ANA PAULA NASCIMENTO DA SILVA

SUBLETHAL EFFECTS OF SYNTHETIC INSECTICIDES ON LIFE HISTORY OF *Myzus persicae* (SULZER) (HEMIPTERA, APHIDIDAE) UNDER VARYING TEMPERATURES

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Orientador

Prof. Dr. Khalid Haddi

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EFEITOS SUBLETAIS DE INSETICIDAS SINTÉTICOS SOBRE A HISTÓRIA DE VIDA DE *Myzus persicae* (SULZER) (HEMIPTERA, APHIDIDAE) SOB TEMPERATURAS VARIADAS

Tese apresentada à Universidade Federal de Lavras, como parte das exigências do Programa de Pós-Graduação em Entomologia, área de concentração em biologia e ecologia de insetos, para a obtenção do título de Doutor.

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RESUMO

Myzus persicae (Sulzer) (1776) (Hemiptera: Aphididae) tem uma ampla distribuição geográfica e é considerado uma praga de importância econômica em várias culturas agrícolas. Este trabalho foi realizado para investigar como a exposição letal e subletal do *M. persicae* aos inseticidas sintéticos (ou seja, neonicotinóides, organofosforados e piretróides), juntamente com a variação de temperatura, afetam os traços biológicos e reprodutivos, e obtendo uma melhor compreensão do papel do estresse oxidativo nas respostas aos afídeos. Para este fim, primeiro melhoramos nosso protocolo de cultivo de pulgões testando o uso potencial de um hidrogel como substrato de criação. Também determinamos as curvas concentração-resposta de cinco inseticidas (clorpirifos, deltametrina, tiametoxam, lambda-cialotrina, tiametoxam + lambda-cialotrina e imidacloprido) para o afídeo verde sob quatro temperaturas diferentes (15, 20, 25, e 28°C). Em seguida, testamos o efeito de baixas concentrações (CL_1, CL_5, CL_{10}, CL_{15}, CL_{20} e CL_{30}) de cada inseticida sobre a longevidade e a fecundidade do *M. persicae*. Em seguida, avaliamos as respostas de estresse oxidativo dos afídeos expostos a diferentes baixas concentrações de imidacloprido, medindo seu conteúdo de malondialdeído (MDA) e H_2O_2 em diferentes pontos de tempo (12 e 48 horas). Os resultados mostraram que as colônias de *M. persicae* podem ser mantidas ao longo do tempo através da produção de descendentes suficientes usando hidrogel. Além disso, as toxidades dos produtos químicos testados e seu efeito estimulante sobre a fecundidade e longevidade variaram de acordo com as temperaturas. Além disso, tal variação na resposta hormética poderia estar ligada, ainda que apenas parcialmente, às respostas ao estresse oxidativo induzido pela exposição a baixas concentrações de inseticidas. Portanto, os resultados obtidos aqui forneceram informações importantes sobre os mecanismos de adaptação desses organismos em ambientes desafiadores, o que será de suma importância para o desenho de estratégias de manejo de *M. persicae* em agroecossistemas.

Palavras-chave: afídeos, ágar, condições de laboratório, fecundidade, hidrogel, hormese, longevidade

GENERAL ABSTRACT

Myzus persicae (Sulzer) (1776) (Hemiptera: Aphididae) has a wide geographic distribution and is considered a pest of economic importance in several agricultural crops. This work was carried out to investigate how lethal and sublethal exposure of *M. persicae* to synthetic insecticides (i.e., neonicotinoids, organophosphate, and pyrethroids) together with temperature variation, affect the biological and reproductive traits , and gaining a better understanding of the oxidative stress role in the aphids responses. To this end, we first improved our aphid-rearing protocol by testing the potential use of a hydrogel a rearing substrate. We also determined the concentration-response curves of five insecticides (chlorpyrifos, deltamethrin, thiamethoxam, lambda-cyhalothrin, thiamethoxam + lambda-cyhalothrin, and imidacloprid) for the green aphid under four different temperatures (15, 20, 25, and 28°C). Then, we tested the effect of low concentrations (LC_1, LC_5, LC_{10}, LC_{15}, LC_{20}, and LC_{30}) of each insecticide on the longevity and fecundity of *M. persicae*. Subsequently, we evaluated the oxidative stress responses of exposed aphids to different low concentrations of imidacloprid by measuring their malondialdehyde (MDA) and H_2O_2 contents at different time points (12 and 48 hours). The results showed that colonies of *M. persicae* can be maintained over time by producing sufficient offspring using hydrogel. Furthermore, the toxicities of the tested chemicals and their stimulatory effect on fecundity and longevity varied according to temperatures. In addition, such variation in hormetic response could be linked, eventhough only partially, to the oxidative stress responses induced by the exposure to low concentrations of insecticides. Therefore, the results obtained here have provided important information on the adaptation mechanisms of these organisms in challenging environments, which will be of paramount importance for the design of management strategies for *M. persicae* in agroecosystems.

Keywords: agar, aphids, fecundity, hormesis, hydrogel, laboratory conditions, longevity

SUMÁRIO

FIRST PART

GENERAL INTRODUCTION

Myzus persicae (Sulzer) (1776) (Hemiptera: Aphididae) known as the green peach aphid, is a polyphagous agricultural pest, feeding on more than 400 plant species, and is associated with crops of great economic importance worldwide (BLACKMAN; EASTOP, 2000; VAN EMDEN, HELMUT F; HARRINGTON, 2017). Through direct feeding, honeydew production and transmission of more than 100 plant viruses, this species generates expressive losses in the yield of crops of great economic importance depending on their level of infestation (BASS *et al.*, 2014; DE LITTLE *et al.*, 2016).

The control of *M. persicae* is frequently based on the use of synthetic insecticides, and among the chemical groups most commonly used are organophosphates, carbamates, pyrethroids, and neonicotinoids. The intensive and widespread use of these insecticides has resulted in the occurence of resistance to many of them, posing a major threat to the efficient and sustainable control of aphids (BASS *et al.*, 2014; MOTA-SANCHEZ; WISE, 2021).

After being sprayed in agricultural areas, pesticides can be degraded over time due to the action of several factors (i.e., drift and degradation) leading to sublethal exposures in insects (BIONDI *et al.*, 2012; DESNEUX; DECOURTYE; DELPUECH, 2007; DUKE, 2014). This exposure can impact the physiological and/or behavioral characteristics of individual insects (DESNEUX; DECOURTYE; DELPUECH, 2007), affecting positively their population dynamics (SIAL *et al.*, 2018; YU *et al.*, 2010). This phenomenon, defined as hormesis, is a biphasic effect resulting from stimulation at low dose and inhibition at high dose after pesticide exposure (CALABRESE; BALDWIN, 2003; GUEDES, RAUL NARCISO C; RIX; CUTLER, 2022).

Aphids in agroecosystems showing the ability to survive exposure to low concentrations/doses of a toxic compound under and manifest sublethal effects has been reported in several studies. The species reported include *M. persicae* (RIX; AYYANATH; CUTLER, 2016; SIAL *et al.*, 2018; TANG *et al.*, 2019; WANG, PAN *et al.*, 2017), *Aphis gossypii* Glover (CHEN *et al.*, 2016; WANG, SIYI *et al.*, 2017), *Aphis craccivora* Koch (FOUAD; EL-SHERIF; MOKBEL, 2022), and *Aphis glycines* Matsumura (QU, YANYAN *et al.*, 2015, 2017). Insecticide-induced hormesis may be disadvantageous in applicability in pest management, as the adaptive mechanism and stress coping abilities of these insects may contribute to the resurgence of pest insects.

Environmental temperature has an important role in both the population dynamics, development rates, and seasonal occurrence of aphids (ALFORD; BLACKBURN; BALE, 2012; CAMPBELL *et al.*, 1974; COCU *et al.*, 2005; SOH *et al.*, 2018), and the performance, properties, and distribution of pesticides used in pest insect management programs (HORN, 2019; JOHNSON, 1990). The influence of temperature on toxicity can be positive or negative depending on the mode of action of the insecticide and the insect species in question, in addition to the route of exposure, thus, the toxicity of products can increase/decrease with varying temperature (DENG *et al.*, 2016; LIU, JIA *et al.*, 2016; SWELAM *et al.*, 2022; WANG, XIAO-YI; SHEN, 2007).

The continuous maintenance of insect populations in laboratory with quality (in small scale for research or in large scale for mass rearing) becomes fundamental (PARRA, JOSÉ ROBERTO POSTALI; COELHO JR, 2022), and due to its economic importance, individuals of *M. persicae* are valuable models for scientific studies and control methods (GAVKARE; GUPTA, 2013; MITTLER; DADD, 1962). Thus, according to the need, methodologies that enable the maintenance and availability of *M. persicae* individuals in the laboratory are developed, increased or adapted.

Given all the above, to date, most studies focus on the efficacy of insecticides in controlling pest aphids without taking into consideration thermal regimes and how these regimes shape their toxicity. The literature also lacks information on how sublethal effects act on the biological characteristics of individuals exposed to insecticides under temperature variations. Thus, it is increasingly important to understand the underlying processes and mechanisms involved in sublethal exposure of pesticides and their hormetic effects on *M. persicae* individuals to guide the development of effective integrated pest management strategies.

This thesis presents four chapters in manuscript form. Chapter 1 is dealing with the methodology of rearing *M. persicae* in the laboratory, relative to the first objective of this study. In chapters 2, 3, and 4, investigations on the sublethal exposure of the species in question to different insecticides, single and in mixtures, under varying regimes of temperatures as well as the resulting potential oxidative responses are presented. A general literature review of the subject is presented in the "Theoretical Framework", followed by a "General Introduction". Thus, each chapter was started with a specific introduction to the investigation developed and its respective summary.

1. THEORETICAL FRAMEWORK

1.1. Insecticides and their application on pest aphids

Aphids are considered one of the main agricultural pests worldwide, and the control methods of these organisms basically focus on the use of agrochemicals within Integrated Pest Management (IPM) due to their effectiveness (ALTIERI; NICHOLLS, 2018; CARVALHO, 2017; GAVLOSKI, 2018; SANDHI; REDDY, 2020), and among the chemical groups most commonly used to control these individuals are: organophosphates, carbamates, pyrethroids, and neonicotinoids (MOTA-SANCHEZ; WISE, 2021).

Organophosphates are one of the most successful chemical pesticides, used for over 70 years in agricultural fields and effective against pests due to their broad-spectrum efficacy. They are esters of phosphoric and thiophosphoric acids and their toxicity depends mainly on their ability to inhibit acetylcholine esterase (AChE) activity (MORADI *et al.*, 2019; PERRY *et al.*, 2020). Chlorpyrifos is a synthetic insecticide from the organophosphorus chemical group that is non-systemic, broad-spectrum, efficient, and has been widely used to control various pests, including aphids (AHMAD; ASLAM, 2005; LI, GUOYONG *et al.*, 2022; SIMON, 2011). This insecticide is an acetylcholinesterase (AChE) inhibitor, induces oxidative stress, and can damage DNA (RASHEED *et al.*, 2020).

Pyrethroids are also commonly used to control many populations of pest aphids. They bind to voltage-dependent sodium channel protein, which alters the function of the pore channel, causing repetitive neurological impulses, thus potentially impairing any nerve activity and resulting in paralysis and death of the insect (HAUG; NAUMANN, 1990; NARAHASHI, 2002; SIMON, 2011; VAIS *et al.*, 2000). Deltamethrin is a synthetic pyrethroid that acts as a rapid neurotoxic agent (GIBSON; RICE; SAWICKI, 1982; MALBERT-COLAS *et al.*, 2020). They are classified as sodium channel modulators, where the molecule induces toxic responses in the central and peripheral nervous system of insects (HAUG; NAUMANN, 1990; SIMON, 2011), stimulating nerve cells to produce repetitive discharges and consequently causing paralysis in the insect (NARAHASHI, 2002; SODERLUND, 2012). Lambda-cyhalothrin is also a pyrethroid classified as a potent neurotoxic agent (DONG, BAO *et al.*, 2022), it acts by interfering with the ionic conductance of nerve membranes by prolonging the Na^+ current leading to insect paralysis and death (CLARK, J MARSHALL, 1997). It has an effective, rapid and

persistent potency and has been highly recommended in the management of pest aphids in agricultural areas (MENGER *et al.*, 2022).

Neonicotinoids are efficient agonists of the nicotinic acetylcholine receptor (nAChR), providing excitatory neurotransmission in the insect central nervous system (MATSUDA *et al.*, 2001). Their success is related to high efficiency compared to organophosphates, carbamates and pyrethroids, low mammalian toxicity, high insect toxicity, unique mode of action and versatile applications (BASS *et al.*, 2015; JESCHKE *et al.*, 2011).

Inside this chemical group, imidacloprid has become the main product used to control sucking insect pests, especially pest aphids (BASS *et al.*, 2015; CUI *et al.*, 2016). Imidacloprid is a systemic insecticide and agonist that activates the nicotinic acetylcholine receptor (nAChR) on postsynaptic membranes, its neurotoxins can cause a variety of behavioral/physiological effects (BUCKINGHAM *et al.*, 1997; LIU, MING-YIE; CASIDA, 1993; MATSUDA *et al.*, 2001). Imidacloprid has been considered a safe insecticide because of its high toxicity to insects and low toxicity to mammals (BUCKINGHAM *et al.*, 1997). Thiamethoxam is also a neonicotinoid insecticide acting as an agonist that binds to nicotinic acetylcholine receptors (nAChRs) in the insect nervous system, causing nerve stimulation, paralysis and death, and widely used in aphid control (CHO *et al.*, 2011; SIMON, 2011; ULLAH *et al.*, 2020).

However, overuse of these chemical groups in agroecosystems led to many cases of increased tolerance and resistance development in aphid populations (FONTAINE; CADDOUX; BARRÈS, 2023; LI, YONG *et al.*, 2016; WANG, ZI-JIAN *et al.*, 2021).

1.2. Sublethal effects and hormesis

Insecticides are intended to control pest insects, and to cover different target sites in the physiology of organisms, they present a large number of chemical classes with various modes of action (GUPTA *et al.*, 2019; SIMON, 2011). An optimal amount is required to effectively reduce crop damage attacked by these pests in agroecosystems (ABD EL-MAGEED; SHALABY, 2011; NETO *et al.*, 2019; RASHEED *et al.*, 2020), however, various abiotic and biotic processes can alter the concentrations of applied insecticides (BANTZ *et al.*, 2018; MÜLLER, 2018; TUDI *et al.*, 2021). Pesticides can have their variable distribution and continuous degradation due to misapplication, drift, and formulation degradation over time in plants, animals, soil, and water (CUTLER *et al.*, 2022; GUEDES, N M P *et al.*, 2010; MÜLLER, 2018; RIX; CUTLER, 2022). As a consequence, these products can cause sublethal exposures in target and non-target

organisms (DONG, JUNFENG *et al.*, 2017; SERRÃO *et al.*, 2022), that is, such exposure does not induce apparent mortality in the population, but potentially causes physiological or behavioral effects in individuals that survive exposure to the insecticide (DENG *et al.*, 2016; DESNEUX; DECOURTYE; DELPUECH, 2007; FOUAD; EL-SHERIF; MOKBEL, 2022; HE *et al.*, 2013; ULLAH *et al.*, 2019).

This phenomenon defined as hormesis is a biphasic adaptive response characterized by stimulation at low doses and inhibitory effects at high doses of pesticides (AGATHOKLEOUS; CALABRESE, 2022; CALABRESE; BALDWIN, 2003; CUTLER *et al.*, 2022). Hormesis has been observed in a multitude of organisms, and can cause various biological changes, including numerous metabolic and molecular processes, cognitive function, and immune response (CALABRESE; BALDWIN, 2003; CUTLER, 2013; DUKE, 2014; RIX; CUTLER, 2022), which can be short-term (improved performance and increased mating success) and long-term (increased longevity and performance in subsequent generations) (BERRY III; LÓPEZ-MARTÍNEZ, 2020). Moreover, hormetic effects are not only limited to chemical stressors, such as pesticides, they can manifest after temperature stress, radiation and food restriction (BERRY III; LÓPEZ-MARTÍNEZ, 2020; CALABRESE; BLAIN, 2011; CUTLER, 2013; FEINENDEGEN, 2005; MIRONIDIS; SAVOPOULOU-SOULTANI, 2010).

The study of insecticide-induced hormesis in pest insects has become of utmost importance due to its potential implications in pest management. The uptake of a small amount of agrochemicals can contribute to a beneficial stimulatory effect on fecundity, fertility, longevity, intrinsic rate of increase, finite rate of increase, and net reproductive rate of pests (CALABRESE; BALDWIN, 2003; SHANG *et al.*, 2021; SIAL *et al.*, 2018; ULLAH *et al.*, 2019). Currently, in the literature there is a range of studies on hormetic responses that cause stimulation under low doses of insecticides, and has been reported in various pest species, such as in aphids (KOO *et al.*, 2015; LU; ZHENG; GAO, 2016; ZENG *et al.*, 2016), caterpillars (DONG, JUNFENG *et al.*, 2017; NOZAD-BONAB *et al.*, 2017), thrips (CAO *et al.*, 2019; KORDESTANI *et al.*, 2021; LIANG, HUA-YU *et al.*, 2021), whitefly (ESMAEILY *et al.*, 2014; QU, CHENG *et al.*, 2017; RAKOTONDRAVELO *et al.*, 2019), and among other arthropods (CUTLER, 2013; GUEDES, RAUL NARCISO C; RIX; CUTLER, 2022; RIX; CUTLER, 2022).

In this context, pesticide-induced hormesis becomes a disadvantageous response in agricultural fields. For being widely observed that after the application of chemicals, there

is the possibility of an increase in the growth of pest insect populations at a higher rate than would otherwise be the case. Thus, hormetic responses can quickly lead to resistance selection, pest resurgence, and/or secondary pest outbreaks (CUTLER, 2013; CUTLER *et al.*, 2022; GUEDES, N M P *et al.*, 2010).

However, although there are many records of stimulatory effects induced by pesticides in the literature, they are usually not termed as hormetics effects, but rather as efficacy failures. Thus, there is a need for studies that deal with the mechanism of different types of hormesis as well as the cost of this adaptive response if insecticides are to continue being applied in the future in an environmentally acceptable way.

1.3. Temperature and its effects on the insect life cycle

As ectothermic organisms, insects are highly susceptible to abiotic changes in the environment (COLINET *et al.*, 2015; GILBERT; RAWORTH, 1996). Temperature has an important role in regulating physiological functions in these organisms such as respiration, immunity, metabolism, growth, and reproduction (GONZÁLEZ-TOKMAN *et al.*, 2020; NEVEN, 2000). In temperate and polar regions, insects may have developed different strategies to survive recurrent environmental conditions that are inappropriate for their development (GILBERT; RAWORTH, 1996; TOUGERON *et al.*, 2020). However, when physiological injuries occur under heat stress, insects may impact their biological fitness, such as, behavior, locomotion, dispersal, longevity, and survival (HOOPER, DAVID U *et al.*, 2012; JOHNSTON *et al.*, 2019; RODRIGUES; BELDADE, 2020).

Insects are vulnerable to high temperatures due to their small size and ectothermic physiology (GILBERT; RAWORTH, 1996; GULLAN; CRANSTON, 2014). In a climate scenario where conditions are extremely high, heat exposure can rapidly raise body temperature to lethal levels for these organisms, and can drive profound consequences in their life history characteristics (HARVEY *et al.*, 2020; REBAUDO; RABHI, 2018). High temperatures can kill insect cells by denaturing proteins, altering membrane and enzyme structures and properties, can cause water loss (dehydration) due to their small size (CHAMPMAN, 1998; GULLAN; CRANSTON, 2014), and metabolic rates can increase and population doubling times can decrease as temperatures increase (GONZÁLEZ-TOKMAN *et al.*, 2020; NEVEN, 2000).

Mironidis and Savopoulou-Soultani (2010) investigated the effects of temperatures on the survival and reproductive parameters of *Helicoverpa armigera* (Hübner) (Lepidoptera:

Noctuidae) adults, and revealed that increasing the duration of exposure to high temperatures resulted in a significant decrease in the survival rate of individuals.

Not only high temperatures are responsible for this variation in the biological fitness of insects, but low temperatures also play an important role in the physiological and biological properties of species (CLARK, MELODY S; WORLAND, 2008; LEE, 2012). Insects can be susceptible to chilling, and due to injury from this condition, these organisms can show abnormalities and may even reach death (MARSHALL; GOTTHARD; WILLIAMS, 2020; TEETS; DENLINGER, 2013).

