaker et al., 1976; Zhang et al., 2018).

Biological control of insects with natural enemies is an important strategy when multiple strategies for pest control are sought for joint implementation as an integrated pest management strategy to alleviate the selection pressure and the non-target effects associated with the sole use of organic insecticides (Baker, Green & Loker, 2020; Lucchi & Benelli, 2018; Zalucki, Adamson & Furlong, 2009). Parasitoids are among the most important natural enemies of insects, as they are capable of reducing populations of their pest hosts below the economic threshold level (Doutt, 1959; Eggleton & Gaston, 1990; Godfray, 1994; Kraaijeveld & Godfray, 2009; Russel, 1989; Thomson et al. 2010; van Lenteren, 2005). The parasitic way of life is found in different orders of insects (Diptera, Coleoptera, Lepidoptera, Trichoptera, Neuroptera and Strepsiptera), but is highly diversified in Hymenoptera, with 80% of the hymenopterans being parasitic wasps (Pennacchio & Strand, 2006).

The relationship established between host and parasitoid is the result of their coevolutionary history, leading to the selection of phenotypes of parasitoids that most successfully overcome the host defenses and exploit their host, and the selection of host phenotypes that carry successful behavioral, chemical, and/or physiological defensive mechanisms to escape parasitoid attack and/or the establishment of parasitization. The evolutionary process between host and parasitoid is explained by the red queen theory, which is based on the continuous development of adaptations to counteract the counter adaptations of the interacting group, resulting in co-evolutionary processes that select the best adapted individuals (Kraaijeveld et al., 2002, van Valen, 1977; Vienne et al., 2013).

The success of parasitoids depends on how elaborate and efficient the processes of host location, selection and exploitation used are to parasitize different hosts (Hafer & Vorburger, 2019; Vinson, 1976; Vinson & Iwantsch, 1980; Vinson, 1984; Godfray, 1994). Parasitoids developed different strategies of parasitization during their evolutionary history for the successful location of their hosts and the establishment and successful development of the offspring allocated to hosts (Vinson, 1976, Vinson 1984; Vinson, 1990). Koinobiont parasitic wasps mastered their weaponry to regulate several of the host's physiological processes in order to attend the physical and nutritional requirements of the immature parasitoid under development in the host (Harvey & Malcicka, 2016; Vinson & Iwantsch, 1980). The processes by which parasitic wasps regulate their hosts result in alterations of the host endocrine system, metabolism, and immune response to facilitate parasitoid successful colonization and development (Digilio et al. 1998; Falabella, 2018; Strand & Pech, 1995; Vinson & Iwantsch, 1980; Vinson, 1984; Vinson, 1990; Vinson et al. 2001).

The strategies host species developed to escape parasitization are based on parasitoid avoidance and immune defense. Host defensive strategies are based on three types of defense: 1) morphological (mimicry, camouflage), 2) behavioral (aggressive behavior), and 3) physiological traits (cellular and/or humoral immune responses) (Godfray, 1994; Greeney, Dyer & Smilanich, 2012; Gross, 1993; Kraaijeveld et al., 1998; Vilmos & Kurucz, 1998; Vorburger, 2014; Zhou, Meng & Li, 2017). Homochromy is a morphological defensive strategy many species of aphids employ to avoid attacks by natural enemies, as demonstrated by the more frequent attacks reddish-colored *Acyrtosiphon pisum* suffers from predatory ladybugs and parasitoids when compared to green-colored aphids (Losey et al. 1997). *Myzus persicae* is also able to avoid detection by natural enemies by adopting a body color similar to most of their host plant species (Gillespie et al., 2009).

There are several behavioral mechanisms associated with host defense against natural enemy attacks (Dixon, 2012; Firlej et al., 2010), and the most common behavioral defenses aphids display are the body rotation around the stylets while still inserted in the plant tissues, walking away, dropping from the plant, antennal jerking movements and/or confrontation (Dixon, 2012; Firlej et al., 2010; Gross, 1993; Le Ralec, et al, 2010; Stadler, Weisser & Houston, 1994). Such defensive mechanisms can be activated by visual perception, olfactory stimuli and/or perception of substrate vibrations, which indicate the presence of a parasitoid and very commonly leads to the release of alarm pheromones (Moayeri et

al., 2014). Release of alarm pheromones triggers a local response in other members of the colony that may respond by evading the area or by remaining in the area and display other defensive behaviors. The decision alarmed aphids take is based on the predicted costs associated with the location a new suitable host plant, which also represents risks for survival and reproduction (Firlej et al. 2010; Gross, 1993; Stadler, Weisser & Houston, 1994).

