An overview of the mitigating mechanisms of the fitness cost of mutations associated with pesticide resistance in arthropods

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Resumo

Uma visão geral dos mecanismos atenuadores do custo de aptidão de mutações associadas à resistência a pesticidas em artrópodes. Alelos que causam resistência a pesticidas também podem trazer algum custo fisiológico para seus portadores, uma vez que afetam outras características como a reprodução e a sobrevivência. Entretanto, trabalhos têm descrito que em alguns artrópodes mecanismos atenuadores do custo fisiológico também têm sido selecionados. Esta revisão traz um levantamento dos mecanismos atenuadores do *fitness* descritos na literatura e que podem contribuir na manutenção da resistência em artrópodes. Consultas foram realizadas junto aos bancos de dados Pubmed e Scopus, durante o período de outubro de 2019 a janeiro de 2020 para busca de referências relacionadas ao tema, utilizando combinações de palavras-chave, o operador Booleano AND e usando critérios de inclusão e exclusão. Foram selecionados 61 artigos científicos, a partir do título e resumo. Foram encontrados diferentes mecanismos de atenuação que foram classificados em nove categorias de acordo com sua forma de ação descrita no artigo. Diptera foi a ordem mais abordada (24 artigos), representada principalmente por mosquitos da família Culicidae (17 artigos). A duplicação gênica, principalmente de genes da acetilcolinesterase, foi o mecanismo mais encontrado (14 artigos), e o que apresentou maior variedade de espécies em que ocorre (oito espécies de três Ordens, incluindo insetos e aracnídeos).

Palavras-chave: Atenuação; Genótipo de resistência; Inseticidas químicos

Abstract

Alleles that cause resistance to pesticides may also result in host physiological cost, as they can impair phenotypes such as reproduction and survival. However, works have described that in some arthropods mechanisms that mitigate the physiological cost have also been selected. This review covers a survey of the fitness attenuating mechanisms reported in the literature that can contribute to the maintenance of resistance in arthropods. Searches were made in the Pubmed and Scopus databases, from October 2019 to January 2020, to find references related to the topic, using combinations of key words, the Boolean operator AND, as well as inclusion and exclusion criteria. Sixty-one scientific articles were obtained, which were selected from the title

and abstract. Different attenuation mechanisms were found and were classified into nine categories according to the mechanism of action described in the article. The Diptera Order was the most addressed in the articles (24 articles), represented mainly by the Culicidae family mosquitoes (17 articles). Gene duplication, mainly of acetylcholinesterase genes, was the mechanism most found (14 articles), and the one with the greatest variety of species in which it occurs (eight species from three Orders, including insects and arachnids).

Key words: Chemical insecticides; Mitigation; Resistance genotype

Introduction

Pesticide resistance is defined as an inheritable change in the sensitivity of a population of pests to a particular compound, which is perceived by the repeated failure of a particular target-vector to reach the expected level of control when the product is used according to the recommendations of control policies (IRAC, 2014). Usually, the emergence of resistance is related to the continuous and indiscriminate use of chemical compounds, such as insecticides, leading to a selective pressure on a vector population, resulting in the survival of only a few phenotypically resistant individuals, which will have the opportunity to pass this phenotype on to the offspring. This will contribute to the increase in the frequency of the genetic variant that confers resistance in overall field population (HEMINGWAY; RANSON, 2000). There are currently over 600 species of arthropods that are resistant to different types of pesticides (ARTHROPOD PESTICIDE RESISTANCE DATABASE, 2020).

Alterations in insecticide sensitivity can be caused by different mechanisms, such as: insensitivity at the target site of the insecticide action (change in the target protein); increase in detoxification or excretion (metabolic mechanisms), reduction in insecticide ability to penetrate and behavioral resistance (HEMINGWAY, 2000). Some mutations in genes that encode proteins that are the target for insecticide action can render them insensitive to these compounds. Most insecticides are neurotoxic, targeting proteins such as acetylcholinesterase, sodium channels and the GABA receptor, so mutations in the genes encoding such proteins can compromise the effectiveness of insecticides. Metabolic resistance involves an increase either in the sequestration or metabolism of the insecticide through the enzyme action from different families, the main

ones being glutathione-S-transferases or GSTs (efficient in the action against pyrethroids, organophosphates and organochlorines), esterases or ESTs (effective in action against organophosphates and carbamates) and cytochrome P450 or CYPs (effective in action against most types of chemical insecticides). Metabolic resistance can be caused by the overexpression of one or more enzymes, changes in the catalytic properties of the enzyme, or both (HEMINGWAY, 2000; HEMINGWAY et al., 2004).