Besides the temperature influencing the population dynamics of organisms, it can also affect the toxicity and relative efficacy of insecticides that are used in agricultural fields (JOHNSON, 1990; MAHMOODI *et al.*, 2020). In this sense, abiotic factors become important, since, temperature influences the physiological processes of organisms involved in the detoxification and excretion of chemical compounds (DONG, BAO *et al.*, 2022; HOOPER, MICHAEL J *et al.*, 2013; ILTIS *et al.*, 2022).

Understanding the mechanisms by which these organisms respond to this variation under thermal stressors is of utmost importance, as there is a great need to plan and formulate adaptation and mitigation strategies in Integrated Pest Management tactics, especially due to the increasing effects of global warming and climate change on natural systems.

1.4. Aphids as pests and above all as study models for entomology and ecotoxicology

The aphids are sucking insects phytophagous, having a size ranging from 2 to 3 mm, and are considered one of the most important pests in numerous crops, both in open field conditions and in protected crops due to thier high reproductive capacity (BLACKMAN; EASTOP, 2000; KENNEDY; STROYAN, 1959; VAN EMDEN, HELMUT F; HARRINGTON, 2017). Their reproduction occurs with several generations per year by thelytocous parthenogenesis; i.e., females giving rise to females; they are very adapted to the exploitation of new and temporary habitats and are also responsible for several direct and indirect damages (BUENO, 2005; NEBREDA; MICHELENA; FERERES, 2005). The direct damage caused by these organisms is due to the suction of sap, which leads to the shortening of the internodes of the plants and to the wilting and yellowing of the leaves, which do not develop normally and end up harming the growth of the host (DEDRYVER; LE RALEC; FABRE, 2010; RABBINGE *et al.*, 1981; VAN EMDEN, HELMUT F; HARRINGTON, 2017). Indirectly, one of the main problems is the spread

of viruses on host plants in the field through a variety of aphid species (GAAFAR; ZIEBELL, 2020; QI *et al.*, 2021; STEVENS; LACOMME, 2017).

Aphids can attack a multitude of crops, causing losses and increasing production expenses (KENNEDY; STROYAN, 1959; VAN EMDEN, HELMUT F; HARRINGTON, 2017). Despite advances in the development of management techniques and sustainable production practices, current aphids' control methods still focus largely on the use of agrochemicals within Integrated Pest Management (IPM) due to their effectiveness (ALTIERI; NICHOLLS, 2018; CARVALHO, 2017; GAVLOSKI, 2018; SANDHI; REDDY, 2020).

Furthermore, besides being one of the major agricultural pests worldwide, and from an applied design, aphids are exceptional models for studying a number of fundamental ecological and evolutionary topics, including reproductive mode variation, insect-plant interactions, virus transmission, phenotypic plasticity, symbiosis, and insecticide resistance (VAN EMDEN, HELMUT F; HARRINGTON, 2017). Studies on this important group of insects are extremely important to provide potential tools for efficient pest management measures.

Among the aphid species, the species *Myzus persicae* Sulzer (1776) (Hemiptera: Aphididae) are associated with crops of great economic importance that are intensely controlled by chemical pesticides, and has developed resistance to almost all insecticides used (VAN EMDEN, H F *et al.*, 1969; WANG, XIAO-YI; SHEN, 2007). Considering the set of direct and indirect damages, these aphids present potential to generate expressive losses in the yield of several crops, depending on the level of infestation (BLACKMAN; EASTOP, 2000; HEIE, 1986; PAVELA, 2018; PIMENTA; SMITH, 1976; SALVADORI, 2000; SINGH; SINGH, 2015; VERESHCHAGINA; GANDRABUR, 2016). The wide distribution of *M. persicae* worldwide is due to its very high adaptability to various environmental conditions, its wide genetic variability and broad phenotypic plasticity.

In view of the above, it is essential that studies aim to evaluate the efficiency of methods of population control of the aphid *M. persicae* and consequent reduction of damage caused in the yield of various crops. However, these studies are still a great challenge, because when conducting studies of biology and behavior, it is essential to structure the conditions provided to the insects, to have individuals in sufficient quantity and quality for the purpose of research (COHEN, 2001; OLIVEIRA *et al.*, 2010; PARRA, J. R. P. *et al.*, 2002). In this perspective, the refinement of these breeding techniques with the

addition of new technologies can enable the expansion and evolution of Integrated Pest Management programs (PARRA, J. R. P., 2012).

1.5.Sublethal/hormesis effects studied on aphids

As a result of the economic importance of pest aphids in agroecosystems, one of the key questions for scientists studying hormesis in the context of ecotoxicology is the consequence of the hormetic response on the biological fitness of these organisms in agricultural fields. Several recent studies are revealing that exposure to low doses of a contaminant can induce a hormetic response in pest aphids (AYYANATH *et al.*, 2013; FOUAD; EL-SHERIF; MOKBEL, 2022; QU, YANYAN *et al.*, 2015; ULLAH *et al.*, 2019). Pesticide-induced hormesis may be a new fundamental pillar in the field of ecotoxicology, as it may promote the evolution of adaptive coping mechanisms in these organisms in challenging environments.

The chemical groups most commonly used for aphid control are organophosphates, carbamates, pyrethroids, and neonicotinoids (MOTA-SANCHEZ; WISE, 2021). However, as stated earlier, these insecticides can cause sublethal exposures to aphids by several factors, including pesticide misapplication, drift, and degradation over time (BIONDI *et al.*, 2012; DESNEUX; DECOURTYE; DELPUECH, 2007; DUKE, 2014). Consequently, these exposures can influence the physiological and/or behavioral characteristics of individuals, such as changes in mortality rate, longevity and fecundity, immune capacity, and/or the sex ratio of specimens (DESNEUX; DECOURTYE; DELPUECH, 2007; LU; ZHENG; GAO, 2016; QU, YANYAN *et al.*, 2015; SIAL *et al.*, 2018; WANG, PAN *et al.*, 2017; YU *et al.*, 2010).

Moreover, these effects may be transgenerational, indirectly affect their offspring, and may induce changes in communities and ecosystem services (CHEN *et al.*, 2016; FOUAD; EL-SHERIF; MOKBEL, 2022; GUO *et al.*, 2013; JU *et al.*, 2022; TANG *et al.*, 2019; WANG, SIYI *et al.*, 2017). The induction of transgenerational hormetic responses may be a mechanism by which the parental generation can adapt the phenotype of their offspring for the adverse conditions they face (ULLAH *et al.*, 2020; YUAN *et al.*, 2017). The occurrence of pesticide-induced hormesis can arise at any time throughout the individual's life, and can be highly dependent on the type of insecticide and the species, as they can develop different adaptive patterns to exposure to low doses (CHO *et al.*, 2011). The consequences of hormetic responses on the biological fitness of these insects, such as increased survival, fecundity, and reproduction after exposure to sublethal

concentrations of insecticides has been reported in several aphid species, such as in *M persicae* (CHRISTOPHER CUTLER *et al.*, 2009; JANMAAT *et al.*, 2011; SIAL *et al.*, 2018; TANG *et al.*, 2019; WANG, PAN *et al.*, 2017; YU *et al.*, 2010; ZENG *et al.*, 2016), *Aphis gossypii* Glover (AMINI JAM *et al.*, 2014; CHEN *et al.*, 2016; CUI *et al.*, 2018; LIANG, PING-ZHUO *et al.*, 2019; MA *et al.*, 2019; SHI *et al.*, 2011; ULLAH *et al.*, 2019), *Sitobion avenae* (F.) (LU; ZHENG; GAO, 2016; MIAO *et al.*, 2014; XIAO *et al.*, 2015; XIN *et al.*, 2019) and *Rhopalosiphum padi* (Linnaeus) (LI, WENQIANG *et al.*, 2018; LU; ZHENG; GAO, 2016; XIN *et al.*, 2019; ZUO *et al.*, 2016). Thus, reproduction-related traits represent one of the most important sublethal parameters that are studied in pesticide toxicology in pest insects due to their crucial outcomes at the population level (CUTLER *et al.*, 2022; GUEDES, RAUL NARCISO C; RIX; CUTLER, 2022; RIX; CUTLER, 2022).

As a result of damage, hormesis becomes a disadvantageous response in agricultural areas, since aphids surviving hormetic effects can accelerate population growth, develop resistance to various insecticides, ensuring resurgence and/or secondary outbreaks of this pest (CUTLER, 2013; CUTLER *et al.*, 2022; GUEDES, N M P *et al.*, 2010). Thus, comprehensive knowledge of the effects of sublethal concentrations on pest aphids is essential to improve sustainable pest management strategies.

1.6. The impact of temperature on sublethal effects on aphids

Global climate change has significant impacts on agroecosystems, and agricultural crops and their corresponding pests are directly and indirectly affected (AMJAD BASHIR *et al.*, 2022; SHRESTHA, 2019; SKENDŽIĆ *et al.*, 2021), and understanding the potential risks and economic losses of agribusiness in many different regions is becoming a determining factor in the strategic planning of agricultural activities in the context of global warming (DUBOVITSKI *et al.*, 2021).

Abiotic disturbances, particularly upper and lower thermal effects, check insect pest multiplication and abundance, generation time, emergence, flight, and dispersal rate (NAEEM-ULLAH *et al.*, 2020; SKENDŽIĆ *et al.*, 2021). In addition, this climate disruption can create new ecological niches that provide opportunities for insect pests to establish and distribute into new geographic regions (GUTIERREZ; PONTI, 2014).

As mentioned earlier, extreme temperature values have an important impact on all levels of a biological organization, from the whole organism to the molecular level (CAMMELL; KNIGHT, 1992; GILBERT; RAWORTH, 1996; TANYI; NGOSONG;

NTONIFOR, 2018), and temperature is also important in the activity and performance of insecticides used in pest insect management programs (HORN, 2019; LI, HAIPING *et al.*, 2006). For pest aphids, high temperatures can have negative impacts on populations by slowing development and reducing fecundity, i.e., high temperatures can be detrimental to embryo development and therefore population growth may be slowed in subsequent generations (ALFORD; BLACKBURN; BALE, 2012; CAMPBELL *et al.*, 1974; COCU *et al.*, 2005; DAVIS; RADCLIFFE; RAGSDALE, 2006; SOH *et al.*, 2018). Interactions between low temperatures and insect physiological responses can also rapidly alter their biological fitness (BAYLEY *et al.*, 2018; MARSHALL; GOTTHARD; WILLIAMS, 2020; OVERGAARD; GERBER; ANDERSEN, 2021; SINCLAIR *et al.*, 2003). Aphids have the ability to rapidly acclimate to low temperatures, and this significantly interferes with the development time, longevity, mortality, and reproduction of individuals (DURAK; DURAK, 2021; MICHAUD; BAIN; ABDEL-WAHAB, 2018). This cold tolerance of aphids can progressively increase over subsequent generations and can be lost as quickly as it is acquired (POWELL; BALE, 2008). Saeidi et al. (2017) showed that a 1- to 3-hour exposure to 0.0°C was sufficient to increase the survival of the Russian wheat aphid, *Diuraphis noxia* (Kurdjumov), by four times.

Heating or cooling can cause accelerated degradation of chemical compounds (HOOPER, MICHAEL J *et al.*, 2013), and as we already know, insecticides in the field can be degraded by these abiotic factors over time, potentially causing sublethal exposures to aphids (BIONDI *et al.*, 2012; DESNEUX; DECOURTYE; DELPUECH, 2007). From an applied perspective, climate change may have the potential to alter the benefits/costs balance of pesticide use in the agricultural context, and in this regard, in the literature there is little detailed knowledge about the thermal modulation of pesticide side effects on pests, especially on aphids. In this sense, it is of utmost importance that further studies are conducted to have a better understanding of the uptake and elimination of insecticides with thermal fluctuation and how these effects affect the biological processes of pest aphids.

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SECOND PART: ARTICLES

ARTICLE I

Hydrogel as an alternative to agar for laboratory rearing of the green peach aphid *Myzus percicae*

Version prepared according to *CABI Agriculture and Bioscience*

Abstract

Background

Proper and cost-effective rearing methodologies are critical for successful insects' production. In this context, standard laboratory rearing of aphids uses plant leaf discs floating on agar layer. Due to high cost of agar, we tested a hydrogel, a synthetic polymer, as an affordable alternative for laboratory rearing of the green peach aphid.

Methods

Initially, we compared the effects of three concentrations of hydrogel (0.3g, 0.6g and 0.9g) and 10% agar on the ability of aphids to complete their life cycle. Then, using age-stage, two-sex life tables, we assessed the suitability of the hydrogel (0.6 gr) as substrate for two different host plants (e.i; *Brassica oleraceae* and *Nicandra physalodes*) under two different temperatures in aphids' production. Subsequently, we tested the response of the produced aphids in toxicological bioassays.

Results

Our findings showed that, similarly to the agar, the hydrogel concentration of 0.6 grs allowed the production of aphids in high numbers without affecting their life cycle parameters or their reproductive outputs. Furthermore, the most significant differences between the evaluated treatments resulted mostly from the combined effects of the host plants and the temperatures. Therefore, colonies of *M. persicae* can be maintained over time producing sufficient offspring using *N. physalodes* leaves on layer of hydrogel (0.6 g) at 20°C. Moreover, in toxicological bioassays the use of higher hydrogel concentrations (0.9 grs) is recommended.

Conclusions

The hydrogel can be adopted as a viable alternative to replace the widely used agar-based methodology for the green peach aphid rearing.

Keywords: hydrogel, aphids rearing, agar, temperature, *Nicandra physalodes*

Background

The rearing of insects for mass production or scientific inquiry is a great challenge. The production and multiplication of affordable and high-quality insects to be employed in the many important purposes of basic and applied research demands to structure the provided insect-rearing conditions (Cohen, 2003, 2001; Oliveira et al., 2010; Parra et al., 2002). One of the key applications of insect rearing is the pest management and obtaining experimental subjects in sufficient quantity and quality that will respond accurately in various bioassays is crucial (Huynh et al., 2021). In this perspective, the refinement of insect-rearing techniques can improve the accuracy of designed and advocated Integrated Pest Management strategies (Parra, 2012).

Aphids, such as the green peach aphid *Myzus persicae* Sulzer (1776) (Hemiptera: Aphididae), are widely distributed pests associated with many crops of economic importance. Due to the direct and indirect damages they cause, aphids have the potential to generate expressive losses in several crops (Blackman and Eastop, 2000; Heie, 1986; Pavela, 2018; Pimenta and Smith, 1976; Salvadori, 2000). Aphids are also frequently used as model organisms to study various fundamental questions related to insect biology, physiology, ecology, and evolution within the basic and applied life sciences. Studies aiming to evaluate the efficiency of pest control methods and leading to consequent reduction of damages caused in the field are some of the examples of aphids' use in applied research (Toledo et al., 2020). Consequently, the rearing and multiplication of these insects are of great importance, and methodologies that enable the production and

maintenance of their colonies in the laboratory have been developed, improved, or adapted according to the research needs.

Several methods for rearing aphids in the laboratory have already been developed, including the use of plants grown in pots, and/or artificial diets (Van Emden and Wild, 2020; Gavkare and Gupta, 2013; Gorham, 1942; Mittler and Dadd, 1962). In 1960, for the purposes of their work on wing polymorphism in aphids, (Johnson and Birks 1960), developed a rearing technique for *Aphis craccivora* Koch using leaf discs of *Vicia faba* L. floating on a modified Hougland-Snyder culture solution. Later on, (Milner 1981) used leaf discs attached to the surface of 1% agar gel and obtained positive results for the maintenance of aphid colonies. Consequently, this method became the aphid reference rearing method and has been since widely used to maintain aphid colonies under laboratory conditions (Conti et al., 2010; Leite et al., 2008; Li and Akimoto, 2018; Michelotto et al., 2005; Simões Santos Rando et al., 2011; Valente et al., 2014).

Agar, used as substrate in Milner's methodology, stands out for its high carbohydrate concentration and nutrient-rich chemical structure, being a substance of gelatinous consistency, which is obtained from red seaweed and formed by a combination of agarose and agaropectin (Armisen and Gaiatas, 2009). The importance and efficiency of agar as a substrate supporting the leaves in aphids' laboratory rearing with different foci is well established in the literature (Michelotto et al., 2005; Simões Santos Rando et al., 2011; Tang et al., 2019; Wang et al., 2018). Although this protocol is feasible, it is important to recognize that cost-effectiveness is of utmost importance for large-scale rearing of aphids aiming different purposes. In that respect, other potential media with gelling properties include hydrophilic gels called hydrogels. They are synthetic polymers and are traditionally prepared using chemical polymerization methods, absorbing large amounts of water without dissolving (it expands to about 200-800 times the original volume (Neethu et al., 2018; Shibayama and Tanaka, 1993). This property has led to many

practical applications of this material, particularly in agriculture, improving water supply to plants. However, there is a huge knowledge gap regarding the real potential, applicability and cost-effectiveness of hydrogels as insects' rearing substrates.

Here, we hypothesized that hydrogel can be used as alternative substrate for the agar in *M. persicae* rearing under laboratory conditions. Firstly, we determined the most adequate concentrations of hydrogel that allowed normal aphid life cycle. Then, we tested its suitability under two different temperatures and using leaf-discs of two different host plants. Finally, we assessed the response of the produced aphid to exposure to a neonicotinoid insecticide.

Results

Establishing the hydrogel concentration

The results of one-way analysis of variance (ANOVA I) showed no statistically significant differences between the three different hydrogel concentrations (0.3g, 0.6g, and 0.9g) and the control (agar at 10%) for female fecundity when reared on *N. physalodes* leaf discs at 15 ± 2°C (p=0.07) (Figure 1A) and at 20°C (p =0.24) (Figure 1B). Similarly, the female's survival was not different between the treatments (Agar, 0.3g, 0.6g and 0.9g) when reared on *N. physalodes* plants at 15 ± 2°C (χ^2= 1.643; df = 3; p = 0.65) (Figure 1C), and at 20 ± 2°C (χ^2= 7.872; df = 3; p = 0.05) (Figure 1D).

Based on these results, the subsequent bioassays were carried out using only two hydrogel concentrations (0.6g, and 0.9g) and the control (agar at 10%). The hydrogel concentration of 0,3g/100ml was desecrated due to the higher viscosity of the substrate layer causing aphids' drowning.

Life table study for two sexes by age and developmental stage of *M. persicae*

The change of stage structure during the life history of *M. persicae* can be observed in the curves of the age-stage survival rate (*Sxj*) (Figure. 2). The survival rate *Sxj* gives the probability that a newborn nymph will survive to age x while in stage *j*. The *lx* is the

probability that a newly hatched nymph survives to age x; in this regard, the *lx* curve is a simplified version of *Sxj* (Chi and Su, 2006).

In the data set evaluated, the survival rate of *M. persicae* for the treatments (BA15, BH15, NA15, NH15, BA20, BH20, NA20, and NH20) was between 75.5% and 100% for the first instar, between 57% and 100% for the second instar, between 51% and 97.9% for the third instar, and between 51% and 91.8% for the fourth instar. The overlaps between different stages during the developmental period demonstrate the varying developmental rates among individuals (Figure 2).

The mean durations of *M. persicae* stages among the different treatments (BA15, BH15, NA15, NH15, BA20, BH20, NA20 and NH20) showed significant differences (paired bootstrap test, $p < 0.05$) as shown in Table 1. In general, shorter nymphal development times, longevity and consequently total duration of the life cycle were found when the aphids were reared under 20 °C compared to 15 °C. When the treatments were compared within the temperature of 20 °C, the total cycle and stages' durations were always longer for the aphids reared on *N. physalodes* plants and using hydrogel as rearing substrate. When the temperature of 15 °C is considered, similar life cycles were found for the aphids reared on the *N. physalodes* plants and using either substrate (Agar or hydrogel) while shorter life cycles were found for the females kept on *B. oleraceae* and on hydrogel compared to agar.

Regarding the reproductive parameters (Table 2), the rearing temperature had little effects as the females' fecundity was similar when they were reared on the same combination of plant and substrate under the two temperatures tested (20 vs. 15 ºC) except when reared on the *B. oleraceae* leaf discs on agar. In addition, females' fecundity was constantly higher when reared on the *N. physalodes* plants independently of the temperatures and the rearing substrate (agar or hydrogel). Similar trends were generally observed for the

other reproductive parameters assessed (effective fecundity, the mean number of days of viviparity, APOP, TPOP…).