The third defensive mechanism consists in the activation and mounting of an immune response to isolate and eliminate the invader. Insects carry an innate immune system capable to provide humoral (melanization and antimicrobial peptides production) and cellular responses (phagocytosis, nodulation and encapsulation) (Gerardo et al. 2010). Encapsulation and melanization are the main mechanisms activated to eliminate multicellular invaders, such as the eggs of parasitoids. Encapsulation of parasitoid eggs by the host immune system results in parasitoid death by asphyxia and/or necrosis by the release of toxic and hydrolytic substances (Gross, 1993; Kraaijeveld et al. 1998; Vilmos & Kurucz, 1998; Vorburger, 2014). In some insects, including aphids, the immune system has been supplemented with toxins produced by associated-secondary symbionts, such as the ribosome inactivating proteins (RIP) produced by *Spiroplasma*, or the shiga-like toxin, the cytolethal distending toxin (CdtB) and the YD-repeat toxin produced by bacteriophages infecting the *Acyrtosiphon pisum* secondary endosymbiont, *Hamiltonella defensa*, which have been shown to improve insect immune response to entomophages and macrophages (Asplen et al. 2014; Ballinger & Perlman, 2019; Cayetano & Vorburger, 2015; McLean, 2019; Oliver et al. 2003; Rothacher et al. 2016; Rouil et al, 2020; Scarborough et al. 2005; Schimid et al. 2012). The mechanisms by which insect-associated bacteria enhance their host immune response against environmental stressors are still poorly understood. Nevertheless, the contribution of associated bacteria to host defense in the best studied system model was indicated to occur through 1) resources competition, in which host-associated microbial symbionts compete with natural enemies for limited nutritional resources (Paredes et al. 2016); 2) competition by interference, in which metabolites produced by the host-associated microbes can directly interfere with the survival and development of the natural enemy (Ballinger et al. 2017; Oliver et al. 2009); and 3) apparent competition, in which the symbiont can activate the host immune system (Kwong et al. 2017).

In aphids, several secondary symbionts were shown to interfere with the successful parasitization of aphids by parasitoids. The APSE bacteriophages infecting the bacterium *H. defensa* produce toxins of three distinct groups of proteins depending on the phage type (shiga-like toxin, CdtB, and YD-repeat toxin), resulting in increased parasitoid immature mortality (Martinez et al. 2016, Rouil et al, 2020). *Serratia symbiotica* is another secondary symbiont that interferes with production and emission of plant volatiles and affects host-plant attractance to aphid parasitoids (Frago et al., 2017; Oliver et al., 2003). The secondary symbionts *Regiella insecticola* (Vorburger et al., 2010) and *Candidatus Fukatsua symbiotica* (Vorburger, 2018) were shown to interfere with the successful parasitization of aphids, but the mechanisms involved remain unknown. Aphids can still harbor several

other secondary symbionts (*Spiroplasma*, *Rickettsia*, *Arsenophonus* and *Wolbachia*), but their contribution to host defense have not yet been reported (Vorburger, 2018).

The defensive adaptations developed by insects to stressors is also associated with energy costs to build such response, which can carry associated fitness costs and affect fitness parameters, such as rate of growth, fecundity, fertility, and longevity (Boots & Begon, 1993; Kraaijeveld & Godfray, 1997; Martinez et al., 2018; Sager & Coley, 1995; Stadler, Weisser & Houston, 1994). Such defense mechanisms can be classified as constitutive or induced, and mechanisms that are constitutively available may require higher energy allocation (Schmid-Hempel, 2005). The existence of trade-offs between efficacious host defensive mechanisms to avoid parasitization and fitness traits due to associated costs can be decisive for the evolution and fixation of such defensive mechanisms in natural populations (Ebert, 2005).