Changes in the composition of the exoskeleton that increase the thickness by adding proteins and lipids generate a reduction in cuticular penetration by the insecticide. The reduction in the insecticide's absorption rate, when compared to the metabolic mechanisms and changes in the target site of action of the insecticide, is considered of secondary importance, since it alone does not cause worrying levels of resistance. However, in combination with other mechanisms it can cause an increase in resistance, compromising control strategies (BRAGA; VALLE, 2007). Some insecticides such as permethrin (a type of pyrethroid) can have a repellent effect and cause behavioral changes, so that the insect becomes less exposed to this compound (MATHENGE et al., 2001).

The selection of a new characteristic can lead to the appearance of the pleiotropy phenomenon which, in a context of resistance, has been related to a gene mutation that can also influence the alteration of other characteristics in addition to providing resistance to insecticides. Studies have shown that some mutations that confer resistance can cause some cost in the fitness of their carriers since they can also alter other characteristics of the organism's life history. These changes are more easily observed in the absence of the selection pressure exerted by the insecticide (COUSTAU et al., 2000).

Mutations conferring high levels of insecticide resistance have been associated with deleterious changes in some characteristics, such as less successful mating, lower fertility and hatchability; reduction of individuals who survive to adulthood; higher susceptibility to natural enemies or higher mortality overwintering, among others (BOIVIN et al., 2001; BERTICAT et al., 2008; KLIOT; GHANIM, 2012). There are several physiological costs that have been associated with resistance, and the intensity of that cost is directly related to the molecular basis of resistance. However, over time, attenuating fitness mechanisms have also been selected in some species of arthropods (FFRENCH-CONSTANT; BASS, 2017), although for most of them the molecular ways of acting to mitigate fitness costs have not yet been elucidated.

The review presented here sought to provide an overview of what has been described in the literature regarding the mechanisms that mitigate the fitness costs and that can contribute to maintaining resistance in different species of arthropods, and in which species this issue has been most investigated.

Material and Methods

Data collect

The search for articles was carried out in the PubMed (NCBI) and Scopus (Elsevier) databases from October 2019 to January 2020. The searches were performed using descriptors associated with the theme of this work. Therefore, 22 combinations of key words (Table 1) and the Boolean operator "AND" were used.

For the term trade-off, different numbers of articles were found in initial searches using the term with or without a hyphen, so both forms were used to ensure broader searches. The searches in the databases were carried out by two researchers independently that read the titles and abstracts of the articles that appeared in the searches with the key words (Table 1), first, to check if they could contain information that would answer the guiding questions of this work; and, second, if they were in agreement with the inclusion criteria. After this selection, the two researchers responsible for these searches made a consensus list of articles for reading. While reading these articles different information was noted as follows: Authors and Year; Title of Article; Species analyzed (Scientific name; Order; Family); Resistance mechanism; Fitness cost observed; Mitigation mechanism described.

TABLE 1: Combination of key words used to search for articles in the PubMed and Scopus databases.

Key words
Fitness cost and insecticide resistance
Fitness cost and pesticide resistance
Fitness cost and acaricides resistance
Fitness cost and pyrethroids
Fitness cost and organophosphates
Fitness cost and carbamates
Fitness cost and mosquitoes and insecticide resistance
Fitness cost and flies and insecticide resistance
Fitness cost and moths and insecticide resistance
Fitness cost and ticks and acaricide resistance
Fitness cost and insecticide resistance and arthropods
Fitness cost and insecticide resistance and insects
Fitness cost and pesticide resistance and arthropods
Fitness cost and pesticide resistance and insects
Amelioration and insecticide resistance
Amelioration and pesticide resistance
Trade-off and insecticide resistance
Tradeoff and insecticide resistance
Trade-off and pesticide resistance
Tradeoff and pesticide resistance
Trade-off and fitness cost and insecticide resistance
Tradeoff and fitness cost and insecticide resistance

Inclusion and exclusion criteria

Inclusion criteria used: studies in Portuguese and English, published until December 2019, that cited or described mechanisms involved in mitigating the fitness cost caused by resistance to some kind of chemical insecticide in arthropods.