At the population level (Table 3), rearing the aphids' females using hydrogel resulted in a higher increase (intrinsic and finite) and reproductive (gross and net) rates independently of the temperature and the rearing host. The generation time was affected mainly by the rearing temperature rather than the other factors (rearing substrates and plants).

Response to neonicotinoid insecticide exposure

The response of *M. persicae* adults to imidacloprid exposure was investigated by foliar discs immersion methods under two different temperatures (20 and 15 °C) and using treated *B. oleraceae* foliar discs deposited on layers of hydrogel (0.6 or 0.9 gr) or 10% agar (Figure 3). The LC_{50} concentrations for imidacloprid in *B. oleraceae* Agar 15°C and *B. oleraceae* Hydrogel 15°C 0.6g and 0.9g (Figure 3A) were 0.212, 0.079 and 0.144 a.i. mg/ml respectively, while their LC_{50} concentrations in *B. oleraceae* Agar 20°C, and *B. oleraceae* Hydrogel 20°C 0.6g and 0.9g (Figure 3B) were 0.088, 0.072 and 0.111 a.i. mg/ml respectively.

Based on the obtained LC_{50}s and the calculated toxicity ratios (TR= LC_{50}s of hydrogel/ LC_{50}s of agar) (Figure 3), the resulting dose-response curves were similar indicating no differences between the responses of aphids within the same temperature regime (Hydrogel-0.9 gr 15°C :TR = 0.7 [0.5 – 1.00]; Hydrogel-0.9 gr 20°C: TR = 1.3 [0.9 – 1.7]; Hydrogel-0.6 gr 20°C: TR = 0.8 [0.4 – 1.6]). The only exception was the Hydrogel-0.6 gr at 15°C (TR = 0.4 [0.2 – 0.8]) that presented an LC_{50} significantly lower than the LC_{50} of the agar under the same temperature (15°C).

Discussion

The importance of insect rearing and multiplication is increasing for basic research and for the more applied field of pest management (Anderson, 2021). Given the need for

laboratory rearing of *M. persicae* for a variety of applications, a standard, cost-effective, reliable, and easy-to-use rearing methodology is necessary to provide the optimal requirements for the insects and thus to produce individuals that manifest normal biological and reproductive characteristics of the species and when used in scientific studies produces reliable responses. Here, we established and tested a low-cost, efficient aphid-rearing methodology using hydrogel. The established methodology allowed the normal production of aphids in high numbers and did not affect either the life cycle parameters or the reproductive outputs of *M. persicae*, being, therefore, a viable alternative to replacing the widely used agar-based methodology.

Agar is a well-known solidifying agent widely used in studies of biological aspects involving aphids', and other insects', rearing (Conti et al., 2010; Michelotto et al., 2005; Valente et al., 2014). Agar has been employed mainly to guarantee, during rearing or experiments period, the turgidity of leaves used as the host for tested insects. For example, agar at 1% was used as a supporting substrate for leaves of *Vicia faba* L. aimed to evaluate the biological aspects of *Acyrthosiphon pisum* Harris ((Li and Akimoto 2018). The use of agar allowed the maintenance of suitable conditions of the leaves for approximately two weeks. However, its cost is high, making its large-scale use unfeasible and highlighting the need to develop more affordable alternatives that reduce this cost without affecting the quality of produced insects.

In agriculture, hydrogels can be used as soil conditioners, where their main function is the retention and availability of water for agricultural crops (Sayed et al., 1991). However, as far as we know, there are no previous reports on the use of hydrogels as substrates for insects rearing. Our initial findings showed that the tested hydrogel dosages were good water retainers, causing the leave discs to stay turgid during the experiment period. The aphids were able to reproduce and develop in the three concentrations of hydrogel tested in a similar way to the aphids reared using the agar. Nevertheless, our observations

indicated that the hydrogel concentration of 0.3 g has a more liquid consistency, which may cause a greater mortality of aphids due to drowning, and the concentration of 0.9 g has a stiffer consistency, which may need a more frequent replacement of the hydrogel layer during the experiment and or rearing times. Thus, the hydrogel concentration of 0.6g was considered to provide better conditions for the leaves and aphids, and thus more viable for use in the subsequent bioassays.

In the present study, we constructed Age-stage, Two-sex life tables to evaluate the fitness of *M. persicae* under the different rearing substrates, temperatures, and host plants. Such life tables have been frequently used to study different aspects of the life history of many insects including aphids (Jahan et al., 2014; Khurshid et al., 2022; Maroofpour et al., 2021; Özgökçe et al., 2018; Zeng et al., 2016). The reproduction results showed that adult females could reproduce in all treatments with high intrinsic (r) and finite growth rate (λ) for *M. persicae* reared on both host plants but using hydrogel under the temperature of 20 °C. Furthermore, the life table analysis indicated that the most significant differences in the development times observed between the evaluated treatments resulted mostly from the combined effects of the host plants and the temperatures while the rearing substrate did not show discrepant impacts on the development of the reared individuals. It is worth noting that fecundity is an important parameter for aphid populations and is usually influenced by a variety of factors, including temperature and host plant quality (Davis et al., 2006; Van Emden et al., 1969; Liu and Meng, 1999; Shu-Sheng, 1991). In addition, aphid development can also be affected due to feeding on different host plants (Ali et al., 2021; Jahan et al., 2014; La Rossa et al., 2013).In general, the mean development time of aphids decreases with increasing temperature depending on the temperature range required for the survival of each species (Baral et al., 2022; Liu et al., 2021). The absence of any negative effect of hydrogel on the reproductive and biological parameters reinforces its suitability as substrate for rearing the aphid *M. persicae* that can be used

under different temperature conditions and with different host-plants. Accordingly, *N. physalodes* combined with hydrogel (0.6g) at a temperature of 20°C proved to be the highest performing and most favorable treatment for rearing *M. persicae*, possessing the ability to produce offspring under controlled conditions and with great potential for mass rearing.

In the toxicology bioassay, the responses of the aphids to exposure to imidacloprid at 20°C were not different between the treatments supported by the similar LC50s obtained and showing no interferences of the hydrogel as rearing substrate in such responses. However, at 15°C we observed a higher mortality when the hydrogel concentration was 0.6 g compared with the hydrogel concentration of 0.9g and agar. Such high mortality was associated with high mortality in the untreated control due to aphids' drowning. Therefore, we recommend the use of slightly higher hydrogel concentrations (0.9 or higher) for bioassays where mortality is the principal assessed endpoint and when experiments' durations are short (up to 72 hours).

Besides being a suitable rearing substrate not interfering with the insect's biological and reproductive output as demonstrated by our findings and taking in consideration its lower cost ($12 per kg) when compared with the conventionally used agar ($114 per kg) as well as the small quantities needed, the hydrogel can be recommended for use in different laboratory experiments targeting the aphids *M. persicae.*

Conclusions

Here, we presented and validated an innovative hydrogel-based methodology of aphid rearing under laboratory conditions, aiming an optimal cost/benefit and that proved to be effective. We highlighted that the hydrogel concentration of 0.6g presented satisfactory conditions to maintain the turgidity of *N. physalodes* leaves, allowing colonies of *M. persicae* be maintained over time producing sufficient offspring with suitable quality for toxicological bioassays. Further investigations are yet to be done to check the possibilities

of scaling-up the methodology for mass-production of aphids, to ascertain the suitability of the produced aphids for wide range of research experiments and to test the suitability of the methods and the potential of its extension and/or adaptation for other insect species.

Materials and methods

Host Plants

As host plants, we tried two species from the Brassicaceae and Solanaceae Families. Thus, entire leaves or foliar disks of the cabbage *Brassica oleraceae* var. *acephala* and the shoo-fly plant *Nicandra physalodes* (L.) Gaert were used for the green aphid rearing and for the subsequent experiments.

Seedlings of *B. oleraceae* (40-day old) and *N. physalodes* (14-day old) were purchased from a local farm supply store in Lavras Brazil, transplanted in 10-liter pots and cultivated under greenhouse conditions. The cultivation substrate (8 kg per pot) consisted of a mixture (2:1) of soil and commercial substrate Carolina Soil®. Plant watering (daily) and weed management (fortnightly) were done manually and no pesticides application was used.

Insect

The *M. persicae* females' are from an established laboratory colony since 2016 at the Entomology Department of Federal University of Lavras (UFLA), and were kept in the Laboratory of Molecular Biology and Ecotoxicology (M.E.E.T) at UFLA. The colony is reared in a climate-controlled chamber, with temperature maintained at 20 ± 2°C, relative humidity at 70 ± 10%, and photophase of 16 hours. The age of aphid females' cohorts was standardized, before experiments, by placing about 100 newly hatched nymphs (less than two days) on leaf discs (12 cm diameter) of *N. physalodes* plants and held for about 8 days to ensure that all aphids are the same age (and growth stage) at the beginning of each bioassay.

Rearing substrates

The hydrogel (Agrogel Gel Hidroretentor – Planting Gel) and the agar (Agar-agar (nacional) 500g - Dinâmica CAS 9002-18-0) were used as rearing substrates by placing entire leaves or leave-disks on approximately 5 mm of either agar or hydrogel layers in Petri dishes. The Agar solution (10% w/v) was prepared by diluting and homogenizing 10 grams of the agar powder in 100mL of distilled water. Then, the solution was heated in a microwave to the boiling point and left to cool (~50 ºC) before being poured (100; 4 and 2 mL) into petri dishes (12; 5.6 and 3cm) as 5 mm high layer and left to solidify before being used. The hydrogel solution was prepared following the same procedure but without the boiling steps. A preliminary test was carried out to determine the most suitable dose of hydrogel to be used (see the following section).

Establishing the hydrogel concentrations

Considering the reported high moisture retention of hydrogel, an optimal mixing ratio is required to obtain maximum effectiveness of the method. In this sense, three hydrogel doses (0.3g, 0.6g and 0.9g) were initially compared to agar (10%) as substrates for aphid rearing under two temperatures (15 and 20 ºC). After weighted, each hydrogel dose was placed inside a glass Petri dish (12 cm diameter) to which a 100mL of distilled water were added. After homogenization, the solution was left to hydrate for half an hour to reach its maximum water absorption capacity and to form the gel-like layer. The agar gel was prepared as described above. A leaf disc (12 cm diameter) of *N. physalodes* was placed with the abaxial surface upwards on the top of the substrate layer. Twenty female adults (less than 48 h old) were randomly collected from the green aphid colony and transferred to each leaf disc (considered a repetition). Each plate was covered with white towel paper, and secured with a rubber band, in order to prevent the aphids from escaping but to allow aeration inside the plate. Five repetitions were made for each treatment (dose x substrate x temperature), thus totalizing 100 adults for each treatment. The plates were placed in

two climate-controlled chambers, with temperatures maintained at 20 ± 2°C or 15± 2°C, under the same conditions of relative humidity (70 ± 10%), and photophase (16 hrs). Twice a week, the surviving females were moved to a new leaf disk in a new petri dish to avoid microorganisms contamination.

Female longevity was evaluated daily until death, and female fecundity was assessed by daily counting and removing the newly hatched nymphs.

Based on the results of this section, the dose of 0.6g was chosen to carry out the bioassays of the life table, and the doses 0.6g and 0.9g to the response of aphids to exposure to synthetic insecticide. In both bioassays, the agar 10% was sued as a control.

Construction of age-stage, two-sex life tables of *M. persicae* under the different rearing substrates, temperatures, and host plants

Following the same methodology described previously, Petri dishes (3 cm diameter) were prepared using 0.6g of hydrogel and 10% agar and containing one leaf disc of *N. physalodes* or of *B. oleraceae*. Newly hatched females (less than 24h) were collected from a same-age colony and in the Petri dishes (3 cm diameter) containing leaf discs (1 nymph/disc). Each plate (repetition) was sealed with plastic film and several small holes were made to allow for gas exchange. Fifty plates were used for each treatment (host-plant x substrate x temperature). The treatments were named as follows: *B. oleraceae* Agar 15ºC (BA15), *B. oleraceae* Hydrogel 15ºC (BH15), *N. physalodes* Agar 15ºC (NA15), *N. physalodes* Hydrogel 15ºC (NH15), *B. oleraceae* Agar 20ºC (BA20), *B. oleraceae* Hydrogel 20ºC (BH20), *N. physalodes* Agar 20ºC (NA20), and *N. physalodes* Hydrogel 20ºC (NH20). The experiments were conducted under the same conditions of photoperiod and relative humidity as previously described.

To follow the development until adults, each aphid was inspected daily. At each change of stadium, the exuvia was removed and discarded. For the adult females obtained, longevity/mortality and the number of nymphs laid per day during the whole life were

recorded in appropriate tables. The collected data were used to construct an age-stage, two-sex life table for each treatment.

Response to neonicotinoid insecticide exposure

To detect the potential effects of the rearing method on the response to insecticide exposure, a toxicity bioassay with the neonicotinoid insecticide imidacloprid (Evidence 700 WG) was performed. The bioassay determined the dose-response curve, under two temperatures (15 and 20 ºC) using the leaf-dip method proposed by the Insecticide Resistance Action Committee (IRAC, 2009). Briefly, leaf discs (5.6 cm in diameter) of cabbage (*B. oleraceae*) were cut and individually immersed for about 6 seconds in the insecticide and control solutions, then placed at room temperature to dry, for about 2 hours. Subsequently, the leaf discs were placed with the abaxial surface downwards in Petri dishes (5.6 cm diameter) on a layer of hydrogel (0.6 or 0.9 gr) and 10% agar and sealed with plastic film. The 0.3g dose was not tested due to its higher viscosity, causing higher mortality of aphids. The following concentrations of insecticide were tested: 0.0028; 0.0084; 0.014; 0.028; 0.084; 0.14; 0.28; 0.42; 0.98; 1.4 and 2.8 a.i. mg/ml. Five replicates of 20 adult aphids (up to 48 hours) were made for each concentration, totalizing 100 adults for each treatment. The insecticide was diluted with distilled water containing 0.01% (v/v) Tween 20, and for the control, only distilled water containing 0.01% (v/v) Tween 20 was used.

After 48 hours of the exposure, aphid mortality was assessed under a magnifying glass (Zeiss Stemi 2000C – Stereo Microscope 1.5x). Aphids that did not respond when poded with a fine brush were considered dead.

Data analysis

Fecundity data were subjected to a one-way analysis of variance (ANOVA), and survival results were subjected to survival analysis using Kaplan-Meier estimators (log-rank method) with SigmaPlot 12.0 (Systat Software, San Jose, CA, USA). The overall similarity between survival times and median survival times (LT_{50} values) was tested using the χ^2 log-rank test, and pairwise comparisons between curves were performed using the Holm-Sidak test ($P < 0.05$).

Life tables were constructed using the TWOSEX-MSCHART Program (Chi, 2004) and were analyzed according to the two-sex life table theory of the age stage (Chi et al., 2020). Briefly, the TWOSEX-MSChart computer program was used to investigate the parameters linked to stage differentiation, longevity, and fecundity; such as the intrinsic rate of increase (*r*), finite rate of increase (λ), net reproductive rate (R_0), mean generation time (*T*), gross reproduction rate (*GRR*), adult pre-viviparity period (*APOP*), total pre-viviparity period (*TPOP*), viviparity days (*Od*), age-stage specific survival rates (*sxj*), age-specific survival rate (*lx*), age-specific fecundity (*mx*), age-specific maternity (*lxmx*), age-stage specific life expectancy (exj), age-stage reproductive value (*vxj*) following (Chi and Liu 1985) and (Chi 1988). The standard errors of the population parameters were estimated via bootstrap technique with 100.000 resampling and the differences between the population parameters of treatments were compared using the paired bootstrap test based on the confidence intervals of differences implemented in TWOSEX-MSChart (Chi et al., 2020; Huang et al., 2018; Huang and Chi, 2013). All figures were constructed using SigmaPlot 12.0 (Systat Software Inc., San Jose, CA, USA).

Lethal concentrations of the insecticide imidacloprid for aphids in the concentration-mortality bioassays were estimated by probit analysis using PROC PROBIT (SAS 9.4; SAS Institute, Cary, NC.) and 95% confidence intervals for resistance ratios were estimated following (Robertson et al., 2017) and considered significant when not

including the value 1. Mortality data were corrected for natural mortality using Abbott's Formula (Abbott, 1925) prior to analysis.

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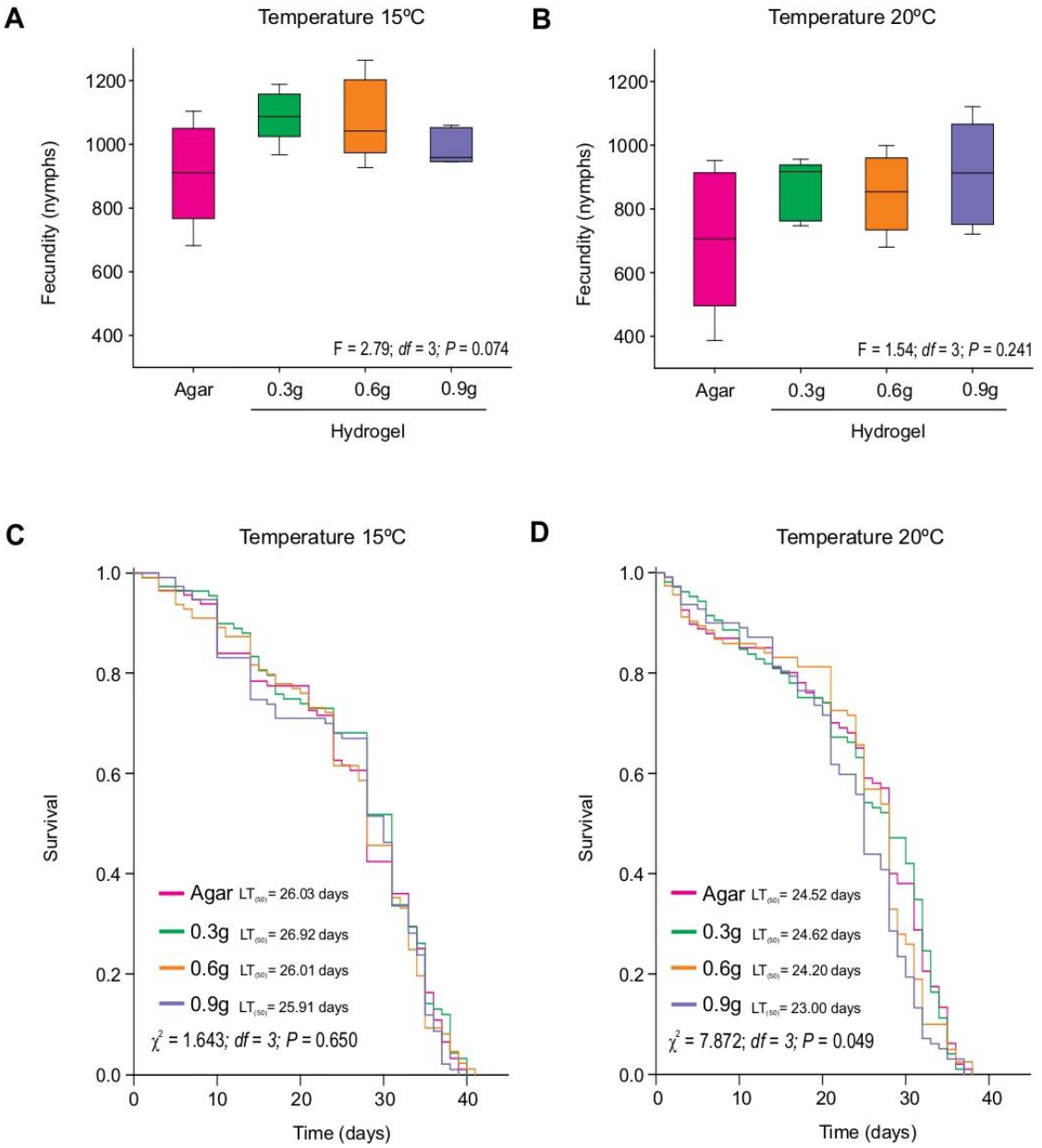

Figure 1. Fecundity (total number of nymphs per female) of *Myzus persicae* at 15 ± 2°C (***A***) and 20 ± 2°C (***B***), and survival curves of *M. persicae* at 15 ± 2°C (***C***) and 20 ± 2°C (***D***), 70 ± 10% RH under *Nicandra physalodes* plants in different concentrations of hydrogel (0.3; 0.6; and 0.9g) and 10% agar.