The use of Classical Biological Control strategies in aphid biocontrol through the importation, multiplication and inoculation of parasitic wasps has been proven an efficient strategy by reestablishing the natural, ecological forces involved in the regulation of host – parasitoid populations, such as the use of parasitic wasps for aphid control in wheat fields in Brazil (Kenis et al, 2019; Stáry, Sampaio & Bueno, 2007; Sampaio et al, 2008). But the existing technologies for the mass production of insects and the establishment of biofabrics of biocontrol agents gave rise to Applied Biological Control strategies through the use of inundative releases of natural enemies (Oliveira et al. 2013). In such system, selected lines of biocontrol agents are successively mass produced and selected by responding to the selection pressures imposed by the rearing system they are exposed (Pinto & Stouthamer, 1994), and used in ways to cause a shock effect on pest populations, representing a strong source of selection acting upon the target pest population. Since the stock population of natural enemies once selected for mass production and commercialization are maintained the same, we would expect that the continuous use of such “stable” selection pressure would lead to the selection of host phenotypes with increased capacity to avoid the successful attack by parasitoids and/or the establishment of parasitism, considering that the genetic variability of parasitoid species used in biological control programs is one of the factors that can determine the selection of defensive mechanisms against parasitization (Tomasetto et al., 2017; Tomasetto et al., 2018).

Palaearctic and Nearctic species of aphids invasive to tropical and subtropical areas will not display the alternance from sexual and asexual reproduction modes naturally observed in the native regions due to the lack of the required environmental stimuli (Blackman & Eastop, 2021; Moran, 1992; Simon, Stockel & Tagu, 2010; Sorensen, 2009; Vorburger, Lancaster & Sunnucks, 2003). Thus, we would expect that populations of aphids invasive to tropical countries that are only reproducing asexually would carry very low genetic variability, once recombination does not occur in aphid parthenogenesis, and daughters are clones of their mothers (Blackman, 1979; Blackman & Eastop, 2021; Moran, 1992; Vorburger, Lancaster & Sunnucks, 2003). Therefore, we would expect to find no variation among sisters of clonal lines in response to parasitism, and that the expected differences among isolines

would allow us to better isolate and investigate the behavioral, physiological and molecular mechanisms behind aphid escape to parasitism.

Thus, in this dissertation we focused in evaluating the intra and interisoline variation in isolines of the invasive *Myzus persicae* in response to parasitization by *Diaeretiella rapae*, and select different phenotypes to investigate the existence of associated fitness costs and behavioral defensive mechanisms in response to parasitoid attack. Our data will contribute to the understanding of the mechanisms of parasitism avoidance aphids use, and support the development of investigations required to improve the successful use of parasitoids in applied biological control programs.

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2. VARIATION IN ISOLINES OF *MYZUS PERSICAE* TO SUCCESSFUL PARASITIZATION BY *DIAERETIELLA RAPAE* AND ASSESSMENT OF BIOLOGICAL TRAITS OF SELECTED LINES

ABSTRACT

Parasitoids are the main natural enemies of most insects, exerting strong selection pressure upon their hosts as successful parasitization implies in the elimination of the genetic pool of the parasitized host from the population genetic pool, once parasitized hosts die much more often before they reach their reproductive stage. The selection pressure imposed by parasitoids upon their living hosts leads to the selection of traits evolved to protect aphids from the successful development of parasitoids, and the exposure of parasitoids to host defense mechanisms leads to the selection of traits developed to counter-resist the host defense strategies. Thus, there are sources of chromosomal and extra-chromosomal variation, such as association with symbionts, that can influence the development of defense mechanisms by the host against natural enemies. The avoidance of parasitization may result in costs associated with the defensive mechanism. The coevolution in host-parasitoid interactions is difficult to observe in field populations since the visualization of resistant individuals depends on the host encounter and on the genotype of the natural enemy. Laboratory tests allow genotypes with greater and/or lesser capacities to avoid parasitoid attacks to be attacked at similar rates, under controlled abiotic and biotic conditions and similar patch structures, allowing host/parasitoid genotypes with selected responses to be studied. The present work evaluated the *Myzus persicae*-*Diaeretiella rapae* interaction under laboratory conditions to investigate the variation of isolines to parasitization and the existence of biological differences among selected clonal lines of *M. persicae* based on their association with secondary symbionts and successful parasitization by the parasitoid *D. rapae*. The observed success of parasitism in *M. persicae* by *D. rapae* varied between 43% and 76% in 14 tested isolines, of which six were selected (three in the first quartile = high parasitization; three in the fourth quartile = low parasitization) for the evaluation of biological parameters. The biological parameters evaluated showed significant differences between isolates of *M. persicae* with different responses to parasitism, and adaptive costs may be associated with the low parasitization observed in the selected isolines from the fourth quartile. The secondary symbiont *Rickettsia* does not provide additional defensive mechanisms to *M. persicae* when parasitized by *D. rapae*. Isolines of *M. persicae* carry variation within and among isolines in response to parasitization by *D. rapae* and in biological traits, but only one isolate clearly had costs associated with aphid capacity to avoid parasitization by *D. rapae*. Aphid infection by *Rickettsia* does not improve the defense response of *M. persicae* to parasitization by *D. rapae*, but it does increase female fecundity.