Exclusion criteria used: studies addressing resistance to biological insecticides (*Bacillus thuringiensis* and *Bacillus sphaericus* toxins) and studies addressing plant resistance to herbicides. Review articles were also not included in the analysis.

Results

After searching the two databases and drawing up a consensus list between the two researchers involved in this step, a total of 61 articles were selected for full reading. All selected articles were the results of the search using two or more key word combinations. Some articles (15) were considered "false positive" because, after reading, it was observed that they did not describe the mechanism for attenuating the fitness cost caused by insecticide resistance mutations (DURON et al., 2006; RIVERO et al., 2011; BRITO et al., 2013; 2019; RIBEIRO et al., 2014; WEETMAN et al., 2015; ZHANG et al., 2015; HSU et al., 2016; BAJDA et al., 2018; DAVID et al., 2018; SOMERS et al., 2018; VERNON et al., 2018; LIEBMAN et al., 2019; MA et al., 2019; YING et al., 2019). Some articles also did not describe which physical, physiological or behavioral characteristics were affected by the insecticide resistance mechanism or cited other articles where the strains or samples used were characterized. However, considering that the focus of this work is mitigation mechanisms, only those that did not describe such information were considered false positive.

Articles involving species of insects from different Orders and arachnid species (mites and ticks) were analyzed. The Order Diptera (Class Insecta) was the most frequent, with 24 articles found, represented mainly by mosquitoes of the Culicidae family (17 articles). For the Ixodida and Thrombidiformes Orders (Class Arachnida), 2 articles of each were found (Table 2).

The different attenuation mechanisms found were classified into nine categories according to what was discussed in the articles. Most of the attenuating mechanisms are repeated in multiple arthropod species. The two attenuation mechanisms with the largest number of articles found are gene duplication (14 articles), with the majority addressing duplications of acetylcholinesterase, and differential protein expression (10 articles), usually involving overexpression (Tables 3 and 4). Gene duplication was also the mechanism most commonly found in species of different arthropod orders, with eight species belonging to three Orders (including insects and arachnids species), followed by compensatory mutations in the same gene, found in seven species of three Orders (all Class Insecta). In two articles

TABLE 2: List and quantity of analyzed articles, organized according to the Order of the target organism of the study.

Order	Number of articles	References
Diptera	24	Davies et al. (1996); Chevillon et al. (1997); Guillemaud et al. (1998); Eritja and Chevillon (1999); Shi et al. (2004); Le Goff et al. (2005); Berticat et al. (2008); Labbé et al. (2005; 2007; 2014); Djogbénou et al. (2009); Jones et al. (2012); Martins et al. (2013); Remnant et al. (2013); Hou et al. (2014); Linss et al. (2014); Diniz et al. (2015); Assogba et al. (2015; 2016; 2018); Liebman et al. (2015); Hawkes et al. (2016); Kasai et al. (2017); Milesi et al. (2017)
Hemiptera	07	Hick et al. (1996); Silva et al. (2012); Germano and Picollo (2015); Gordon et al. (2015); Zhang et al. (2016); Wang et al. (2017); Yang et al. (2018)
Coleoptera	06	Arnaud and Haubruge (2002); Araújo et al. (2008a; 2008b); Lopes et al. (2010); Silva et al. (2010a; 2010b)
Lepidoptera	05	Zhao et al. (2000); Dai et al. (2016); Xia et al. (2018); Gutiérrez et al. (2019); Li et al. (2019)
Ixodida	02	Temeyer et al. (2012; 2013)
Trombidiformes	02	Kwon et al. (2010; 2012)
Total	**46**	

(ARNAUD; HAUBRUGE, 2002; KWON et al., 2012), the authors cite two mechanisms as possible fitness attenuators (Table 3). The two classes of insecticides most cited in the articles, and possibly related to the selection of different mechanisms to mitigate the fitness cost in resistant arthropods are organophosphates and pyrethroids (Table 4).

TABLE 3: List of mitigation mechanisms and articles where they were found, with the number of articles for each Order that describes the mechanism.