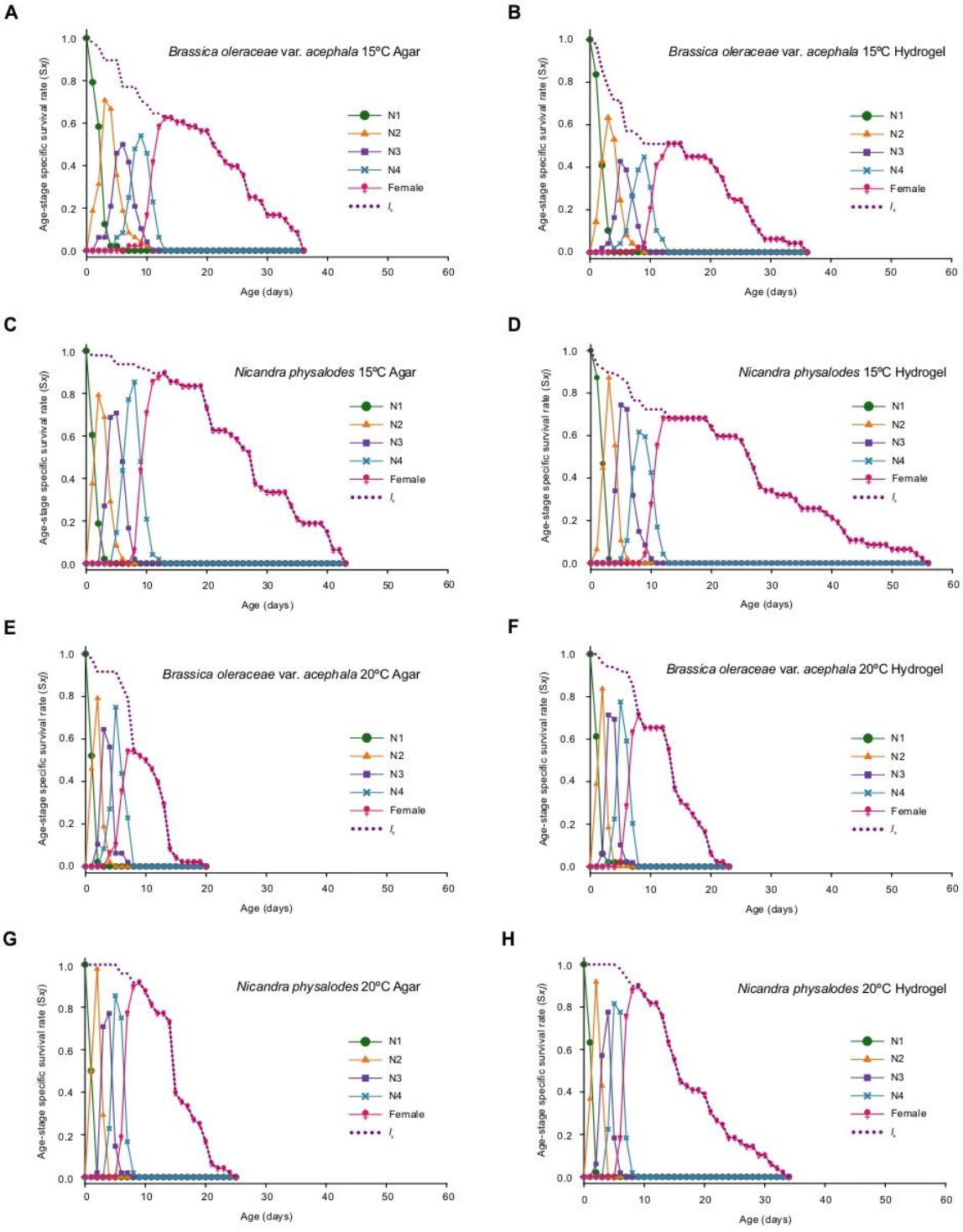

Figure 2. Graphs of survival rate by age and developmental stage (*Sxj*) of *Myzus persicae* and the survival rate of the total cohort (*lx*). (***A***) *Brassica oleracae* Agar 15ºC (***B***) *Brassica oleraceae* Hydrogel 15ºC (***C***) *Nicandra physalodes* Agar 15ºC (***D***) *Nicandra physalodes* Hydrogel 15ºC (***E***) *Brassica oleraceae* Agar 20ºC (***F***) *Brassica oleraceae* Hydrogel 20ºC (***G***) *Nicandra physalodes* Agar 20ºC (***H***) *Nicandra physalodes* Hydrogel 20ºC.

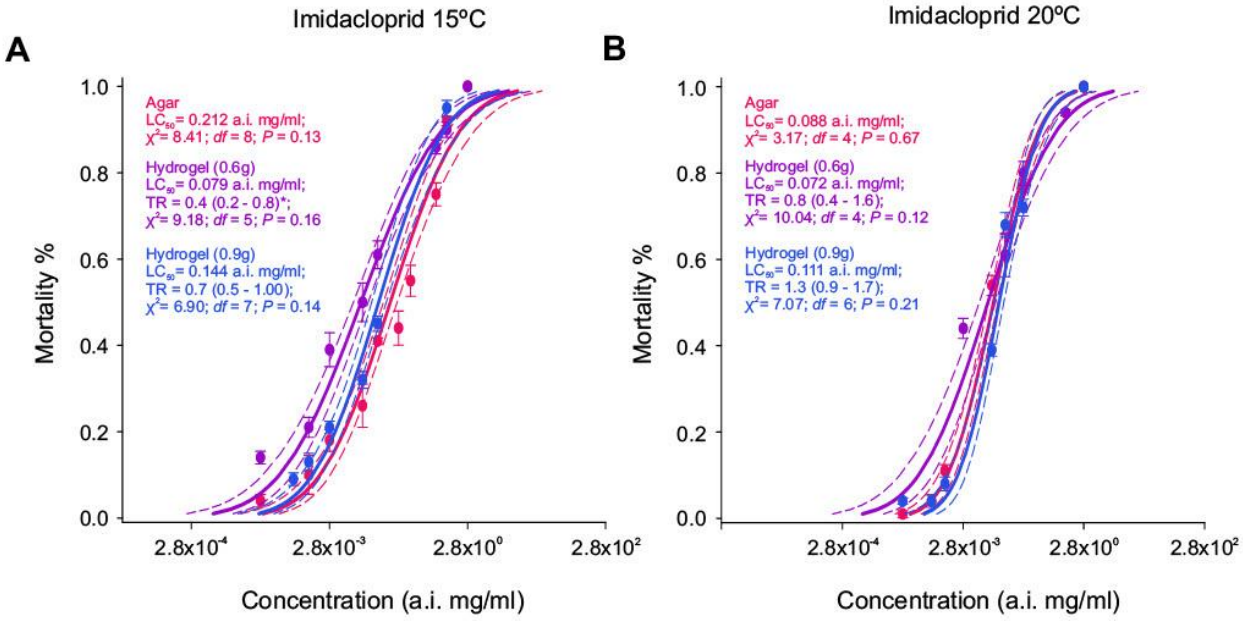

Figure 3. Concentration-mortality curves showing susceptibility of *Myzus persicae* adults using leaf immersion bioassay. (***A***) Curves of *Brassica oleraceae var. acephala* Agar and Hydrogel 15°C. (***B***) Curves of *Brassica oleraceae var. acephala* Agar and Hydrogel 20°C.

Table 1. Developmental periods, adult longevity and life cycle duration of *Myzus persicae* reared on leaf disks of the cabbage *Brassica oleraceae* and the shoo-fly plant *Nicandra physalodes* on agar or hydrogel layers under two different temperatures (15 and 20 °C)

		15°C								20°C							
		AGAR				HYDROGEL				AGAR				HYDROGEL			
		B. oleraceae		*N. physalodes*		*B. oleraceae*		*N. physalodes*		*B. oleraceae*		*N. physalodes*		*B. oleraceae*		*N. physalodes*	
Parameters	**Stage**	**N**	**Mean ± SE**	**N**	**Mean ± SE**	**N**	**Mean ± SE**	**N**	**Mean ± SE**	**N**	**Mean ± SE**	**N**	**Mean ± SE**	**N**	**Mean ± SE**	**N**	**Mean ± SE**
Development time (days)	N1	44	2.57 ± 0.16 a	47	1.83 ± 0.11 b	37	2.24 ± 0.13 a	42	2.45 ± 0.41 a	45	1.53 ± 0.08 c	48	1.50 ± 0.07 c	45	1.6 ± 0.08 bc	49	1.65 ± 0.07 bc
	N2	36	2.58 ± 0.14 a	46	2.30 ± 0.12 a	28	2.61 ± 0.13 a	41	2.32 ± 0.10 a	44	1.57 ± 0.1b c	48	1.77 ± 0.07 b	45	1.53 ± 0.07 b	49	1.71 ± 0.07 bc
	N3	32	2.72 ± 0.15 a	44	2.48 ± 0.08 a	25	2.56 ± 0.17 a	38	2.82 ± 0.15 a	42	1.52 ± 0.09 b	47	1.66 ± 0.09 b	43	1.7 ± 0.07 b	47	1.57 ± 0.07 b
	N4	30	3.30 ± 0.19 a	43	3.16 ± 0.12 a	25	3.40 ± 0.22 a	33	3.15 ± 0.16 a	32	1.94 ± 0.12 b	44	2.05 ± 0.05 b	41	2.05 ± 0.10 b	45	2.09 ± 0.07 b
	N1-N4	30	11.3 ± 0.21 a	43	9.72 ± 0.16 b	25	10.80 ± 0.23 a	33	10.79 ± 0.16 a	32	6.41 ± 0.20 d	44	6.98 ± 0.10 c	41	6.83 ± 0.12 cd	45	7 ± 0.10 c
Longevity (days)	Female	30	16.1 ± 1.13 b	43	19.3 ± 1.32 ab	25	14.08 ± 1.05 bc	33	22.73 ± 1.96 a	32	5.97 ± 0.53 e	44	9.45 ± 0.56 d	41	7.76 ± 0.66 d	45	12.18 ± 1.02 c
Life cycle*	N1 - Female	48	19.46 ± 1.61 b	48	26.65 ± 1.54 a	49	14.84 ± 1.58 cde	47	25.19 ± 2.32 a	48	10.21 ± 0.59 f	48	15.65 ± 0.63 d	49	13.08 ± 0.77 e	49	18.18 ± 1.05bc

N = number of specimens at each developmental stage; N1 = 1st instar aphid, N2 = 2nd instar aphid, N3 = 3rd instar aphid, and N4 = 4th instar aphid; (*): Mean total life history for females. Developmental stage, longevity and life cycle are given as Means(days) ± SE. Different letters in the same line indicate statistical differences based on paired bootstrap test.

Table 2. Reproductive parameters of females of *Myzus persicae* reared on leaf disks of the cabbage *Brassica oleraceae* and the shoo-fly plant *Nicandra physalodes* on agar or hydrogel layers under two different temperatures (15 and 20 °C)

	15°C								**20°C**							
	AGAR				**HYDROGEL**				**AGAR**				**HYDROGEL**			
	B. oleraceae		***N. physalodes***		***B. oleraceae***		***N. physalodes***		***B. oleraceae***		***N. physalodes***		***B. oleraceae***		***N. physalodes***	
Parameters	**N**	**Mean ± SE**	**N**	**Mean ± SE**	**N**	**Mean ± SE**	**N**	**Mean ± SE**	**N**	**Mean ± SE**	**N**	**Mean ± SE**	**N**	**Mean ± SE**	**N**	**Mean ± SE**
Total fecundity	30	27.43 ± 2.90 b	43	32.26 ± 2.33 c	25	16.24 ± 2.09 ab	33	33.33 ± 3.05 ab	32	8.53 ± 1.73 d	44	36.55 ± 2.80cd	41	11.93 ± 1.63 a	45	39.71 ± 3.54 a
Effective fecundity	30	27.43 ± 2.90 b	43	32.26 ± 2.33ab	24	16.92 ± 2.06 c	33	33.33 ± 3.05 ab	26	10.5 ± 1.94 d	44	36.55 ± 2.80 a	35	13.97 ± 1.68cd	44	40.61 ± 3.51 a
Viviparity (days)	30	11.87 ± 1.02 ab	43	13.02 ± 0.89 a	24	8.5 ± 0.84 bc	33	14.73 ± 1.15 a	26	4.54 ± 0.49 e	44	8.18 ± 0.53 c	35	6.49 ± 0.56 d	44	10.2 ± 0.87 b
APOP	30	1.23 ± 0.16 ab	43	1.07 ± 0.10 ab	24	1.46 ± 0.22 a	33	1.24 ± 0.01 ab	26	1.85 ± 0.24 a	44	0.86 ± 0.07 b	35	1.14 ± 0.15 ab	44	0.95 ± 0.06 b
TPOP	30	12.27 ± 0.22 a	43	10.79 ± 0.18 b	24	12.38 ± 0.26 a	33	12.03 ± 0.17 a	26	8.23 ± 0.23 c	44	7.84 ± 0.08 c	35	8.03 ± 0.13 c	44	7.95 ± 0.12 c
Maximum total fecundity*	-	70	-	76	-	44	-	75	-	37	-	76	-	40	-	84
Maximum daily fecundity*	-	7	-	8	-	7	-	7	-	9	-	10	-	6	-	12

Effective fecundity: only those females that performed viviparity; APOP: Pre-viviparity period of the adult female; TPOP: Total pre-viviparity period (from N1 to adult female); (*): nymphs/female; All reproductive parameters are given as Means ± SE. Different letters in the same line indicate statistical differences based on paired bootstrap test.

Table 3. Population parameters of *Myzus persicae* reared on leaf disks of the cabbage *Brassica oleraceae* and the shoo-fly plant *Nicandra physalodes* on agar or hydrogel layers under two different temperatures (15 and 20 °C)

	15°C				**20ºC**			
	AGAR		**HYDROGEL**		**AGAR**		**HYDROGEL**	
	B. oleraceae	***N. physalodes***	***B. oleraceae***	***N. physalodes***	***B. oleraceae***	***N. physalodes***	***B. oleraceae***	***N. physalodes***
Population parameter	**Mean ± SE**	**Mean ± SE**	**Mean ± SE**	**Mean ± SE**	**Mean ± SE**	**Mean ± SE**	**Mean ± SE**	**Mean ± SE**
Intrinsic rate of increase, *r* (day^{-1})	0.15 ± 0.01 d	0.12 ± 0.01 e	0.20 ± 0.01 b	0.16 ± 0.01 d	0.15 ± 0.02 cde	0.19 ± 0.01 bc	0.29 ± 0.01 a	0.28 ± 0.01 a
Finite rate of increase, λ (day^{-1})	1.16 ± 0.01 c	1.12 ± 0.01 d	1.22 ± 0.01 b	1.17 ± 0.01 c	1.16 ± 0.02 cd	1.21 ± 0.01 bc	1.34 ± 0.01 a	1.33 ± 0.01 a
Gross reproductive rate (offspring per individual), *GRR*	40.39 ± 2.94 b	22.35 ± 3.24 bc	46.02 ± 2.81 b	47.65 ± 3.15 b	22.73 ± 5.67 c	32.47 ± 6.15 c	57.46 ± 3.17 a	64.28 ± 2.96 a
Net reproductive rate (offspring per individual), R_0	17.14 ± 2.60 b	8.28 ± 1.56 cd	28.89 ± 2.50 ab	23.40 ± 3.06 b	5.68 ± 1.27 d	9.97 ± 1.48 c	33.5 ± 2.92 a	36.46 ± 3.56 a
The mean length of a generation, *T* (days)	18.62 ± 0.31 a	17.54 ± 0.45 b	16.77 ± 0.33 b	19.53 ± 0.40 a	11.12 ± 0.25 d	11.89 ± 0.32 cde	11.87 ± 0.15 e	12.42 ± 0.21 c

Different letters in the same line indicate statistical differences based on paired bootstrap test.

ARTICLE II

Sublethal effects of organophosphate and pyrethroid under temperature variations on biological characteristics of *Myzus persicae* (Sulzer) (Hemiptera, Aphididae)

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Highlights

- The variation in thermal regimes influenced the toxicity of chlorpyrifos and deltamethrin.
- Exposure of *M. persicae* to mild insecticidal stress can result in stimulatory (hormetic) effects.
- Sublethal concentrations of chlorpyrifos and deltamethrin combined with temperature variation induced stimulation in female reproduction and survival.

Abstract

Aphids in agroecosystems are exposed to numerous forms of stresses. Exposure of these organisms to low doses of agrochemicals can induce biological stimulation (hormesis), resulting in implications for the management of these insects in agricultural fields. To discuss issues related to the nature of dose-response and possible consequences on the biological fitness of the pest aphid *Myzus persicae* (Sulzer) (Hemiptera, Aphididae), the aim of the work was to investigate the lethal and sublethal exposure of organophosphate and pyrethroid along with temperature variation on the behavior of this species. Two insecticides (chlorpyrifos and deltamethrin) and four different temperatures (15, 20, 25 and 28°C) were selected for the toxicity bioassays. For this, the dose-response curve of the insecticides was determined, and after that, the sublethal concentrations (LC_1, LC_5, LC_{10}, LC_{15}, LC_{20} and LC_{30}) were selected for the evaluations of the biological characteristics of *M. persicae*. The results showed that high temperature (28°C) induced higher toxicities at lower concentrations of chlorpyrifos and deltamethrin. We also observed that there were significant changes in fecundity and survival of individuals exposed to low concentrations of the insecticides when compared to unexposed individuals. These results report that hormesis in the individuals was induced by these stressors, however, the hormetic effects on the individuals varied between temperature

regimes. We highlight the importance of considering the hormetic effects of insecticides together with temperature variation under the response mechanisms of *M. persicae*, therefore, these findings contribute to a better understanding of the impact on the future management of this pest.

Keywords: aphids, fecundity, hormesis, longevity, pesticides

1. Introduction

Myzus persicae (Sulzer) is a pest aphid, popularly known as the green peach aphid, is a polyphagous species that exhibits wide distribution worldwide, feeds on more than 400 plant species from about 40 different families, and transmits more than 100 plant viruses (Blackman and Eastop, 2000; Meng et al., 2014; van Emden and Harrington, 2017). The intensive infestation of these organisms in the field can cause serious damage to plants and generate significant yield losses in economically important crops (Bass et al., 2014; de Little et al., 2016; Saljoqi et al., 2009).

As a result of its economic importance, *M. persicae* is one of the most studied species and target of intense chemical control programs worldwide. Thus, aphid populations are exposed to large amounts of pesticides in agricultural systems, and among the chemical groups most commonly used to control these individuals are: organophosphates, carbamates, pyrethroids, and neonicotinoids (Mota-Sanchez and Wise, 2021).

However, their doses can differ in space and time by several factors, including misapplication, drift, and degradation over time (Biondi et al., 2012; Desneux et al., 2007; Duke, 2014). Consequently, this sublethal exposure can cause a range of effects on physiological and/or behavioral characteristics of individuals (Cutler et al., 2022; Guedes et al., 2022; Sial et al., 2018).

Hormesis is a biphasic phenomenon resulting from low dose stimulation and high dose inhibition following insect exposure to insecticides (Calabrese and Baldwin, 2003; Guedes et al., 2022). Individuals that survive exposure to a toxic compound at concentrations, low or sublethal doses (Desneux et al., 2007), may exhibit an improvement in their biological fitness, such as, longevity rate, fecundity, immune capacity, and/or sex ratio of specimens (Lu et al., 2016; Qu et al., 2015; P. Wang et al., 2017). Agrochemical-induced hormetic effects have been reported in several aphid species, such as in *M persicae* (Zeng et al., 2016), *Aphis gossypii* Glover (Koo et al., 2015), *Sitobion avenae* (F.), and *Rhopalosiphum padi* (Linnaeus) (Lu et al., 2016). In view of this, hormesis becomes a disadvantageous response, since, it can favor species increase, resurgence and/or secondary outbreaks of pests (Cutler, 2013; Guedes et al., 2022; Rix and Cutler, 2022).