Keywords: Resilience to parasitism; Host-parasitoid interaction; Biological costs; Defensive symbiont.

2.1. Introduction

Ecological interactions are ubiquitous to all macro and microorganisms. Ecological interactions can be established between individuals of the same species (intraspecific interactions) or between individuals of different species (interspecific interactions). The main effects that an individual has on other individuals during their interactions can be positive for both, as in mutualism; negative for

both, as exemplified in competition, or positive for one and negative for the other individual, as in relationships of predation and parasitism (Leung & Poulin, 2008; Schoener, 1988; Travis et al. 2005).

In insect-insect relationships, parasitoids are the main entomophagous, and are exploited for conservative or applied biological control of different agricultural pests (Russell, 1989; Silveira et al. 2019). The term parasitoid was first used in 1913 by Reuter to define organisms with intermediate characteristics between predators and parasites (Eggleton & Gaston, 1990; van Lenteren, 2005; Vinson, 1976). Later, Doult (1959) highlighted the major traits to differ parasitoids from parasites, arguing that parasitoids differ from parasites as they i) kill their hosts once they complete their immature development; ii) parasitize hosts belonging to their own taxonomic class; iii) are relatively smaller than their hosts; iv) mostly present a parasitic way of life only at their immature stage; v) complete their full development by exploiting a single host; and vi) regulate their host population in a way similar to predatory organisms (Vinson, 1976).

Unlike true parasites, parasitoids exert strong selection pressure on their hosts, because successful parasitization and parasitoid development leads to the elimination of the genetic pool the parasitized host represents from the population genetic pool, once parasitized hosts die much more often before they reach their reproductive stage (Godfray, 1994; Koltz et al., 2019; Kraaijeveld & Godfray, 2009; Moore et al., 2021). Thus, the selection pressure hosts face when exposed to parasitoids will lead to the selection of hosts that were unattractive to parasitoids and/or hosts that escaped parasitoid attack and survived parasitoid development. Insects that survive the attack and development of the parasitoid are observed mainly in interactions with koinobiont parasitoids than that presented with idiobionts, once idiobiont parasitoids induce the interruption of the host's development immediately after parasitism, or attack hosts in sessile stages such as eggs or pupae, while koinobionts, on the contrary, keep the host alive and in development, allowing host to feed and grow (Godfray, 1994; Harvey et al. 1994).

Koinobiosis is advantageous as parasitoids can attack hosts at earlier stages of development, favoring successful parasitization. Young hosts are known to have lower mechanical, physiological and behavioral defensive capacity, and the fact they are allowed to grow after parasitization permits the host to develop into a suitable nutritional resource even when they are parasitized at a sub-optimal nutritional stage (Jervis & Ferns, 2011; Kraaijeveld et al. 1998). But koinobiosis is also disadvantageous to parasitoids once parasitoids cannot access the future availability of nutritional resources to their hosts, and young hosts are more vulnerable to biotic and abiotic mortality factors. Nevertheless, parasitoids that keep their hosts alive can also count on the use of the host's defensive strategies to protect the allocated parasitoid progeny to natural enemy attacks (Brodeur & Boivin, 2004; Jervis & Ferns, 2011; Kraaijeveld et al. 1998; Weinersmith, 2019). An example of this is the strategy used by most immature koinobiont parasitoids, in which the immature avoids carrying out processes that compromise the host's defense and mobility capacity, keeping the host functional until the end of its immature development (Brodeur & Boivin, 2004; Brodeur & McNeil, 1989; Weinersmith, 2019). Also, parasitoids can induce

behavioral changes in their hosts for their own benefit (Broudeur & McNeil, 1989; Brodeur & McNeil, 1990; Brodeur & Boivin, 2004; Muller, 1994; Weinersmith, 2019).