Mechanisms classification	Order and number of articles[#]	References
Gene duplication	Diptera: 10 Trombidiformes: 2 Ixodida: 2	Labbé et al. (2007; 2014); Djogbénou et al. (2009); Kwon et al. (2010; 2012[a]); Martins et al. (2013); Remnant et al. (2013); Temeyer et al. (2012; 2013); Liebman et al. (2015); Assogba et al. (2015; 2016; 2018); Milesi et al. (2017)
Differential protein expression	Diptera: 1 Coleoptera: 5 Hemiptera: 4	Hick et al. (1996); Araújo et al. (2008a; 2008b); Hou et al. (2014); Silva et al. (2012); Silva et al. (2010a; 2010b); Lopes et al. (2010); Wang et al. (2017); Yang et al. (2018)
Compensatory mutations in the same gene	Diptera: 5 Hemiptera: 1 Lepidoptera: 1	Shi et al. (2004); Le Goff et al. (2005); Jones et al. (2012); Linss et al. (2014); Dai et al. (2016); Zhang et al. (2016); Kasai et al. (2017)
Substitution in overexpression of esterase loci over time	Diptera: 4	Chevillon et al. (1997); Guillemaud et al. (1998); Eritja and Chevillon (1999); Labbé et al. (2005)
Change at some development stage	Diptera: 1 Hemiptera: 2 Lepidoptera: 1	Diniz et al. (2015); Germano and Picollo (2015); Gordon et al. (2015); Gutiérrez et al. (2019)
Fitness modifier gene	Diptera: 2 Coleoptera: 1	Davies et al. (1996); Arnaud and Haubruge (2002 [b]); Hawkes et al. (2016)
Substitutive mutation in the same gene	Lepidoptera: 1	Zhao et al. (2000)
Differences in the microbial community	Lepidoptera: 2	Xia et al. (2018); Li et al. (2019)
Mutation in another gene	Diptera: 1	Berticat et al. (2008)
Total	**46**	

a: Article also cites another mechanism: Compensatory mutations in the same gene; b: Article also cites another mechanism: Substitutive mutation in the same gene; #: This column describes the number of articles found for each Order that describe the mechanism.

TABLE 4: Mitigation mechanisms of the fitness cost, number of articles and class of insecticide tested or possibly responsible for the selection of the mechanism.

Mechanisms classification	Number of articles	Class of insecticide[a]
Gene duplication	14	Organophosphate (7); Pyrethroid (1); Organochlorine (1); Organophosphate and Carbamate (5);
Differential protein expression	10	Pyrethroid (6); Organophosphate (3); Pyrethroid, Organophosphate and Carbamate (1)
Compensatory mutations in the same gene	07	Pirazol (Fipronil) (2); Carbamate(1); Organophophate and carbamate (1); Pyrethroid (3)
Substitution in overexpression of esterase loci over time	04	Organophosphate (4)
Change at some development stage or life history traits (or both)	04	Organophosphate (1); Pyrethroid (2); Neonicotinoid+Pyrethroid (1)[b]
Fitness modifier gene	03	Organophosphate (2); ND (1)[c]
Substitutive mutation in the same gene	01	Pyrethroid
Differences in the microbial community	02	Semicarbazone (1); Organophosphate (1)
Mutation in another gene	01	Pyrethroid and Carbamate
Total	**46**	

a: the number inside the parentheses in this column indicates the number of articles that cites the class of insecticide; b: resistance to a product containing a mixture of compounds from both classes of insecticides; c: ND = Not determined (Hawkes et al., 2016): are used homozygous populations for an allele (*Cyp6g*1) that provides metabolic resistance to different classes of insecticides

Discussion

Overall the studies analyzed by our work targeted species with significant importance for livestock or agriculture affecting the local economy, and public health policies, due to transmission of arboviruses. The Culicidae family, Order Diptera, is the one that has been most investigated, represented mainly by the species *Aedes aegypti*, *Anopheles gambiae*, *Anopheles albimanus*, *Culex pipiens* and *Culex quinquefasciatus*, which are vectors of different arboviruses (CONSOLI; OLIVEIRA, 1994), which should justify the largest number of studies focusing on these organisms. Understanding the mechanisms that provide resistance in environments where different types of insecticides are present, will lead to better strategies to control the populations of these vector species, since for many of the diseases they transmit there are no vaccines available and the only way to preventing epidemic outbreaks is controlling the size of their populations (WEAVER; REISEN, 2010). Although they are not considered disease vector species or cause economic damage, *Drosophila melanogaster* and *Drosophila simulans* species (Diptera; Drosophilidae) have also been used as a model organism in insecticide resistance and mitigation mechanism studies. Three articles were found that used *Drosophila melanogaster* (SHI et al., 2004; REMNANT et al., 2013; HAWKES et al., 2016) and one with *Drosophila simulans* (LE GOFF et al., 2005) as an organism of study. Due to the multiplicity of consequences in biological characteristics, caused by resistance alleles, negatively affecting the ability of survival, reproduction (or both) of resistant individuals, different mitigation mechanisms were selected and will be discussed below. Some articles did not present an experiment indicating or providing the action of an attenuation mechanism; its existence was suggested in the discussion of the results.