The stimulatory effects induced by hormetic exposures to pesticides can be highly dependent on chemical group and insect species (Cho et al., 2011). Chlorpyrifos is a synthetic insecticide of the organophosphorus chemical group that is non-systemic, broad-spectrum, efficient, and has been widely used to control various pests, including aphids (Ahmad and Aslam, 2005; Li et al., 2022; Simon, 2011). This insecticide is an acetylcholinesterase (AChE) inhibitor, induces oxidative stress, and can damage DNA (Rasheed et al., 2020). Sublethal concentrations of chlorpyrifos under individuals of *Daphnia carinata* King (Cladocera) resulted in reproductive hormetic effects in the second generation (Zalizniak and Nugegoda, 2006). On the other hand, sublethal concentrations of chlorpyrifos affected the population dynamics of *Rhopalosiphum padi* (Linnaeus), decreasing the population development rate, survival and fecundity of these individuals (Duan et al., 2015; Guiying et al., 2014).

Deltamethrin is a synthetic agrochemical of the pyrethroid group and acts as a rapid neurotoxic agent (Gibson et al., 1982; Malbert-Colas et al., 2020). They are classified as sodium channel modulators, where the molecule induces toxic responses in the central and peripheral nervous system of insects (Haug and Naumann, 1990; Simon, 2011), stimulating nerve cells to produce repetitive discharges, and consequently causes paralysis in the insect (Narahashi, 2002; Soderlund, 2012). Guedes et al. (2010) evaluated concentrations of deltamethrin on a resistant strain of *Sitophilus zeamais* Motsch. (Coleoptera: Curculionidae), and showed that exposure to this insecticide rapidly boosted reproduction, generating significant increases in population growth, and increasing the frequency of resistant insects in this population.

Aphids are affected not only by agrochemical exposure in agricultural systems, but can also be significantly impacted by climate change in that environment. Temperature is a key abiotic factor that regulates population dynamics, development rates, and seasonal occurrence of aphids (ectothermic organisms) (Alford et al., 2012; Campbell et al., 1974; Cocu et al., 2005; Soh et al., 2018). In addition, temperature also affects the activity, performance, and properties of insecticides, such as differences in product volatility and stability (Horn, 2019; Johnson, 1990). In this sense, knowledge of the interaction of temperature on insecticide exposure against pest insects is of utmost importance, given the impacts on possible decision-making in pest management.

The economic implications of aphids in agriculture raise many questions about how their population dynamics might change under varying stressors, however, most studies focus on the efficiency/effectiveness of insecticides in controlling these pests. Information is still lacking in the literature on how these individuals might react from agrochemical exposure in relation to temperature changes. Therefore, the aim of this work was to investigate how lethal and sublethal exposure of insecticides of different chemical groups

(organophosphate and pyrethroid) together with temperature variation affect the biological characteristics of *M. persicae*.

2. Material and Methods

2.1.Insect, insecticide and temperature variation

The experiment was carried out in the Laboratory of Molecular Biology and Ecotoxicology (M.E.E.T) at the Entomology Department of UFLA.

The *M. persicae* colony was established from an already established laboratory rearing. Aphid age standardization was performed with about 100 newly hatched nymphs, which were placed on leaf discs (12 cm diameter) of *Nicandra physalodes* plants under 6% hydrogel, and held for about 8 days. This method was used to ensure that all aphids are the same age (and growth stage) at the beginning of each bioassay. The colony was kept in a climate-controlled chamber, with temperature maintained at 20 ± 2°C, relative humidity at 70 ± 10%, and photophase of 16 hours.

The insecticides evaluated were organophosphate (CAPATAZ®) and pyrethroid (DELTAMAX 25 CE). Serial dilutions were prepared using distilled water containing 0.01% (v/v) Tween 20, and were used immediately after preparation in order to minimize any chemical decomposition.

To evaluate the effects of temperature variation on responses to insecticide exposure, four temperatures were selected: 15, 20, 25, and 28ºC. All bioassays were maintained in climate-controlled chambers (BOD, ELETRO*lab*) with relative humidity at 70 ± 10% and and photophase of 16 hours.

Temperature coefficients were calculated as the ratio of the highest to lowest LC_{50} values, and were considered positive if toxicity increases with increasing temperature, negative if toxicity decreases with increasing temperature, and no effect if unaffected by increasing

temperature (Sparks et al., 1982). The effects of temperature coefficients were "no effect" = < 2, "slight" = (2-5), and "strong" = > 5, following the methodology of (Liu et al., 2016).

2.2.Exposure to insecticide by foliar immersion

The evaluation of insecticide toxicity was based on dose-response curves and evaluated by the foliar immersion method proposed by the Insecticide Resistance Action Committee (IRAC, 2009).

Initial preliminary tests were conducted to determine the range of experimental doses for each insecticide. Then, at least seven concentrations were used to establish dose-response curves with a target mortality ranging from 0% to 100%. Concentrations ranged from $0.028x10^{-6}$ to $0.028x10^{2}$ a.i. mg/ml for chlorpyrifos and from $0.015x10^{-6}$ to $0.015x10^{2}$ a.i. mg/ml for deltamethrin. The insecticides were diluted with distilled water containing 0.01% (v/v) Tween 20, and for the control, only distilled water containing 0.01% (v/v) Tween 20 was used.

Leaf discs (5.6 cm in diameter) of *Brassica oleraceae* var. acephala were cut and individually dipped for about 6 seconds in the insecticide and control solutions, and then placed at room temperature to dry for about 2 hours. The leaf discs were placed with the abaxial surface upwards in Petri dishes (5.6 cm diameter) under 10% agar, and sealed with plastic film, with several small holes made to allow gas exchange and humidity stabilization.

Five replicates were made for each bioassay, and each replicate was inoculated with 20 adult aphids aged up to 48 hours, totaling 100 adults for each treatment. Aphid mortality was assessed under a magnifying glass (Zeiss Stemi 2000C – Stereo Microscope 1.5x) after 48 hours of the exposure period. Aphids that did not move their legs when touched with a fine brush were considered dead.

2.3.Sublethal exposure bioassay of the parental generation

For sublethal exposure, the concentrations LC_1, LC_5, LC_{10}, LC_{15}, LC_{20} and LC_{30} of the insecticides were selected (Table 1). Leaf discs were dipped into the insecticide solutions and the control solution, and were placed in Petri dishes as described above. 100 adult females were casually distributed on the treated leaves.

After 48 hours, 50 females were removed from the Petri dishes and individualized in a Petri dish with a new leaf disc not treated with insecticide. The dishes were filled with 10% agar and sealed with plastic wrap. The leaf discs not treated with insecticide were replaced every 5 days during the experiment, and kept in climate-controlled chambers.

The experiment had fifty repetitions for each treatment. The initial fecundity rates of the aphid during the pre-treatments with the sublethal doses of the insecticide were not recorded, because the nymphs were not removed during this period. After exposure, fecundity and longevity of the adults were checked daily during their life. The newly hatched nymphs were counted and removed daily.

2.4.Statistics

The mortality rate of adults was corrected for the natural mortality observed in controls (i.e., cabbages treated with distilled water) prior to analysis. Dose-mortality curves were estimated by probit analyses using the PROC PROBIT procedure (SAS Institute, Cary, NC, USA), with a Probit regression method analysis, to obtain 95% confidence intervals. Sublethal concentrations between LC_1 and LC_{30} were calculated using SAS - Statistical Analysis Systems. Fecundity data were subjected to one-way analysis of variance (ANOVA), and survival results were subjected to survival analysis, which was performed using Kaplan-Meier estimators (log-rank method) with SigmaPlot 12.0 (Systat Software,

San Jose, CA, USA). The overall similarity between survival times and median survival times (LT_{50} values) was tested using the χ^2 log-rank test, and pairwise comparisons between curves were performed using the Holm-Sidak test ($P < 0.05$).

3. Results

3.1.Exposure to the insecticide by leaf dipping

The toxicity of chlorpyrifos and deltamethrin to *M. persicae* adults was investigated 48 hours after exposure to leaf immersion. The effects of treatments on aphid responses to increasing concentrations of imidacloprid depended on the classes of insecticides and the different temperatures, resulting in significant differences in the LC_{50s} of the treatments. In general, higher temperatures induced greater toxicities at lower concentrations. The dose-response curves represented in Figure 1 showed the different responses found for each temperature evaluated.

The toxicity of chlorpyrifos at different temperatures on adults of *M. persicae* showed that there was a significant difference in the LC_{50} of the temperatures (Figure 1a – Table 2). It was found that the increase in temperature caused a decrease in the concentration (a.i. mg/ml) to kill 50% of the population. At 15°C, chlorpyrifos had a LC_{50} = 0.0086, at 20°C a LC_{50} = 0.0070, at 25°C a LC_{50} = 0.0017, and at 28°C a LC_{50} = 0.0020. We can see that temperatures 15°C and 20°C did not show significant differences between each other, but differed from 25°C and 28°C, and temperatures 25°C and 28°C did not show significant differences between each other.

The toxicity of deltamethrin also showed that there was a significant difference in LC_{50} between temperatures (Figure 1b – Table 2). It was found that increasing the temperature caused the concentration (a.i. mg/ml) to decrease to kill 50% of the population. For the temperature of 15°C, deltamethrin showed a LC_{50}= 0.0042, for 20°C it showed a LC_{50}=

0.0046, for 25°C it obtained a LC_{50}= 0.0053, and for 28°C it obtained a LC_{50}= 0.0011. We can see that the temperatures 15°C, 20°C, and 25°C did not show significant differences among themselves, but they show significant differences at 28°C.

Table 3 shows the temperature coefficients between the ranges of 15-20°C, 15-25°C, 15-28°C, 20-25°C, 20-28°C and 25-28°C in adult females of *M. persicae* exposed to chlorpyrifos and deltamethrin. In the chlorpyrifos, the ranges between 15-25°C, 15-28°C, 20-25°C and 20-28°C showed a slight temperature coefficient, and the other temperatures had no effect. The ranges between 15-28°C, 20-28°C and 25-28°C of deltamethrin showed slight temperature coefficient, and the other temperatures no effect.

3.2.Parental generation sublethal exposure bioassay

3.2.1. Chlorpyrifos

Exposure within 48 hours in *M. persicae* adults to chlorpyrifos LCs had a significant effect on the longevity and fecundity of exposed individuals (F0 generation) (Figure 2). Initial aphid fecundity rates during pretreatment with the sublethal doses of the insecticide were not recorded because no nymphs were removed during this period. After exposure, fecundity was evaluated daily.

Fecundity of females with the sublethal doses of chlorpyrifos (a.i. mg/ml) compared to the control at 15°C temperature (Figure 2a) was significantly increased after exposure to LC_1, LC_5, and LC_{20}, while LC_{15} and LC_{30} was significantly reduced (H = 8.607; df = 6; $P < 0.001$). At 20°C temperature (Figure 2c), fecundity was significantly reduced at LC_{20} and LC_{30}, on the other hand, when females were exposed to LC_{10} fecundity was significantly increased when compared to the control (H = 50.959; df = 6; $P < 0.001$). At 25°C (Figure 2e), the increase in fecundity occurred when the aphids were exposed to LC_{15} and LC_{20}, and the reduction occurred at LC_{30} (H = 9.195; df = 6; $P < 0.001$). At 28°C

(Figure 2g), the highest fecundity occurred in the control, resulting in a decrease at concentrations LC_1, LC_5, LC_{10}, LC_{20} and LC_{30} ($H = 49.518$; $df = 6$; $P < 0.001$).

Compared to the control group (13.66 days) at 15°C temperature (Figure 2b), adult longevity of F0 was significantly increased at all concentrations, where LC_1 showed the highest mean lethal time (21.58 days) ($\chi^2 = 35.854$; $df = 6$; $P < 0.001$). At 20°C temperature (Figure 2d), longevity was significantly increased at LC_{10} (12.29 days) when compared to the control (9.38 days), on the other hand, there was no significant difference when females were exposed to LC_1, LC_{20} and LC_{30} (9.32, 9.25 and 8.59 days respectively) when compared to the control ($\chi^2 = 22.727$; $df = 6$; $P < 0.001$). At 25°C temperature (Figure 2f), no significant difference was found in the longevity of females ($\chi^2 = 9.903$; $df = 6$; $P < 0.129$). Finally, at 28°C temperature, there was no significant difference between control (5 days) and LC_{15} (4.30 days), on the other hand, there was a decrease in survival at LC_1, LC_5, LC_{10}, LC_{20} and LC_{30} (3.84, 3.64, 3.95, 4.14 and 3.98 days respectively) ($\chi^2 = 47.404$; $df = 6$; $P < 0.001$) (Figure 2h).

3.2.2. Deltamethrin

In relation to pyrethroid, 48-hour exposure of *M. persicae* adults to LCs of deltamethrin also had a significant effect on the longevity and fecundity of exposed individuals (F0 generation) (Figure 3).

Fecundity of females with the sublethal doses of deltamethrin (a.i. mg/ml) compared to the control at 15°C temperature (Figure 3a) was significantly reduced after exposure at LC_5, LC_{10}, LC_{15}, LC_{20} and LC_{30}, while the LC_1 concentration there was no significant difference compared to the control ($H = 74.448$; $df = 6$; $P < 0.001$). At 20°C temperature (Figure 3c), fecundity was significantly reduced at LC_5, LC_{10}, LC_{15} and LC_{30}, on the other hand, when females were exposed to LC_1 and LC_{20} fecundity was significantly increased

when compared to the control (H = 57.071; df = 6; P < 0.001). At 25°C (Figure 3e), the increase in fecundity occurred when aphids were exposed to LC_5 and LC_{10}, and the reduction occurred at LC_{15}, LC_{20} and LC_{30} (H = 133.048; df = 6; P < 0.001). At 28°C (Figure 2g), fecundity increased in LC_1, LC_5, LC_{10} and LC_{20}, and decreased significantly in LC_{30} (H = 31.165; df = 6; P < 0.001).

Compared with the control group (9.82 days) at 15°C temperature (Figure 3b), adult longevity of F0 was significantly increased at LC_1 and LC_5 (10.95 and 10.39 days), and decreased at LC_{10}, LC_{15} and LC_{20} (8.16, 8.36 and 8.32 days) (χ^2 = 22.323; df = 6; P < 0.001). At 20°C temperature (Figure 3d), longevity increased significantly by LC_1 (14.07 days) when compared to control (12.23 days), on the other hand, survival decreased by LC_{30} (9.91 days) (χ^2 = 31.622; df = 6; P < 0.001). At 25°C temperature (Figure 3f), longevity increased significantly by LC_5 (6.30 days) when compared to the control (5.35 days), and decreased by LC_{20} (3.98 days) (χ^2 = 38.545; df = 6; P < 0.001). At 28°C temperature there was a significant increase in concentrations LC_1, LC_5, LC_{10}, LC_{15}, LC_{20} and LC_{30} (5.04, 5.0, 5.02, 5.02, 5.09 and 4.48 days) compared to the control (4.08 days) (χ^2 = 31.908; df = 6; P < 0.001) (Figure 3h).

4. Discussion

Sublethal effects of agrochemicals on fecundity, longevity, and even behavior change are commonly observed in agricultural pests after exposure to low concentrations of insecticides in agricultural areas (Cutler et al., 2022; Ding et al., 2018; Fouad et al., 2022). In the present study, we investigated how the thermal regime molds the toxicity of chemicals, and selected different sublethal concentrations of two insecticides from different chemical groups (organophosphate and pyrethroid) along with different temperatures (15, 20, 25, and 28°C) to ascertain the biological consequences of the

hormetic responses of the aphid species *M. persicae*. The results obtained revealed that the toxicity of the insecticides was influenced by the different temperature levels. Moreover, it was observed that low concentrations of chlorpyrifos and deltamethrin combined with heat stress significantly affected the development and reproduction of *M. persicae*. Thus, comprehensive knowledge about the negative impact of sublethal concentrations of insecticides are of utmost importance for optimal management strategies against pest aphids.

The assessment of lethal concentrations is a very useful tool to compare the toxicity of chemicals with different active ingredients and different formulations. It is known that the toxicity and relative efficacy of insecticides can vary due to several factors, such as the difference in their mode of action and chemical structure of different active ingredients (Mahmoodi et al., 2020), and even temperature, which can affect their physical and chemical properties, such as stability, vaporization, penetration, activity, degradation, absorption and translocation (Johnson, 1990).

The results of this work showed that the different responses of aphids were due to the influence of the four different temperature levels on the toxicity of the chemicals. According to the LC_{50} of the insecticides, high temperature (28°C) induced high toxicity at lower concentrations of chlorpyrifos and deltamethrin (Figure 1).

The analysis shows that increased temperature (25 and 28°C) may be sufficient to cause a significant increase in chlorpyrifos toxicity (see values in Table 2) than milder temperatures (15 and 20°C). This, in turn, will increase the mortality of individuals at lower concentrations of the product at higher temperatures. In the case of deltamethrin, the significant increase in toxicity occurred only at the highest temperature (28°C) (table 2). The reason for this is that, organophosphate and pyrethroid are very effective against sucking insect pests (Golvankar et al., 2019; Haddi et al., 2018, 2012; Irshaid and Hassan,

2011; Shang et al., 2021; Todorova et al., 2020), and may exhibit positive temperature coefficient, i.e., the toxicity of the insecticide increases with increasing temperature (Li et al., 2006; Mansoor et al., 2015). Previous investigations have shown that the toxicity of insecticides against agricultural pests depends on the thermal regime, for example, Khan and Akram (2014) explored the relationship between temperature and the toxicity of insecticides of different classes under *Musca domestica* L., (Diptera: Muscidae), and observed that the toxicity of chlorpyrifos, profenofos, emamectin, and fipronil had a direct relationship with the temperature range tested, showing positive temperature coefficient, on the other hand, cypermethrin and deltamethrin showed negative association with temperature, where there was a decrease in pyrethroid toxicity at higher temperatures. Jaleel et al. (2020) showed that there was positive correlation between temperature and toxicity of deltamethrin under *Plutella xylostella* L. (Lepidoptera: Plutellidae), and revealed that temperature in the range of 20-25 °C is ideal for the management program of these individuals.

Although the relationship between the insecticides and temperature tested in this study was clearly shown, the exact mechanism has not been fully investigated. In this sense, it is of utmost importance that further studies be conducted to have a better understanding of the uptake and elimination of insecticides with temperature change and how these effects affect the biological processes of *M. persicae*.

In addition to direct exposure with chemical insecticides, it is already well known in the literature that agricultural pests are often exposed to low doses of the products in the field due to their variable distribution and continuous degradation (Cutler et al., 2022; Guedes et al., 2022; Rix and Cutler, 2022). This exposure causes several sublethal effects to organisms and is defined as hormesis. Hormesis is a biphasic phenomenon resulting from low dose stimulation and high dose inhibition following insect exposure to insecticides

(Cutler et al., 2022; Duke, 2014; Rix and Cutler, 2022). The study of pesticide-induced hormetic responses in insects has become extremely important due to its potential in implicating pest management. The uptake of a small amount of insecticides after exposure to these concentrations may contribute to a beneficial stimulatory effect on the biological fitness of organisms, such as, fecundity, fertility, longevity, intrinsic rate of increase, finite rate of increase, and net reproductive rate of pest aphids (Calabrese and Baldwin, 2003; Shang et al., 2021; Sial et al., 2018; Ullah et al., 2019).

In the literature several authors have reported insecticide-induced hormesis on pest aphids, including *M. persicae* (Rix et al., 2016; Sial et al., 2018; Tang et al., 2019; P. Wang et al., 2017), *Aphis gossypii* Glover (Chen et al., 2016; S. Wang et al., 2017), *Aphis craccivora* Koch (Fouad et al., 2022), *Aphis glycines* Matsumura (Qu et al., 2017, 2015). However, little is known in the literature about how the sublethal effects of insecticides of different active ingredients in conjunction with temperature variation interferes with the biological aspects of aphids, especially *M. persicae*. The results obtained in the present study showed that significant changes occurred in fecundity and survival in females of *M. persicae* due to the two combined stressors (temperature and insecticides), and the hormetic responses of each product varied within the thermal regimes (Figure 2 and 3). Stimulatory effects on the development and reproduction of individuals observed here, can also be observed in other studies. Shang et al. (2021) evaluated the effect of low concentration deltamethrin (LC_{30}) under five successive generations of *Aphis gossyppi* after the initial aphid (G0) was exposed for 24 hours, and concluded that fecundity values were significantly increased, and offspring population growth of these two generations was significantly promoted.