The selection pressure imposed by parasitoids upon their living hosts leads to the selection of traits evolved to protect aphids from the successful development of parasitoids, and the exposure of parasitoids to host defense mechanisms leads to the selection of traits developed to counter-resist the host defense strategies (Kraaijeveld & Godfray, 2009). The resulting coevolutionary processes during the evolutionary history of host – parasitoid interactions are reinforced by the theory of the red queen, in which each adaptation developed by one species faces a counter adaptation developed by the interacting species, in ways that the survival of both species depends on the continuous development of defense and attack strategies (arms-race) (Brockhurst et al. 2014; Kraaijeveld et al. 2002; van Valen, 1977; Vienne et al., 2013).

The coevolution in host-parasitoid interactions is frequency-dependent (Hamilton, 1990). Parasitoids are selected to avoid mechanisms of defense of common host genotypes, contributing to the selection of hosts with rare resistance genotypes (Carius et al. 2001). This process is difficult to observe in field populations since the visualization of resistant individuals depends on the host encounter and on the genotype of the natural enemy. Host genotypes with a lower capacity to circumvent the encounter with natural enemies are more frequently attacked. But genotypes of parasitoids will face similar selection pressure, resulting in a process of selection that will occur without the fixation of extreme genotypes of hosts or parasitoids (Barret, 1988; Carius et al. 2001). Laboratory tests allow genotypes with greater and/or lesser capacities to avoid parasitoid attacks to be attacked at similar rates, under controlled abiotic and biotic conditions and similar patch structures, allowing host/parasitoid genotypes with selected responses to be studied (Stiling, 1987).

The genetic variability that allows the manifestation of phenotypes with different capacities to respond to parasitoid attacks, as well as parasitoids with phenotypes with different levels of success in host parasitization, may be generated from many factors such as mutations, increased gene flow, genetic drift, inbreeding depression, and selection (Amos, 1998; Vellend & Geber, 2005). However, the main and quickest way to obtain genetic variability is through sexual reproduction, which allows the perpetuation of mutations in the population, as well as genetic recombination between chromosomes (Crow, 1994; Gerber & Kokko, 2016).

Aphids developing in regions of temperate climates display seasonal reproductive polyphenism, alternating from asexual to sexual reproduction in order to produce eggs that will diapause during the winter (Moran, 1992; Vorburger, Lancaster & Sunnucks, 2003; Shah et al., 2018). But under tropical conditions, aphid species such as *Myzus persicae* will reproduce exclusively by ameiotic (apomictic) parthenogenesis, in which chromosomal division occurs by mitosis. In apomixis reproduction the resultant progeny will be genetically identical to their mothers, and the sources of genetic variation to promote phenotypic expression are now limited to rare genomic events, such as mutations, chromosomal rearrangements, and mitotic recombination, and the interaction with

endosymbionts (Guerrero et al. 2013; Oliver et al. 2006; Sunnucks et al. 1996). Chromosomal rearrangements and interactions with symbionts have been shown to serve as sources of genetic variation leading to the manifestation of insecticide-resistant and host-plant adapted phenotypes (Blackman et al., 1995; Brown & Blackman, 1988; Kikuchi et al., 2012; Oliver et al., 2008; Russell & Moran, 2006). Thus, there are sources of chromosomal and extra-chromosomal variation that can influence the development of defense mechanisms by the host against natural enemies, which invariably carry associated adaptive costs (Gwynn et al. 2005; Rigby et al., 2002; Sunnucks et al., 1996; Vorburger et al. 2008).

Thus, we selected the association *M. persicae* - *Diaeretiella rapae* to investigate i) the variation of clonal lines to parasitization, ii) the association with secondary bacterial symbionts that could interfere in the host response to parasitization, and iii) and the existence of biological differences among selected clonal lines of *M. persicae* based on their association with secondary symbionts and successful parasitization by *D. rapae*.