Gene duplication

Gene duplication allows individuals to carry an extra copy containing mutations related to insecticide resistance, and another copy without changes, attenuating some of the deleterious effects of the presence of only the mutant allele. These duplications can generate permanent heterozygosity facilitating the maintenance and dissemination of resistance mutations (OTTO; YONG, 2002; MILESI et al., 2017). All gene duplications described here involve genes encoding proteins that are the target of insecticide action. Duplication in the sodium channel protein gene, which is the target of pyrethroid and DDT action, has been reported in Brazilian samples of *Aedes aegypti*, with variation in the number of copies, and in a strain selected in the laboratory for resistance to deltamethrin (MARTINS et al., 2013). In *Drosophila melanogaster* duplication was found in the gene encoding the GABA receptor, allowing individuals with mutations in one of the copies to have intermediate levels of resistance to the organochlorine dieldrin (REMNANT et al., 2013). Duplications of acetylcholinesterase genes, target of the action of organophosphates and carbamates, have been found in resistant samples of the mite *Tetranychus urticae* (KWON et al., 2010; 2012), in the ticks *Rhipicephalus* (*Boophilus*) *microplus* and *Ixodes scapularis* (TEMEYER et al., 2012; 2013) and in the mosquitoes *Culex pipiens* (LABBÉ et al., 2007; 2014; MILESI et al., 2017), *Anopheles albimanus* (LIEBMAN et al., 2015) and *Anopheles gambiae* (DJOGBÉNOU et al., 2009; ASSOGBA et al., 2015; 2016; 2018). In samples of natural populations of *Tetranychus urticae*, variation in the number of copies of the acetylcholinesterase gene was found (KWON et al., 2010), and the same was verified for *Anopheles gambiae* populations, including different combinations of wild and resistant copies (ASSOGBA et al., 2016; 2018).

Differential protein expression

The vast majority of articles address the differential expression of enzyme function proteins. The advantage of the differential expression of some proteins lies in the greater efficiency of digestion processes causing a greater increase in energy reserves. Higher energy reserves can provide the necessary energy for the production of detoxification proteins without necessarily reducing the energy that is used in other physiological processes. In addition, greater efficiency in protein digestion can also provide additional energy resources as well as increase the availability of amino acids for protein synthesis (SILVA et al., 2010a). Larvae of Diptera *Bactrocera dorsalis*, exposed to treatment with the pyrethroid β-cypermethrin, showed greater serine protease activity, which was considered a possible mechanism to mitigate the stress caused by exposure to the insecticide and could, therefore, mitigate the fitness costs caused for resistance (HOU et al., 2014). In pyrethroid resistant strains of coleoptera *Sitophilus zeamais*, greater activity of some enzymes was found, such as α-amylase (LOPES et al., 2010), trehalases, lipases (ARAÚJO et al., 2008a), cysteine proteinase (ARAÚJO et al., 2008b; SILVA et al., 2010b), cellulase (ARAÚJO et al., 2008b) and trypsin-like proteinases (SILVA et al., 2010a), either by increasing gene expression or by expressing isoforms with greater activity. In *Nilaparvata lugens* (Hemiptera) it was found that the resistant strain R9 had fewer effects on relative fitness than the susceptible S9 at a lower temperature (R9 had a higher copulation rate at 18°C). The authors argue that this difference found at a lower temperature between strains may be related to differences in the expression of heat shock proteins, although they have not carried out experiments to prove differential expression (YANG et al., 2018). A *Laodelphax striatella* strain (Hemiptera) resistant to chlorpyrifos showed better fitness at a higher temperature (30°C) than at a lower temperature (24ºC), when compared to the fitness of the susceptible strain. It was found that the resistant strain had two HSPs 70 genes that were more expressed in relation to susceptible strain, which enhanced the thermotolerance of the former, contributing to the improvement of relative fitness (WANG et al., 2017). In highly resistant clones of Hemiptera *Myzus persicae* (R3), inactivation of the expression of E4 esterase genes (which act in the metabolization of some insecticides) occurs through differential methylation, avoiding the overexpression of these genes in periods of absence of pressure by the insecticides (HICK et al., 1996). In another study with the same species, susceptible and resistant strains carrying one or two resistance mechanisms were analyzed, all created in a host plant