The ability of *M. persicae* to cope with diverse stressors may be achieved by physiological and biochemical mechanisms. The reason for this is that, sublethal exposure of

insecticides at different temperatures may have caused adaptive responses that increased the cellular defenses of individuals, and consequently increased performance (reproduction and survival) beyond that observed in untreated individuals. In addition to this fact, we observed that as the temperature increased, the range of sublethal concentrations that caused the hormetic responses in the organisms shifted. It is worth noting that at 20°C the sublethal effects occurred due to chemical stress, as the aphids were reared at this temperature. Against this backdrop, it is important that further studies be conducted to understand why hormetic effects at certain temperatures are directly linked at specific sublethal concentration ranges and how the defense mechanisms of individuals are affected by this relationship between stressors.

As stated earlier, the toxicity of chemicals and the development and behavior in insects, can be strongly affected by changes in temperature (Johnson, 1990; Neven, 2000). When physiological injuries occur under different stressors, insects exhibit impacts on their biological and physiological fitness during their lifetime (González-Tokman et al., 2020; Neven, 2000). In this study, the combination of chemical stress (chlorpyrifos and deltamethrin) and high temperatures (25 and 28°C) imposed a higher fitness and physiological cost on this species decreasing its tolerance and performance. The importance of considering these stressors in insects is corroborated by other authors. The results of the study by Deng et al. (2016) showed that low concentrations of chlorpyrifos stimulated the development and fecundity of *Plutella xylostella* (L.), however, the variation of temperature (25 and 38°C) determined a significant high fitness cost among strains of this species.

In our comparisons, we observed that aphid responses were influenced by increasing concentrations of the insecticides and temperature variation. The toxicity of the insecticides was influenced by temperature variation. Based on the present study,

fecundity and survival values of these individuals were significantly increased when compared to the control, however, high temperatures with chemical stress caused a higher adaptive cost for this species, decreasing the efficiency of individuals to survive and produce offspring. Therefore, these findings suggest that short-term exposure to sublethal concentrations of chlorpyrifos and deltamethrin may induce hormesis in *M. persicae*.

In conclusion, the continued degradation of insecticides in fields results in frequent exposure of sublethal concentrations under pest insects, and may contribute to the resurgence of pest insects. This exposure results in organisms with improved stress coping abilities and resilience. Therefore, it is of utmost importance that further ecotoxicological studies are conducted, to provide a fundamental contribution to our understanding of how pesticide-induced stimulation is influenced by temperature variation.

5. Conclusions

The increase in temperature influenced the toxicity of chlorpyrifos and deltamethrin on *M. persicae* individuals, showing that these agrochemicals have a positive temperature coefficient. It is very important to know the relation of temperature with the toxicity of chemicals for integrated pest management, this will allow the selection of products to be more effective in certain environmental conditions.

Due to the variable distribution and degradation of pesticides in the field, aphids are exposed to low concentrations of the product. The increased survival and fecundity of *M. persicae* subjected to insecticides of different chemical groups under temperature variation exemplifies the adaptive nature of hormesis induced by these stressors.

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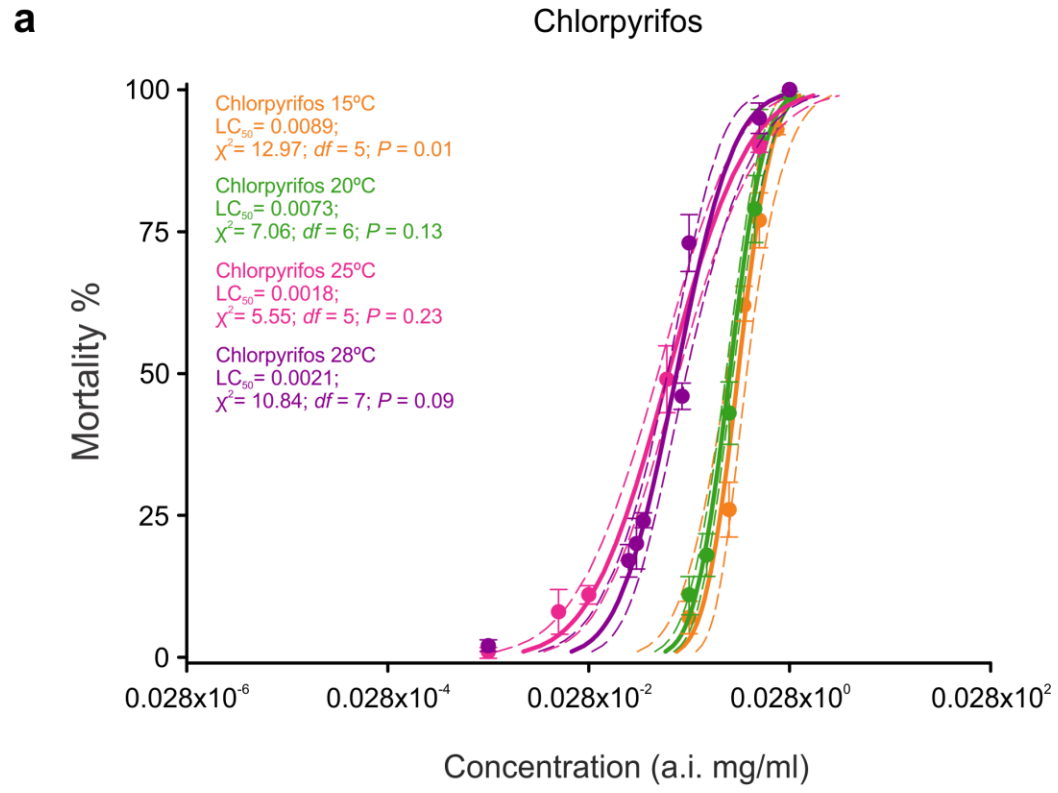
Figures captions

Figure 1. Toxicity of Chlorpyrifos (***a***), and Deltamethrin (***b***) to *Myzus persicae* adults under four temperature regimes (15, 20, 25 and 28°C). The lines denote the estimated lethal concentration (LC) values based on concentration-mortality bioassays using probit analyses. Symbols show the mean mortality for each insecticide concentration applied to each *M. persicae* population. Vertical bars represent the standard error of the mean (SE).

Figure 2. Effects of sublethal exposure to the organophosphate chlorpyrifos on fecundity (a, c , e ,g) and longevity (b, d, f , h) of *Myzus persicae* females to at 15°C (***a ; b***), 20°C (***c ; d***), 25°C (***e ; f***) and 28 ± 2°C (***g ; h***)

Figure 3. Effects of sublethal exposure to the pyrethroid deltamethrin on fecundity (a, c , e ,g) and longevity (b, d, f , h) of *Myzus persicae* females to at 15°C (***a ; b***), 20°C (***c ; d***), 25°C (***e ; f***) and 28 ± 2°C (***g ; h***).


a
Chlorpyrifos
Chlorpyrifos 15ºC
LC50= 0.0089;
χ2= 12.97; df = 5; P = 0.01
Chlorpyrifos 20ºC
LC50= 0.0073;
χ2= 7.06; df = 6; P = 0.13
Chlorpyrifos 25ºC
LC50= 0.0018;
χ2= 5.55; df = 5; P = 0.23
Chlorpyrifos 28ºC
LC50= 0.0021;
χ2= 10.84; df = 7; P = 0.09
Mortality %
100
75
50
25
0
0.028x10-6
0.028x10-4
0.028x10-2
0.028x100
0.028x102
Concentration (a.i. mg/ml)

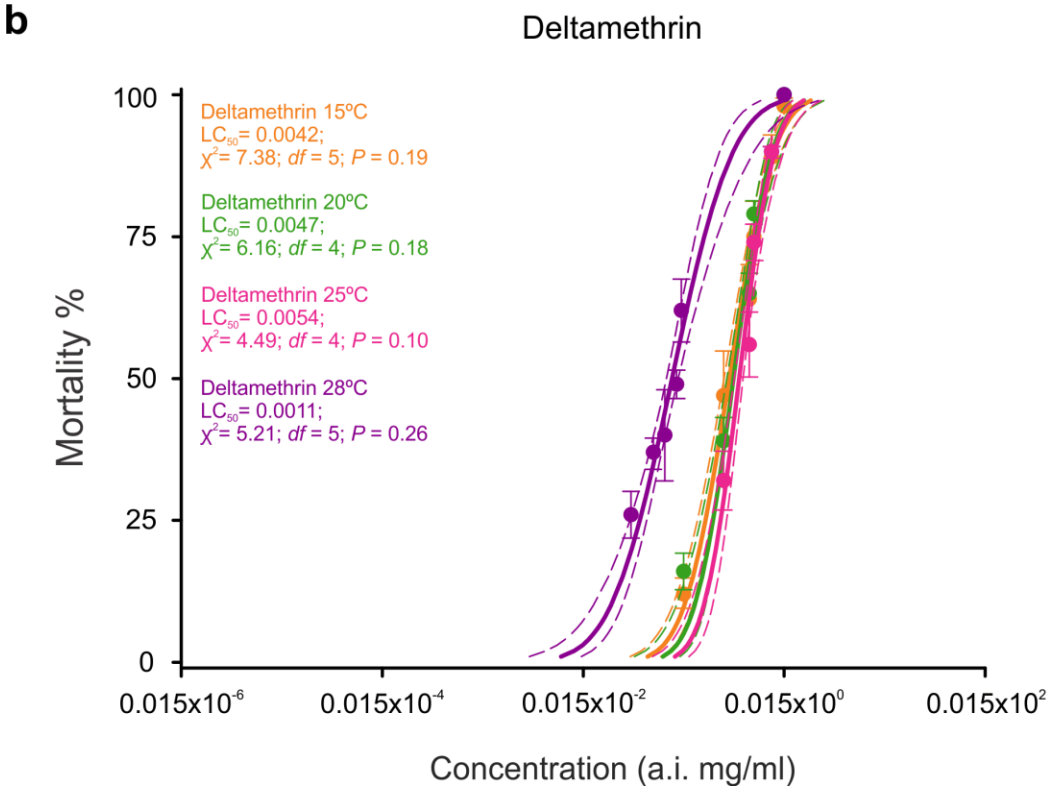

b
Deltamethrin
Deltamethrin 15ºC
LC50= 0.0042;
χ2= 7.38; df = 5; P = 0.19
Deltamethrin 20ºC
LC50= 0.0047;
χ2= 6.16; df = 4; P = 0.18
Deltamethrin 25ºC
LC50= 0.0054;
χ2= 4.49; df = 4; P = 0.10
Deltamethrin 28ºC
LC50= 0.0011;
χ2= 5.21; df = 5; P = 0.26
Mortality %
100
75
50
25
0
0.015x10-6
0.015x10-4
0.015x10-2
0.015x100
0.015x102
Concentration (a.i. mg/ml)

FIGURE 1

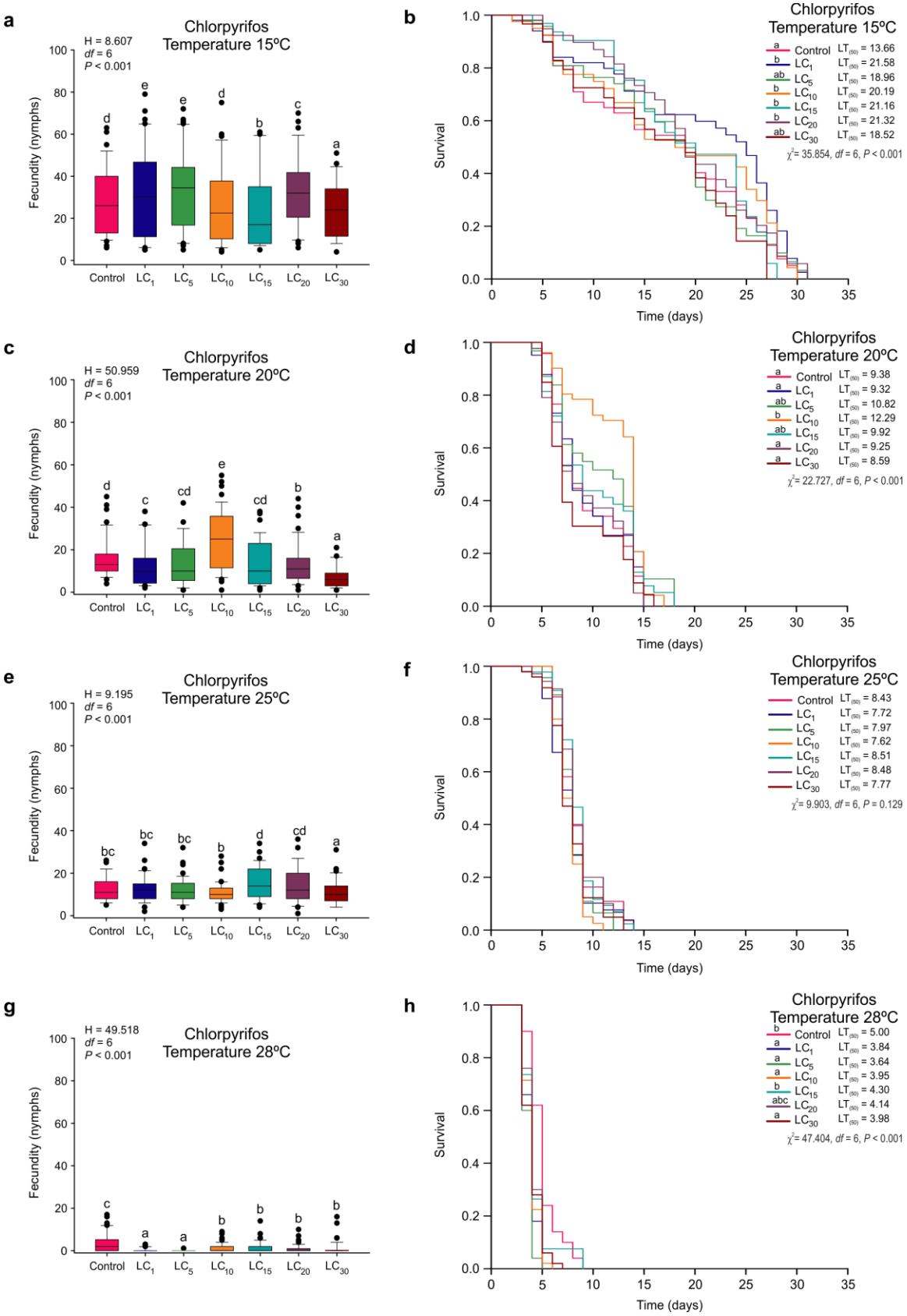

a
H = 8.607
df = 6
P < 0.001
Chlorpyrifos
Temperature 15ºC
Fecundity (nymphs)
Control LC1 LC5 LC10 LC15 LC20 LC30
b
Survival
Time (days)
Chlorpyrifos
Temperature 15ºC
Control LT(50) = 13.66
LC1 LT(50) = 21.58
LC5 LT(50) = 18.96
LC10 LT(50) = 20.19
LC15 LT(50) = 21.16
LC20 LT(50) = 21.32
LC30 LT(50) = 18.52
χ2 = 35.854, df = 6, P < 0.001
c
H = 50.959
df = 6
P < 0.001
Chlorpyrifos
Temperature 20ºC
d
Chlorpyrifos
Temperature 20ºC
Control LT(50) = 9.38
LC1 LT(50) = 9.32
LC5 LT(50) = 10.82
LC10 LT(50) = 12.29
LC15 LT(50) = 9.92
LC20 LT(50) = 9.25
LC30 LT(50) = 8.59
χ2 = 22.727, df = 6, P < 0.001
e
H = 9.195
df = 6
P < 0.001
Chlorpyrifos
Temperature 25ºC
f
Chlorpyrifos
Temperature 25ºC
Control LT(50) = 8.43
LC1 LT(50) = 7.72
LC5 LT(50) = 7.97
LC10 LT(50) = 7.62
LC15 LT(50) = 8.51
LC20 LT(50) = 8.48
LC30 LT(50) = 7.77
χ2 = 9.903, df = 6, P = 0.129
g
H = 49.518
df = 6
P < 0.001
Chlorpyrifos
Temperature 28ºC
h
Chlorpyrifos
Temperature 28ºC
Control LT(50) = 5.00
LC1 LT(50) = 3.84
LC5 LT(50) = 3.64
LC10 LT(50) = 3.95
LC15 LT(50) = 4.30
LC20 LT(50) = 4.14
LC30 LT(50) = 3.98
χ2 = 47.404, df = 6, P < 0.001

FIGURE 2

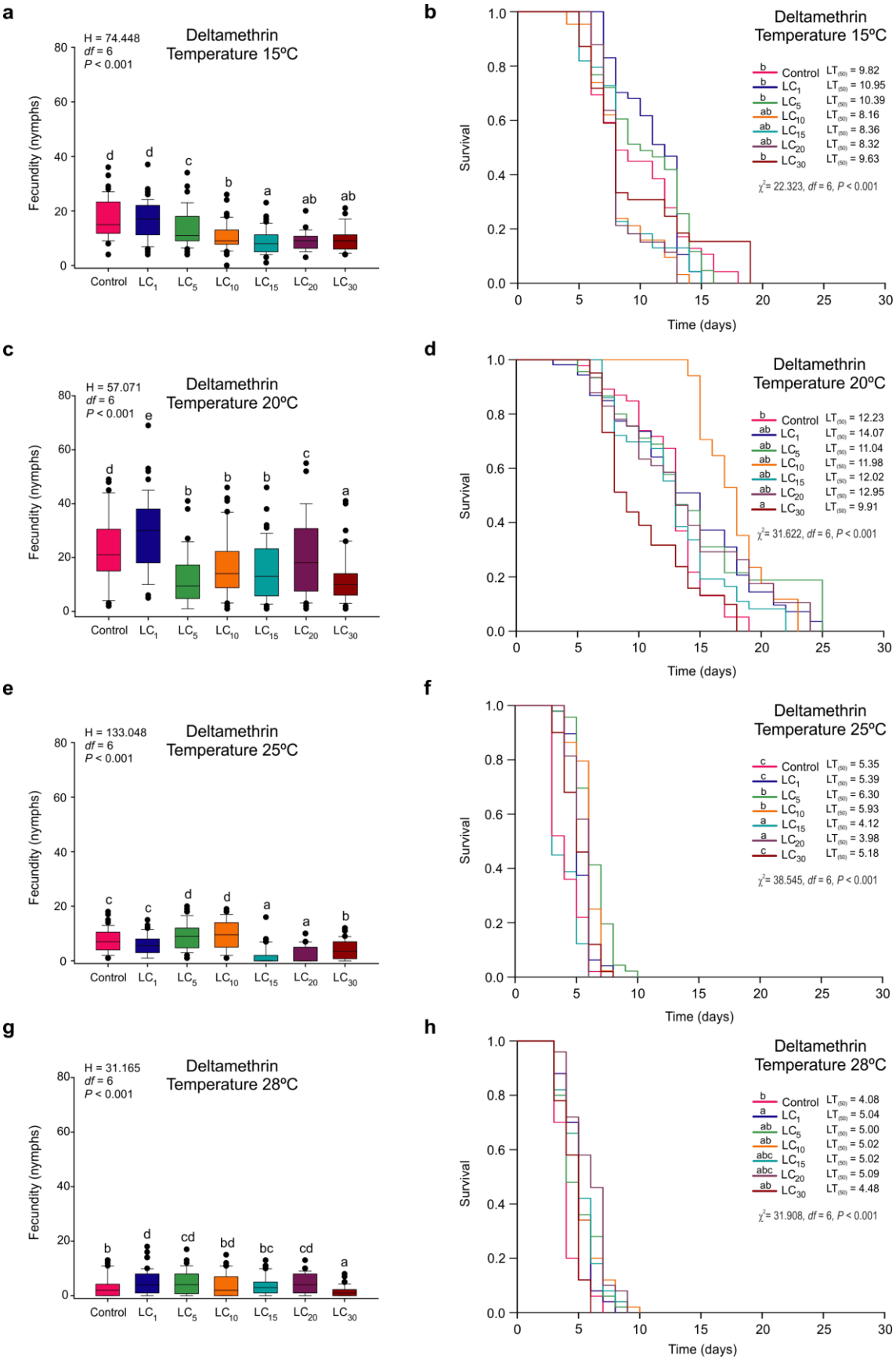

a
H = 74.448
df = 6
P < 0.001
Deltamethrin
Temperature 15ºC
Fecundity (nymphs)
Control LC1 LC5 LC10 LC15 LC20 LC30
b
Deltamethrin
Temperature 15ºC
Survival
Time (days)
Control LT(50) = 9.82
LC1 LT(50) = 10.95
LC5 LT(50) = 10.39
LC10 LT(50) = 8.16
LC15 LT(50) = 8.36
LC20 LT(50) = 8.32
LC30 LT(50) = 9.63
χ2 = 22.323, df = 6, P < 0.001
c
H = 57.071
df = 6
P < 0.001
Deltamethrin
Temperature 20ºC
d
Deltamethrin
Temperature 20ºC
Control LT(50) = 12.23
LC1 LT(50) = 14.07
LC5 LT(50) = 11.04
LC10 LT(50) = 11.98
LC15 LT(50) = 12.02
LC20 LT(50) = 12.95
LC30 LT(50) = 9.91
χ2 = 31.622, df = 6, P < 0.001
e
H = 133.048
df = 6
P < 0.001
Deltamethrin
Temperature 25ºC
f
Deltamethrin
Temperature 25ºC
Control LT(50) = 5.35
LC1 LT(50) = 5.39
LC5 LT(50) = 6.30
LC10 LT(50) = 5.93
LC15 LT(50) = 4.12
LC20 LT(50) = 3.98
LC30 LT(50) = 5.18
χ2 = 38.545, df = 6, P < 0.001
g
H = 31.165
df = 6
P < 0.001
Deltamethrin
Temperature 28ºC
h
Deltamethrin
Temperature 28ºC
Control LT(50) = 4.08
LC1 LT(50) = 5.04
LC5 LT(50) = 5.00
LC10 LT(50) = 5.02
LC15 LT(50) = 5.02
LC20 LT(50) = 5.09
LC30 LT(50) = 4.48
χ2 = 31.908, df = 6, P < 0.001