2.2. Conclusions

- There is variation in *Myzus persicae* isolines response to parasitization by *Diaeretiella rapae* within and among isolines;
- The selected aphid isolines have different biological traits, but only isolate Iso11 clearly had costs associated with aphid capacity to avoid parasitization by *D. rapae*;
- *Rickettsia* does not improve the defense response of *M. persicae* to parasitization by *D. rapae*, but it does increase female fecundity.

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3. BEHAVIORAL ANALYSIS OF THE INTERACTION OF *MYZUS PERSICAE* ISOLINES - *DIAERETIELLA RAPAE*

ABSTRACT

Aphids are sap-feeders of agricultural importance, damaging cultivated plants worldwide and causing millions of dollars of economic losses. Natural enemies are important regulators of aphid populations and are commonly used in applied biological control programs of aphids. Natural enemies impose high selection pressure to the evolution of defense mechanisms to avoid predation or parasitism, since predators and parasitoids influence the density and population growth of prey/host insects. Throughout the evolution of host-parasitoid interactions, morphological (camouflage), physiological (humoral and cellular immunity) and behavioral (kicking, wiggling, confrontation) processes were developed by hosts to avoid parasitization. Host defense against natural enemies can also be enhanced by associated symbiotic bacteria, which can inhibit parasitoid development. In the same way aphids developed defense mechanisms against their natural enemies, parasitoids have also developed strategies to circumvent the host defense tactics to successfully locate and exploit their hosts. Understanding the mechanisms of aphid defense and parasitoid strategies to attack is essential for the successful selection and utilization of parasitoids in biological control programs. In order to investigate the phenotypic diversity in behavioral responses during host-parasitoid interactions, this work evaluated isolines of the aphid *Myzus persicae* that differ in the successful rates of parasitization by *Diaeretiella rapae* in order to test the predictions that the differences in successful parasitization are regarded to direct alterations in host defensive behavior or host alterations that would interfere with the attractance and/or suitability of *M. persicae* to *D. rapae*. Investigations were realized by recording the parasitoid patch exploitation and the defensive behaviors of aphids in patches composed of three-host aphids under controlled conditions ($25\pm 2^{\circ}\text{C}$; $70\pm 10\%$ RH). The behavior of parasitoids and aphids was evaluated according to parameters commonly analyzed in the literature and associated to defensive behaviors and patch exploitation. We detected significant differences in isolines with low and high parasitism when we evaluated the occurrence of host evaluation. *Rickettsia*-infection influenced the intensity and duration of host body wiggling behavior and the number and duration of host attack by *D. rapae* females. Thus, *Rickettsia*-infections affect the defensive behaviors of *M. persicae* aphids, but also interfere with the host selection process of the parasitoid *D. rapae*.

Keywords: Defensive behavior; Host-parasitoid interactions; Defensive symbiont; Patch exploitation.

3.1. Introduction

Aphids are sap-feeders of agricultural importance to cultivated plants worldwide (Blackman & Eastop, 2021; Dedryver et al., 2010; Ellis et al., 1996; Hartbauer, 2010; Katis et al., 2007). The characterization of these insects as pests of cultivated plants is associated with the intensity of the damage caused, which can be directly (malformation of plant tissues, nutritional deficiency) or indirectly associated with insect feeding (reduced photosynthetic capacity, pathogen transmission) (Blackman & Eastop, 2021; Dixon, 1981; Ellis et al., 1996; Hartbauer, 2010; Ortiz et al., 2005). The parthenogenic reproduction of aphids allows fast population growth in a short period of time, increasing the damage to plants and making it difficult to control (Hartbauer, 2010; Simon et al. 2010; Sunnucks & Hales, 1996).

Natural enemies are important regulators of aphid populations and are commonly used in applied biological control programs of aphids. Understanding the mechanisms of aphid defense and parasitoid strategies to attack potential host is essential for the successful selection and utilization of parasitoids in biological control programs (Hartbauer, 2010).

Natural enemies impose high selection pressure to the evolution of defense mechanisms to avoid predation or parasitism, since predators and parasitoids influence the density and population growth of prey/host insects by eliminating those that have been successfully attacked, allowing the selection of phenotypes that have developed morphological, physiological and/or behavioral strategies of defense against natural enemies (Braendle & Weisser, 2001; Ninkovic et al. 2013). Recent studies have shown that aphids have developed defensive mechanisms in response to the risk of attack by natural enemies (Boullis et al. 2017; Fan et al. 2018; Schuett et al. 2015).