considered favorable (pepper) and another that was not favorable (radish). A favorable and non-favorable host refers to the skill of the aphid to overcome allelochemical defense systems presented by plants. When reared in the favorable host, resistant genotypes presented less reproductive fitness compared to susceptible genotype strains. However, when created in the non-favorable host, resistant genotypes showed the best reproductive fitness in relation to susceptible ones. Resistant genotypes did not show significant differences in reproductive fitness in different hosts, but susceptible genotypes did. The analysis of the expression of six genes, including some enzymes that act in the detoxification of xenobiotics, in all strains showed that the resistant genotypes, in general, exhibited less transcriptional plasticity using diferente host plants. The authors argue that the results suggest a greater constitutive expression of the genes analyzed in these strains, including possible paralogous, and even of other related genes. Additionally, aphids living under pressure from insecticides or on plants with chemical defenses may present similar adaptive solutions for two selective agents (SILVA et al., 2012).

Compensatory mutations in the same gene

This mechanism involves the accumulation of mutations that act synergistically to confer resistance, but also minimize fitness costs for the carrier. A mutation that causes resistance can have a cost by decreasing the catalytic efficiency of the enzyme. Over time, different mutations can accumulate in the same locus so that catalytic efficiency can be restored, or the compensation can come from an increase in the affinity of binding to the substrate. One or more compensatory mutations have been described in the GABA receptor gene of *Drosophila simulans* (LE GOFF et al., 2005) and *Nilaparvata lugens* (ZHANG et al., 2016), in the acetylcholinesterase gene from *Chilo suppressalis* (DAI et al., 2016), *Drosophila melanogaster* (SHI et al., 2004) and *Tetranychus urticae* (KWON et al., 2012), and in the gene of the sodium channel (*Kdr* mutations) from *Musca domestica* (KASAI et al., 2017), *Aedes aegypti* (LINSS et al., 2014) and *Anopheles gambiae* (JONES et al., 2012).

Substitution in overexpression of esterase loci over time

The articles describe overproduction of protein esterases in *Culex pipiens* populations, which confer resistance to organophosphates and carbamates. Changes in the regulatory mechanism and co-amplification of duplicated genes are related to the overproduction of these esterases (GUILLEMAUD et al., 1998). In natural populations of *Culex pipiens* in Europe, changes in the frequency of the alleles from two loci, related to overproduction of esterases, have been observed over time, alternating between variants that have a higher cost in fitness and others having a lower cost. This alternation of alleles was related to the heterogeneity of environments and intensity of insecticide treatments (CHEVILLON et al., 1997; GUILLEMAUD et al., 1998; ERITJA; CHEVILLON, 1999; LABBÉ et al., 2005).

Change at some development stage or life history traits (or both)

In a population of *Tecia solanivora* (Lepidoptera), which presented multiple resistance to insecticides of different classes, a shorter time of larval development was verified, which could be an adaptation to reduce the time of contact with tubers and soil (or both) contaminated with insecticides, which are usually the substrates treated to wrestle it. In addition, a delay in the peak of oviposition was also found in this population, which has been suggested as a possible adaptation to compensate for the nutrients needed to complete the egg's development (GUTIÉRREZ et al., 2019). In *Aedes aegypti*, resistant to temephos, an extension in the time of larval development was found, which was interpreted as a compensation mechanism for the loss of energy reserves - caused by resistance -, reducing deficits related to female survival and reproduction (DINIZ et al., 2015). In the vector of Chagas disease, *Triatoma infestans*, longer duration and variability in the last nymph stage was found in the resistant samples, which could reflect an advantage since nymphs molting later can remain in refuges, avoiding exposure to insecticides (GERMANO; PICOLLO, 2015). In populations of the bedbug *Cimex lectularius*, with a history of exposure to insecticides,

the generation time was significantly reduced, in relation to populations without this history. However, why this change was considered a benefit was not discussed exactly (GORDON et al., 2015).