FIGURE 3

Table 1. Sublethal concentrations of chlorpyrifos and deltamethrin used under varying temperatures

Chlorpyrifos		Deltamethrin	
LCs	**Active ingredient (mg/ml)**	**LCs**	**Active ingredient (mg/ml)**
15°C		**15°C**	
LC_1	0.002164	LC_1	0.000658
LC_5	0.003269	LC_5	0.001137
LC_{10}	0.004074	LC_{10}	0.001522
LC_{15}	0.004725	LC_{15}	0.001853
LC_{20}	0.005317	LC_{20}	0.002167
LC_{30}	0.006443	LC_{30}	0.002795
20°C		**20°C**	
LC_1	0.001677	LC_1	0.000932
LC_5	0.002581	LC_5	0.001497
LC_{10}	0.003247	LC_{10}	0.001927
LC_{15}	0.003791	LC_{15}	0.002284
LC_{20}	0.004288	LC_{20}	0.002616
LC_{30}	0.005240	LC_{30}	0.003261
25°C		**25°C**	
LC_1	0.000064	LC_1	0.001227
LC_5	0.000170	LC_5	0.001890
LC_{10}	0.000286	LC_{10}	0.002379
LC_{15}	0.000407	LC_{15}	0.002779
LC_{20}	0.000537	LC_{20}	0.003144
LC_{30}	0.000846	LC_{30}	0.003844
28°C		**28°C**	
LC_1	0.000194	LC_1	0.000090
LC_5	0.000393	LC_5	0.000190
LC_{10}	0.000573	LC_{10}	0.000283
LC_{15}	0.000738	LC_{15}	0.000370
LC_{20}	0.000903	LC_{20}	0.000457
LC_{30}	0.001254	LC_{30}	0.000647

LCs: lethal concentrations.

Table 2. Relative toxicity of organophosphate and pyrethroid (i.e., chlorpyrifos and deltamethrin) to individuals of *Myzus persicae*.

Insecticides	Temperatures	N	LC_{50} (95% CI) mg (a.i)/ml	χ^2	*P*	*TR*
Chlorpyrifos	15°C	100	0.0086 (0.0067 – 0.0105) a	12.97	0.01	5.05
	20°C	100	0.0070 (0.0065 – 0.0076) a	7.06	0.13	4.11
	25°C	100	0.0017 (0.0013 – 0.0022) b	5.55	0.23	-
	28°C	100	0.0020 (0.0017 – 0.0026) b	10.84	0.09	1.17
Deltamethrin	15°C	100	0.0042 (0.0037 – 0.0047) a	7.38	0.19	3.81
	20°C	100	0.0046 (0.0040 – 0.0052) a	6.16	0.18	4.18
	25°C	100	0.0053 (0.0047 – 0.0058) a	4.49	0.10	4.81
	28°C	100	0.0011 (0.0010 – 0.0013) b	5.21	0.26	-

N: number of individuals tested.
LC_{50} (95%): lethal concentration to cause mortality in 50% of individuals
CI: confidence intervals.
a.i.: active ingredient
$\chi 2$: Chi-square for lack-of-fit to the probit model.
P: Probability associated with the chi-square statistic.
TR= calculated by dividing the LC_{50}s of the different temperatures by the smallest LC_{50}

Table 3. Temperature coefficients of chlorpyrifos and deltamethrin on *Myzus persicae* adults.

Chlorpyrifos		
Temperatures	**Temperature coefficients**	
15 – 20°C	1.21	No effect
15 – 25°C	4.94	Slight
15 – 28°C	4.09	Slight
20 – 25°C	4.07	Slight
20 – 28°C	3.38	Slight
25 – 28°C	1.20	No effect
Deltamethrin		
Temperatures	**Temperature coefficients**	
15 – 20°C	1.10	No effect
15 – 25°C	1.25	No effect
15 – 28°C	3.70	Slight
20 – 25°C	1.14	No effect
20 – 28°C	4.08	Slight
25 – 28°C	4.66	Slight

The effects of temperature coefficients were "no effect" = < 2, "slight" = (2-5), and "strong" = > 5

Appendices

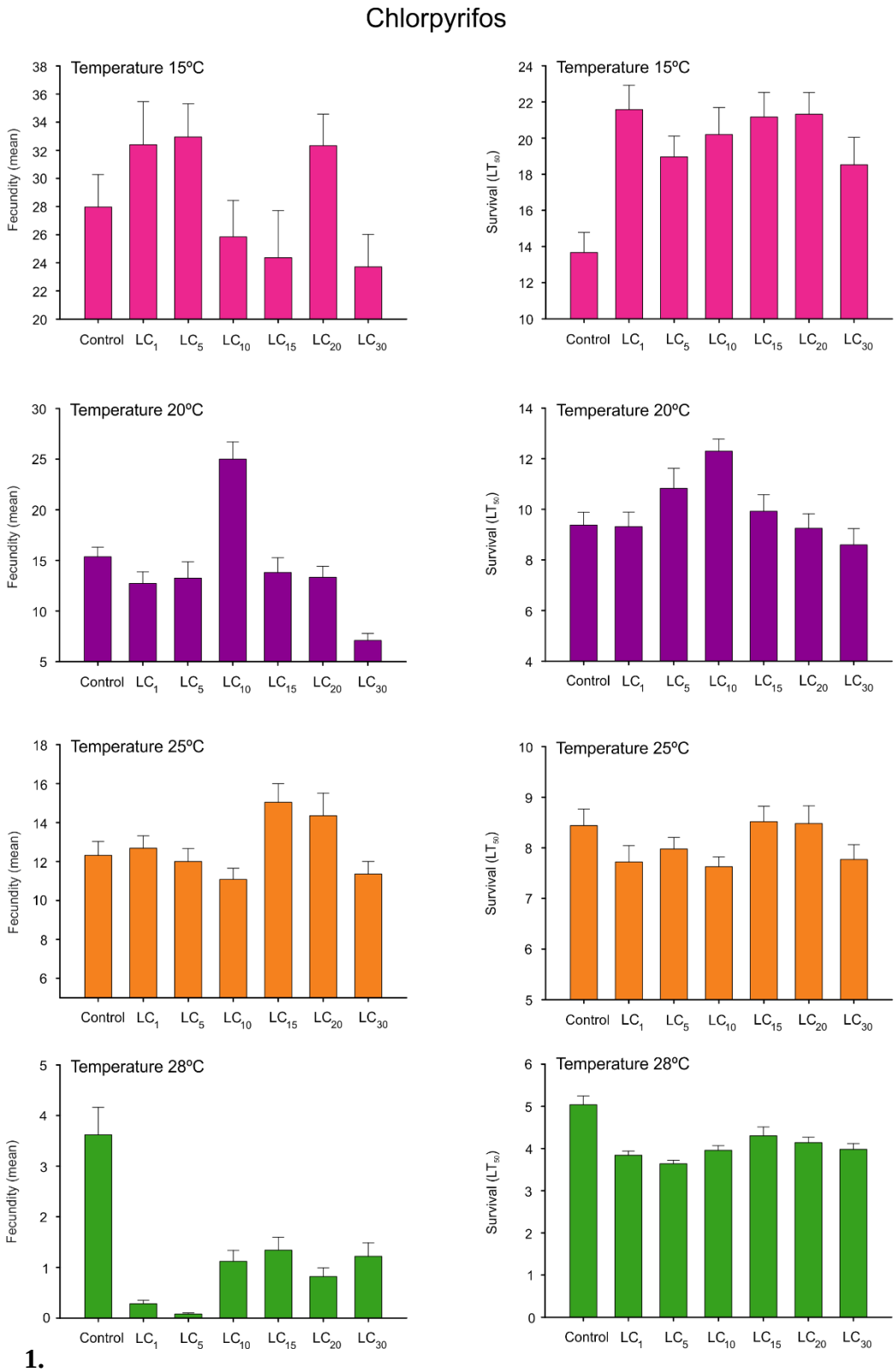


Figure 1.
Mean fecundity and survival (LT_{50}) of *Myzus persicae* females under varying temperatures after exposure to low concentrations of chlorpyrifos

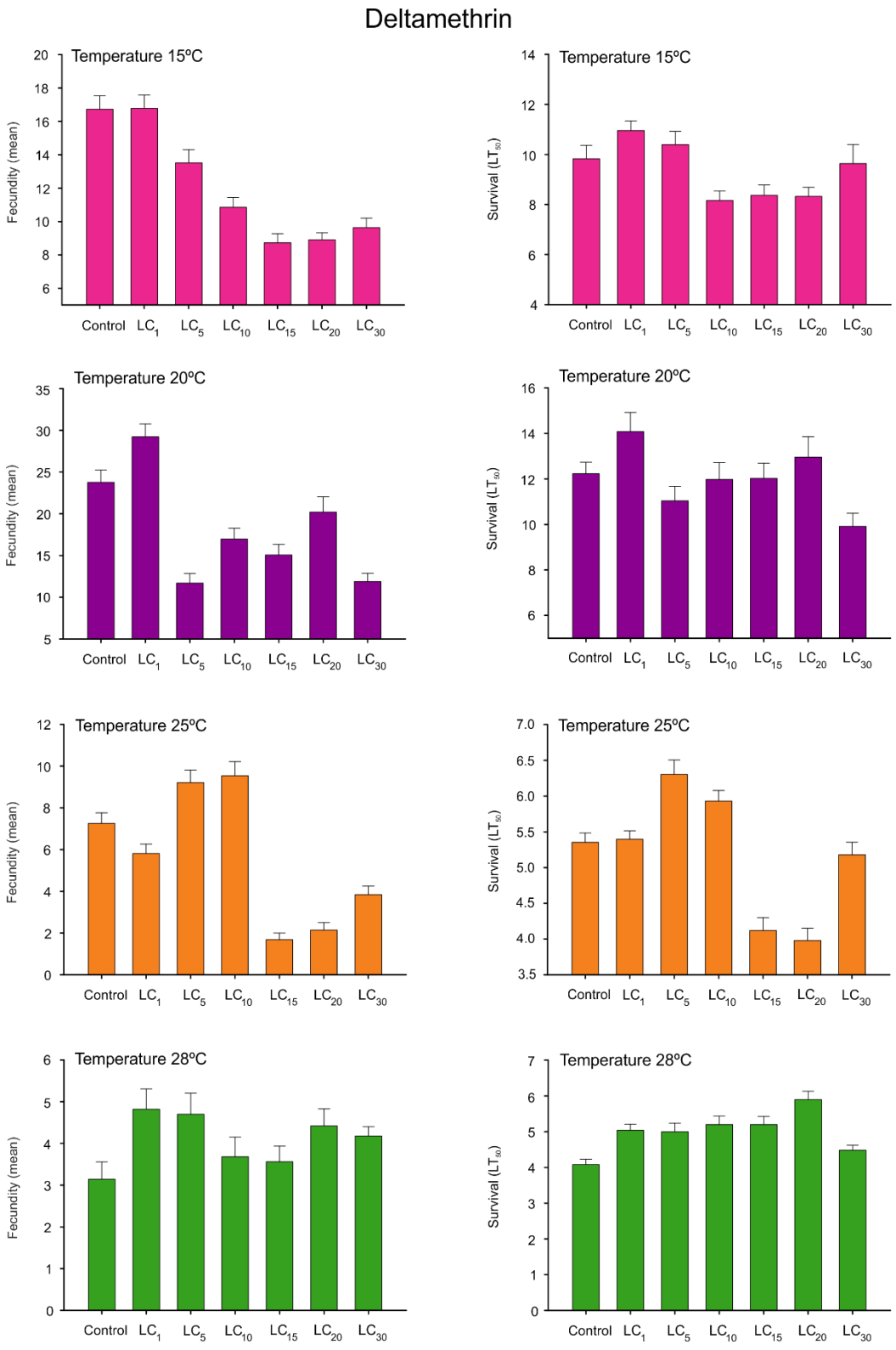


Figure 2. Mean fecundity and survival (LT_{50}) of *Myzus persicae* females under varying temperatures after exposure to low concentrations of deltamethrin.

ARTICLE III

Lethal and Sublethal effects of thiamethoxam and lambda cyhalothrin, used isolated or in mixture, under temperature variations on the biological traits of *Myzus persicae* (Sulzer) (Hemiptera, Aphididae)

Version prepared according to *Ecotoxicology*

Abstract

Myzus persicae (Sulzer) is one of the most destructive and cosmopolitan insects in agroecosystems, and current management of this pest relies mainly on insecticides applications. Given that aphids in agricultural environments are often exposed to sublethal doses of insecticides, the aim of the work was to investigate the lethal and sublethal (LC_1, LC_5, LC_{10}, LC_{15}, LC_{20} and LC_{30}) exposure of a mixture of neonicotinoid and pyrethroid (thiamethoxam + lambda cyhalothrin), and their isolated ingredients under temperature variation (15, 20, 25 and 28°C) on the biological characteristics of *M. persicae*. The results showed that the mixture caused a synergistic effect and induced greater efficacy compared to the active ingredients alone. The toxicity of the mixture on female responses was influenced by the different temperature levels, the high temperature (28°C) induced high toxicity at lower concentrations of thiamethoxam + lambda-cyhalothrin. Stimulatory effects, i.e. hormesis, on reproduction and survival duration of the parental generation were observed in both the mixture and the isolated products under the temperature regimes. Our results contributed to a better understanding of the mechanism of the stimulatory response of *M. persicae* under chemical and thermal stress, and how they affect the biological organization of this species, thus, these findings are useful and could help optimize the use of these insecticides against this pest.

Key words: aphids, biological traits, hormesis, pesticides, toxicology.

Introduction

Advances in various integrated pest management (IPM) programs have been significant in agroecosystems, however, control of *Myzus persicae* (Sulzer) continues to be predominantly accomplished using chemical insecticides (Saljoqi et al. 2009; Bass et al. 2014). The intensive infestation of *M. persicae* in the field causes serious damage to

plants and generates expressive losses in the yield of economically important crops (Van Emden et al. 1969; Zagonel et al. 2002; de Little et al. 2016; Özgökçe et al. 2018). This species feeds on over 400 plant species from about 40 different families, and transmits over 100 plant viruses (Blackman and Eastop 2000; Meng et al. 2014; Van Emden and Harrington 2017). At present, the neonicotinoid and pyrethroid chemical groups are among the most widely used for the control of this species (Nauen and Denholm 2005; Bass et al. 2014; Tang et al. 2017; Sretenovic et al. 2019).

In agricultural areas, insecticides can have variable distribution due to misapplication, drift, and formulation degradation over time (Guedes et al. 2010; Müller 2018; Cutler et al. 2022; Rix and Cutler 2022). As damage, these agrochemicals can cause sublethal exposures in organisms (Dong et al. 2017; Serrão et al. 2022), that is, sublethal doses of insecticides can result in a wide range of effects on the population dynamics of individuals (physiological or behavioral) (Desneux et al. 2007; He et al. 2013; Deng et al. 2016; Ullah et al. 2019a; Fouad et al. 2022).

This case can be defined as hormesis, that is a biphasic phenomenon resulting from low dose stimulation and high dose inhibition following insect exposure to insecticides (Calabrese and Baldwin 2003; Guedes et al. 2022). Hormetic responses in individuals that survive exposure to a toxic compound at sublethal concentrations/doses (Desneux et al. 2007), can cause an improvement in their biological fitness, such as, longevity rate, fecundity, immune capacity, and/or sex ratio (Qu et al. 2015; Lu et al. 2016; Wang et al. 2017a). In the literature, hormesis has been reported in various pest species, such as in aphids (Koo et al. 2015; Lu et al. 2016; Zeng et al. 2016), caterpillars (Dong et al. 2017; Nozad-Bonab et al. 2017), thrips (Cao et al. 2019; Kordestani et al. 2021; Liang et al. 2021), whiteflies (Esmaeily et al. 2014; Qu et al. 2017a; Rakotondravelo et al. 2019). In this view, hormesis is relevant in agricultural systems, because insecticide-induced

hormetic effects may favor species increase, resurgence and/or secondary outbreaks of pests (Guedes et al. 2010; Cutler 2013; Cutler et al. 2022).

In agroecosystems, insecticide mixtures are often used to improve the efficacy and reduce the cost of pest treatment in crops (Abd El-Mageed and Shalaby 2011; Kandil et al. 2022). The use of pyrethroids in combination with neonicotinoid insecticides is common (Stanneck et al. 2012; Wang et al. 2015; Zhu et al. 2017), and among the mixtures that have been used against a variety of pests around the world is lambda-cyhalothrin together with thiamethoxam (Fazolin et al. 2016; Barros et al. 2019; Kambrekar et al. 2021). Wang et al. (2020) showed that combining a lethal dose of thiamethoxam with lambda cyhalothrin showed synergistically increased toxicity to bees, the mortality of the mixture was greater than the sum of the mortalities induced by each insecticide separately.

Thiamethoxam is an insecticide from the neonicotinoid chemical group and acts as an agonist that binds to nicotinic acetylcholine receptors (nAChRs) in the insect nervous system, causing nerve stimulation, paralysis, and death (Cho et al. 2011; Simon 2011; Ullah et al. 2020). Lambda-cyhalothrin is a pyrethroid classified as a potent neurotoxic agent (Dong et al. 2022). Pyrethroids bind to voltage-dependent sodium channel protein, which alters the function of the pore channel, causing repetitive neurological impulses, thus potentially impairing any nerve activity (Haug and Naumann 1990; Narahashi 2002; Simon 2011). Thus, it can be deduced that the activation of the nervous system by neonicotinoids facilitates the interaction of pyrethroids by the complementary mode of action of these two classes of insecticides, which results in this synergistic effect (Taillebois and Thany 2022)

It has been generally recognized that temperature can affect both the population dynamics, development rates, and seasonal occurrence of insects (Campbell et al. 1974; Cocu et al. 2005; Alford et al. 2012; Soh et al. 2018), as well as the activity, performance,

properties of insecticides, and their distribution (Johnson 1990; Horn 2019). (Khan and Akram 2014) evidenced that the toxicity of chlorpyrifos, profenofos, emamectin and fipronil to *Musca domestica* L. revealed a direct relationship with the temperature range tested, and an inverse relationship between temperature and toxicity of cypermethrin, deltamethrin and spinosad was observed. Bearing in mind the need, knowledge of the combination of temperature on the toxicity of insecticides against agricultural pests is of utmost importance for the development of effective pest management plans based on the various seasons of the year.