The defensive strategies developed by aphids against natural enemies can have short- or long-term responses (Fan et al. 2018). Short-term responses include activities that can be performed momentarily by herbivorous insects, and aim to alarm the colony and prevent parasitoid attacks, such as the release of alarm pheromone to warn other colony members and alter the natural enemies foraging behavior (Nault, 2013); body wiggling and kicking the parasitoid with the hind legs (Dixon, 1958); interruption of feeding activities and dispersion from the location threatened by the natural enemy (Dill et al., 1990); detachment of the plant to avoid the attack or reduce the probability of development of the immature inside the host (Dill et al. 1990; Muratori et al. 2014); grouping individuals to reduce the attack through the dilution effect (Chacón & Heimpel, 2010); and select plants with no natural enemies at the time of colonization (Hopkins & Dixon, 1997). On the other hand, long-term responses consist of defense strategies to prevent future attacks by natural enemies, including, for example, ingestion of toxic allelochemicals to natural enemies (Verkerk et al., 1998), increase the development of winged individuals to improve the colony dispersion capacity (Sloggett & Weisser, 2002), and in some species, increase the production of soldiers for colony defense (Stern & Foster, 1996).

Short- and long-term responses can still be classified into morphological, physiological, or behavioral mechanisms (Gross, 1993; Schuett et al. 2011; Weisser et al. 1999). Morphological adaptations range from homochromy camouflage (Gross, 1993) to behavioral adaptations that include physical actions, such as kicking, wiggling and confrontation or escape from the attack of the natural enemy (Dill et al., 1990; Gross, 1993; Vorburger, 2014). Physiological adaptations evolved to avoid the establishment of parasitoids are targeted to eliminate invaders (bacteria, eggs, larvae) through the activation of cellular and/or humoral immune response pathways (Gross, 1993; Kraaijeveld et al., 1998; Vilmos & Kurucz, 1998; Vorburger, 2014).

Host defense against natural enemies can also be enhanced by associated symbiotic bacteria, which can inhibit parasitoid development by competing for limited resources available in the host (*eg.* lipids) (Paredes et al. 2016), producing toxins (Ballinger et al. 2017), and/or by the pre-activation of the host immune system (Kwong et al. 2017). Despite studies already carried out on defense mechanisms

against attack by natural enemies, physiological and behavioral strategies, as well as interaction with secondary symbionts, are still poorly understood (Desneux et al. 2009).

The host behavioral defense mechanisms can be activated upon contact with the natural enemy, detection of vibrations in the plant resulting from the foraging activity of the parasitoid/predator, and/or by the detection of alarm pheromone released by a colony-mate (Firlej et al., 2010; Losey et al., 1997). In the same way aphids developed defense mechanisms against their natural enemies, parasitoids have also developed throughout the coevolutionary history among these taxa, strategies to circumvent the host defense tactics to successfully locate and exploit their hosts (Vinson & Iwantsch, 1980). Successful parasitization depends on the efficacy of a sequence of processes associated with host location, selection, and parasitization, and the regulation of the host physiology to allow parasitoid immature development inside the host (Abram et al., 2019). The execution of the steps involving successful parasitization can impact host fitness, altering its behavior, development and reproduction, but it often results in the death of the host (Abram et al., 2019; Rehman & Powell, 2010).

Thus, we investigated isolines of the aphid *Myzus persicae* with different successful rates of parasitization by *Diaeretiella rapae* in order to test the predictions that differences in the successful parasitization are regarded to 1) direct alterations in host defensive behavior or 2) host alterations that would interfere with the attractance and/or suitability of *M. persicae* to *D. rapae*.

3.2. Conclusions

- Infection of *Myzus persicae* by *Rickettsia* affects the aphid defensive behavior against *Diaeretiella rapae*;
- Infection of *M. persicae* by *Rickettsia* affects the patch time exploitation of *D. rapae*;
- Rickettsia*-infected aphids wiggle their bodies more intensively, but for a shorter period of time than *Rickettsia*-free aphids as a response to *D. rapae* attack;
- The more intense body wiggling of aphids can be the factor influencing the successful parasitization of aphids belonging to the group with low parasitism by *D. rapae*.

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