Fitness modifier gene

In two articles, one with *Drosophila melanogaster* (HAWKES et al., 2016) and another with the beetle *Tribolium castaneum* (ARNAUD; HAUBRUGE, 2002), the authors do not mention which fitness modifier gene it would be, only suggesting the existence of one that would improve the fitness of resistant individuals. However, in the article with *Lucilia cuprina* (DAVIES et al., 1996) the authors cite the *Scalloped wings* (*Scl*) gene, homologous to the *Drosophila melanogaster Notch* gene, as the fitness attenuator of diazinon-resistant strains. This resistance was associated with changes in the locus *Rop1*, which codes for carboxylesterase E3. The existence of an interaction between the products of *Rop-1* and *Scl* has been proposed, probably involving signaling pathways related to cell adhesion, so that developmental stability and the asymmetries observed in the resistant ones are restored.

Substitutive mutation in the same gene

The occurrence of a new allele that also provides resistance to insecticides can bring less cost to its carrier, possibly causing fewer changes in the properties of the protein, and over time replacing the variant that had the highest cost. This mechanism was verified in the sodium channel (*Kdr* mutations) of *Heliothis virescens* resistant to pyrethroids (ZHAO et al., 2000) and cited as a possible fitness costs attenuator of malation-resistant *Tribolium castaneum* (ARNAUD; HAUBRUGE, 2002).

Differences in the microbial community

The MR strain of *Plutella xylostella*, resistant to metaflumizone, showed significantly greater bacterial richness and diversity in the intestine, compared to the susceptible SS strain, and the authors suggest that a greater diversity of microorganisms may bring benefits to the host's metabolism, although they do not detail how this would happen (LI et al., 2019). In the article

by Xia et al. (2018), also with *Plutella xylostella*, the authors argue that symbionts of the intestine are known to contribute to the development of the insect immune system; thus, these symbionts would contribute to prevent or restore possible damage to the immune system of the host caused by insecticides.

Mutation in another gene

Four strains of *Culex quinquefasciatus* were analyzed, one susceptible and three resistant: one resistant strain presented a mutation only in acetylcholinesterase, another only in the sodium channel gene, and another with a mutation in both genes. The BCSR strain, which had mutations in both acetylcholinesterase and sodium channel genes (insensitive to carbamates and pyrethroids), showed better fitness than the strain that only had mutations in acetylcholinesterase (insensitive to carbamates). Sodium channel and acethylcolinesterase are proteins that act in the transmission of the nervous impulse. The authors argue that the relatively higher costs caused by the malfunction of acetylcholinesterase, due to the presence of a resistance mutation, causing a reduced degradation of acetylcholine in the postsynaptic terminal region, can be partially offset by a slower release of acetylcholine due to the presence of the mutation in the sodium channel (BERTICAT et al., 2008).

Negative pleiotropic effects caused by resistance may not be easy to identify. However, testable hypotheses may arise when the molecular basis of the changes is discovered (COUSTAU et al., 2000). The phenotype of an organism is shaped by several selective pressures present in the environment in which it lives and, therefore, large phenotypic changes are expected to be harmful when the environment returns to what it was before the presence of the insecticide. Producing an enzyme that acts in the detoxification of the insecticide, for example, expends a lot of energy: a reallocation of energy used in other metabolic and development processes occurs, so an individual that produces it in excess in the absence of the insecticide would have a higher disadvantage in terms of energy (KLIOT; GHANIM, 2012; FFRENCH-CONSTANT; BASS, 2017). There are many characteristics of an organism's life history that can be analyzed to verify the existence

of fitness costs in a population, and these analyses were carried out using both samples from natural populations and laboratory strains. However, there are certain aspects of fitness that can only be analyzed outside of laboratory conditions; for example, studies of survival overwintering, as well as the influence of the presence of entomopathogens, natural enemies, and the availability of different host plants with their chemical defense mechanisms (KLIOT; GHANIM, 2012).

Changes caused by resistance can also influence rates of infection, development and transmission of pathogens. A resistance mechanism can have opposite effects on each of these components, that is, it can increase the life span of the vector and interfere with the development of the parasite (RIVERO et al., 2010). In the absence of a fitness cost, resistance will remain, and the only alternative will be the introduction of a new chemical class to which no pre-existing mechanism can confer cross resistance (FFRENCH-CONSTANT; BASS, 2017).

Acknowledgments

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