Most studies have focused on the efficacy of insecticides in controlling pest aphids without taking into consideration how thermal regimes shape their toxicity. In the literature, information is also lacking on how sublethal effects act on the biological characteristics of individuals exposed to mixtures of insecticides and their active ingredients alone under temperature variation. Therefore, the aim of the work was to investigate how sublethal exposure of a mixture of thiamethoxam + lambda-cyhalothrin and their isolated active ingredients, together with temperature variation, affect the behavior of *M. persicae*. The results of this study may be useful for optimizing Integrated Pest Management (IPM) programs and understanding the recorded outbreaks of *M. persicae* in agricultural areas.

Material and Methods

Insect, insecticides and temperature variation

The experiment was carried out in the Laboratory of Molecular Biology and Ecotoxicology (M.E.E.T) at the Entomology Department of UFLA.

The *M. persicae* colony was established from an already established laboratory rearing. Aphid age standardization was performed with about 100 newly hatched nymphs, which

were placed on leaf discs (12 cm diameter) of *Nicandra physalodes* plants under 6% hydrogel, and held for about 8 days. This method was used to ensure that all aphids are the same age (and growth stage) at the beginning of each bioassay. The colony was kept in a climate-controlled chamber (BOD, ELETRO*lab*), with temperature maintained at 20 ± 2°C, relative humidity at 70 ± 10%, and photophase of 16 hours.

The insecticides evaluated were a neonicotinoid and pyrethroid mixture: thiamethoxam + lambda-cyhalothrin (ENGEO PLENO™ S), a neonicotinoid: thiamethoxam (ACTARA® 250 WG), and a pyrethroid: lambda-cyhalothrin (Termimax Lambda 10.6% SC). Serial dilutions were prepared using distilled water containing 0.01% (v/v) Tween 20, and were used immediately after preparation in order to minimize any chemical decomposition.

To evaluate the effects of temperature variation on responses to insecticide exposure, four temperatures were selected: 15ºC, 20ºC, 25ºC, and 28ºC. All bioassays were maintained in a climate-controlled chamber with relative humidity at 70 ± 10% and and photophase of 16 hours.

Temperature coefficients were calculated as the ratio of the highest to lowest LC_{50} values, and were considered positive if toxicity increases with increasing temperature, negative if toxicity decreases with increasing temperature, and no effect if unaffected by increasing temperature (Sparks et al. 1982). The effects of temperature coefficients were "no effect" = < 2, "slight" = (2-5), and "strong" = > 5, following the methodology of (Liu et al. 2016).

Exposure to insecticide by foliar immersion

The evaluation of insecticide toxicity was based on the dose response curve and evaluated by the leaf dipping method proposed by the Insecticide Resistance Action Committee (IRAC 2009).

Initial preliminary tests were conducted to determine the experimental dose range for each insecticide. Next, at least seven concentrations were used to establish dose-response curves with a target mortality ranging from 0% to 100%. Concentrations of the commercial product ranged from $1.2e^{-4}$ to $2.2e^{-2}$ ml/ml for thiamethoxam + lambda-cyhalothrin, from 0.15 to 150 mg/ml for thiamethoxam, and from 0.9 to $9e^{-4}$ ml/ml for lambda-cyhalothrin. The insecticides were diluted with distilled water, and for the control only distilled water was used.

Leaf discs (5.6 cm in diameter) of *Brassica oleraceae* var. acephala were cut and individually dipped for about 6 seconds in the insecticide and control solutions, and then placed at room temperature to dry for about 2 hours. The leaf discs were placed with the abaxial surface upwards in Petri dishes (5.6 cm diameter) under 10% agar, and sealed with plastic film, with several small holes made to allow gas exchange and humidity stabilization.

Five replicates were made for each bioassay, and each replicate was inoculated with 20 adult aphids aged up to 48 hours, totaling 100 adults for each treatment. Aphid mortality was assessed under a magnifying glass (Zeiss Stemi 2000C – Stereo Microscope 1.5x) after 48 hours of the exposure period. Aphids that did not move their legs when touched with a fine brush were considered dead.

Sublethal exposure bioassay of the parental generation

For sublethal exposure, the concentrations LC_1, LC_5, LC_{10}, LC_{15}, LC_{20} and LC_{30} of the insecticides were selected (Table 1). Leaf discs were dipped into the insecticide solutions and the control solution, and were placed in Petri dishes as described above. 100 adult females were casually distributed on the treated leaves.

After 48 hours, 50 females were removed from the Petri dishes and individualized in a Petri dish with a new leaf disc not treated with insecticide. The dishes were filled with 10% agar and sealed with plastic wrap. The leaf discs not treated with insecticide were replaced every 5 days during the experiment, and kept in climate-controlled chambers. The experiment had fifty repetitions for each treatment. The initial fecundity rates of the aphid during the pre-treatments with the sublethal doses of the insecticide were not recorded, because the nymphs were not removed during this period. After exposure, fecundity and longevity of the adults were checked daily during their life. The newly hatched nymphs were counted and removed daily.

Statistics

The mortality rate of adults was corrected for the natural mortality observed in controls (i.e., cabbages treated with distilled water) prior to analysis. Dose-mortality curves were estimated by probit analyses using the PROC PROBIT procedure (SAS Institute, Cary, NC, USA), with a Probit regression method analysis, to obtain 95% confidence intervals. Sublethal concentrations between LC_1 and LC_{30} were calculated using SAS - Statistical Analysis Systems. Fecundity data were subjected to one-way analysis of variance (ANOVA), and survival results were subjected to survival analysis, which was performed using Kaplan-Meier estimators (log-rank method) with SigmaPlot 12.0 (Systat Software, San Jose, CA, USA). The overall similarity between survival times and median survival times (LT_{50} values) was tested using the $\chi 2$ log-rank test, and pairwise comparisons between curves were performed using the Holm-Sidak test ($P < 0.05$).

Results

Exposure to the insecticide by leaf dipping

The toxicity of thiamethoxam + lambda-cyhalothrin, thiamethoxam, and lambda-cyhalothrin to adults of *M. persicae* was investigated 48 hours after exposure to leaf immersion. The effects of treatments on aphid responses to increasing concentrations of insecticides depended on the chemical groups and the different temperatures. The dose-response curves represented in Figure 1 showed the different responses found for each insecticide evaluated.

The toxicity of the mixture (thiamethoxam + lambda-cyhalothrin) at different temperatures on adults of *M. persicae* showed that there was a difference in the LC_{50s} of the different temperatures (Figure 1A – Table 2). It was found that increasing the temperature to 28°C caused the concentration needed to kill 50% of the population (ml/ml) to decrease. For the temperature of 15°C, the mixture showed a $LC_{50}= 8.34x10^{-4}$, for 20°C it showed a $LC_{50}= 5.54x10^{-4}$, for 25°C it obtained a $LC_{50}= 7.14x10^{-4}$, and for 28°C it obtained a $LC_{50}= 4.77x10^{-5}$.

The toxicity of thiamethoxam also showed that there was a difference in the LC_{50} of the temperatures (Figure 1B – Table 2). It was found that the temperature 25°C caused an increase in the concentration (ml/ml) to kill 50% of the population compared to the other temperatures. For the temperature of 15°C, thiamethoxam showed a $LC_{50}= 1.24$, for 20°C it showed a $LC_{50}= 1.29$, for 25°C it obtained a $LC_{50}= 3.16$, and for 28°C it obtained a $LC_{50}= 1.08$.

The toxicity of lambda-cyhalothrin also showed variation in LC_{50} across temperatures (Figure 1C – Table 2). For the temperature of 15°C, lambda cyhalothrin showed a $LC_{50}=$ 0.161 ml/ml, for 20°C it showed a $LC_{50}= 0.163$ ml/ml, for 25°C it obtained a $LC_{50}= 0.078$ ml/ml, and for 28°C it obtained a $LC_{50}= 0.126$ ml/ml.

Table 3 shows the temperature coefficients between the ranges of 15-20°C, 15-25°C, 15-28°C, 20-25°C, 20-28°C and 25-28°C in adult females of *M. persicae* exposed to mixture

(thiamethoxam + lambda-cyhalothrin), thiamethoxam and lambda-cyhalothrin. In the mixture, the ranges between 15-28°C, 20-28°C and 25-28°C showed a strong temperature coefficient, and the other temperatures had no effect. The ranges between 15-25°C, 20-25°C and 25-28°C of thiamethoxam showed mild temperature coefficient, and the other temperatures no effect. And in lambda-cyhalothrin, the ranges between 15-25°C and 20-25°C showed mild temperature coefficient, and the other temperatures no effect.

Parental generation sublethal exposure bioassay

Thiamethoxam + lambda-cyhalothrin

Exposure within 48 hours in *M. persicae* adults to the LCs in the mixture resulted in a significant effect on the longevity and fecundity of exposed individuals (F0 generation) (Figure 2). The fecundity of females with the sublethal doses of the mixture compared to the control at 15°C temperature (Figure 2A) there was no significant difference after exposure at LC5, while on exposure at LC_1, LC_{10}, LC_{15}, LC_{20} and LC_{30} was significantly reduced ($H = 63.270$; $df = 6$; $P < 0.001$). At 20°C temperature (Figure 2C), fecundity was significantly reduced at LC_{15} and LC_{30}, on the other hand, when females were exposed to LC_1 and LC_5 fecundity was significantly increased when compared to the control ($H = 104.993$; $df = 6$; $P < 0.001$). At 25°C (Figure 2E), there was no difference when aphids were exposed to LC_1 and LC_5, and the reduction occurred at LC_{10}, LC_{15}, LC_{20} and LC_{30} ($H = 132.216$; $df = 6$; $P < 0.001$). At 28°C (Figure 2G), the highest fecundity occurred at LC_5 ($H = 25.154$; $df = 6$; $P < 0.001$).

Compared to the control group (11.09 days) at 15°C temperature (Figure 2B), the mean longevity (LT_{50}) of adults was increased by LC_1, LC_{20} and LC_{30} (12.65, 12.23 and 11.92 days respectively), and significantly reduced by LC_5, LC_{10} and LC_{15} (11.04, 10.74 and 9.49 days) ($\chi^2 = 17.709$; $df = 6$; $P < 0.001$). At 20°C temperature (Figure 2D), longevity

increased by LC_5, LC_{20}, LC_1 and LC_{10} (15.20, 15.0, 13.49 and 12.80 days respectively) when compared to the control (11.89 days), on the other hand, longevity decreased significantly by LC_{15} and LC_{30} (10.66 and 8.82 days) (χ^2 = 100.174; *df* = 6; *P* < 0.001). At 25°C temperature (Figure 2F), compared to the control (6.50 days) longevity increased by LC_5 (7.12 days), and significantly reduced at LC_1, LC_{10}, LC_{15}, LC_{20} and LC_{30} (6.45, 5.80, 5.82, 5.02 and 4.79 days) (χ^2 = 45.601; *df* = 6; *P* < 0.001). Finally, at 28°C temperature, the highest mean longevity was at exposure at LC_5 (5.96 days), and longevity decreased at LC_1, LC_{10}, LC_{15}, LC_{20} and LC_{30} (5.43, 5.02, 5.26, 5.17 and 5.58 days) compared to the control (5.83 days) (χ^2 = 17.262; *df* = 6; *P* < 0.001) (Figure 2H).

Thiamethoxam

In relation to the neonicotinoid, 48-hour exposure in *M. persicae* adults to LCs of thiamethoxam also had a significant effect on the longevity and fecundity of exposed individuals (F0 generation) (Figure 3).

The fecundity of females exposed to the sublethal doses of thiamethoxam compared to the control at temperature 15°C was significantly reduced after exposure at LC_{10}, LC_{15}, LC_{20}, and LC_{30}, while concentration LC_1 and LC_5 had no significant difference from the control (H = 213.092; *df* = 6; *P* < 0.001) (Figure 3A). At 20°C temperature (Figure 3C), there was no significant difference at LC_1, on the other hand, when females were exposed to LC_5, LC_{10}, LC_{15}, LC_{20} and LC_{30} fecundity decreased significantly when compared to the control (H = 209.224; *df* = 6; *P* < 0.001). At 25°C temperature (Figure 3E), there was no significant difference when aphids were exposed to LC_1 and LC_5, and the reduction occurred at LC_{10}, LC_{15}, LC_{20} and LC_{30} (H = 248.912; *df* = 6; *P* < 0.001). At 28°C (Figure 2G), fecundity was significantly decreased at all concentrations LC_1, LC_5, LC_{10}, LC_{15}, LC_{20} and LC_{30} (H = 26.289; *df* = 6; *P* < 0.001).

Regarding the average lethal time (LT_{50}), compared to the control group (12.90 days) at 15°C temperature (Figure 3B), the average longevity of adults was increased by LC_1 (13.42 days), and significantly reduced by LC_5, LC_{10}, LC_{15}, LC_{20} and LC_{30} (12.13, 8.89, 7.95, 8.17 and 5.61 days respectively) (χ^2 = 181.261; *df* = 6; *P* < 0.001). At 20°C temperature (Figure 3D), longevity was reduced at all concentrations LC_1, LC_5, LC_{10}, LC_{15}, LC_{20} and LC_{30} (9.42, 9.69, 7.59, 8.61, 8.10 and 5.09 days respectively) when compared to the control (9.95 days) (χ^2 = 105.181; *df* = 6; *P* < 0.001). At 25°C temperature (Figure 3F), compared to the control (6.37 days) longevity increased by LC_1 and LC_5 (7.49 and 7.14 days), and decreased significantly by LC_{10}, LC_{15}, LC_{20} and LC_{30} (4.54, 4.96, 4.50 and 4.50 days) (χ^2 = 103.309; *df* = 6; *P* < 0.001). At 28°C temperature, longevity was reduced at all concentrations LC_1, LC_5, LC_{10}, LC_{15}, LC_{20} and LC_{30} (5.88, 5.24, 5.06, 5.80, 6.02 and 5.12 days respectively) when compared to the control (6.14 days) (χ^2 = 12.915; *df* = 6; *P* < 0.001) (Figure 3H).

Lambda-cyhalothrin

In relation to pyrethroid, 48-hour exposure in *M. persicae* adults to the CLs of lambda-cyhalothrin also had a significant effect on the longevity and fecundity of exposed individuals (F0 generation) (Figure 3).

Fecundity of females with the sublethal doses of lambda-cyhalothrin compared to the control at temperature 15°C (Figure 4A) was significantly reduced after exposure at all concentrations LC_1, LC_5, LC_{10}, LC_{15}, LC_{20} and LC30 (H = 56.611; *df* = 6; *P* < 0.001). At 20°C temperature (Figure 4C) it was also significantly reduced after exposure at all concentrations LC_1, LC_5, LC_{10}, LC_{15}, LC_{20} and LC_{30} (H = 166.873; *df* = 6; *P* < 0.001). At 25°C temperature (Figure 4E), there was no significant difference when aphids were exposed to LC_1, fecundity increased significantly at LC_5 and LC_{30}, and reduction occurred

at LC_{10}, LC_{15} and LC_{20} (H = 100.969; *df* = 6; *P* < 0.001). At 28°C (Figure 4G), fecundity increased significantly at LC_1, LC_5, LC_{10} and LC_{15}, while at concentrations LC_{20} and LC_{30} there was no significant difference from the control (H = 21.352; *df* = 6; *P* < 0.001).

In relation the average lethal time (LT_{50}) compared to the control group (13.13 days) at 15°C temperature (Figure 4B), the average longevity of adults was decreased at all concentrations LC_1, LC_5, LC_{10}, LC_{15}, LC_{20} and LC_{30} (9.02, 9.61, 9.63, 11.55, 7.90 and 8.57 days respectively) (χ^2 = 46.847; *df* = 6; *P* < 0.001). At 20°C temperature (Figure 4D), longevity increased by LC_5 (12.22 days), and decreased by LC_1, LC_{10}, LC_{15}, LC_{20} and LC_{30} (9.34, 10.25, 5.85, 4.38 and 6.43 days respectively) when compared to the control (11.29 days) (χ^2 = 175.192; *df* = 6; *P* < 0.001). At 25°C temperature (Figure 4F), compared to the control (6.46 days) longevity increased at all concentrations LC_1, LC_5, LC_{10}, LC_{15}, LC_{20} and LC_{30} (6.69, 7.81, 7.55, 6.78, 7.19 and 9.11 days respectively) (χ^2 = 28.045; *df* = 6; *P* < 0.001). At 28°C temperature compared to the control (4.64 days) longevity also increased at all concentrations LC_1, LC_5, LC_{10}, LC_{15}, LC_{20} and LC_{30} (6.55, 6.42, 7.22, 5.80, 5.49 and 5.02 days respectively) (χ^2 = 35.608; *df* = 6; *P* < 0.001) (Figure 4H).

Discussion

The use of agrochemicals remains one of the main pest management strategies in agriculture, and while the application of insecticides (lethal doses) can cause rapid death of target pests, the variable distribution and continuous degradation of insecticides can expose insects to reduced doses/concentrations, leading to various sublethal effects, involving physiological and behavioral changes in individuals (Desneux et al. 2004; Guedes et al. 2010; Cutler et al. 2022; Rix and Cutler 2022). Furthermore, using insecticide mixtures is an advantageous way both to decrease the amount of chemicals

used and to delay the emergence of pest resistance mechanisms (Darriet and Chandre 2013; Taillebois and Thany 2016, 2022). In the present study, we investigated the biological consequences of *M. persicae* under different lethal and sublethal concentrations along with different temperatures of a mixture of a neonicotinoid and pyrethroid (thiamethoxam + lamda-cylaothrin) and their isolated active ingredients. It was observed that the aphid responses in the lethal evaluations were due to the synergistic effect of the combination of the insecticides, and by the influence of four different temperature levels on the toxicity of the insecticides (Figure 1). It was also observed that low concentrations of the mixture and its active ingredients alone with heat stress significantly affected the development and reproduction of *M. persicae* (Figure 3 and 4). It is known that the toxicity and relative efficacy of insecticides can vary due to several factors, such as the difference in their mode of action, chemical structure of different active ingredients (Mahmoodi et al. 2020), and also by abiotic factors (temperature), which can affect their physical and chemical properties, such as stability, vaporization, penetration, activity, degradation, absorption and translocation (Johnson 1990). Furthermore, the use of two or more insecticides (with different or similar modes of action) in a mixture can have synergistic or additive effects (Darriet and Chandre 2013; Zhu et al. 2017; Shojaei et al. 2018; Alvim and dos Reis Martinez 2019). In the present study, we clearly observed that by LC_{50} of the mixture of thiamethoxam with lambda cyhalothrin induced greater efficacy on *M. persicae* individuals than when they were applied alone. This synergistic effect found in the present study may be linked to the complementary modes of action of these two classes of insecticides (pyrethroids and neonicotinoids). Pyrethroids act on sodium channels (Haug and Naumann 1990; Field et al. 2017) and neonicotinoids act as agonists of nicotinic acetylcholine receptors (Matsuda et al. 2001), but which also overstimulates voltage-sensitive sodium channels (Bodereau-

Dubois et al. 2012). Pyrethroids are known to preferentially bind to open (activated) sodium channels and keep them open after binding (Vais et al. 2000). Therefore, activation of the nervous system by neonicotinoids facilitates the interaction of pyrethroids with their molecular target (Taillebois and Thany 2022).

The effect of using a mixture of compounds is characterized as synergism (if the combined toxicity is greater than the sum of the toxicity of each individual ingredient), additive (if the combined toxicity is equal to the sum of the toxicity of each individual ingredient), or antagonism (if the combined toxicity is less than the sum of the toxicity of each individual ingredient) (Mahmoodi et al. 2020; Taleh et al. 2021; Taillebois and Thany 2022). Previous investigations have shown that the toxicity of mixtures with thiamethoxam + lambda-cyhalothrin against agricultural pests can show different effects, for example, Kambrekar et al. (2021) evaluated aphid populations in wheat after application of Thiametoxam + Lambda cyhalothrin, Thiamethoxam, Lambda cyhalothrin, Quinalphos and Dichlorvos, and revealed that the mixture was superior in reducing aphid populations during spray schedules. On the other hand, Neto et al. (2019) performed laboratory evaluation of seven insecticide formulations against *M. persicae*, and base on the LC_{50} values showed that chlorfenapyr was the most toxic, followed by lambda-cyhalothrin, pymetrozine, thiamethoxam + lambda-cyhalothrin, thiamethoxam + chlorantraniliprole, thiamethoxam and chlorantraniliprole. In this view, we propose that the toxicity of combinations of neonicotinoids and pyrethroids can be very different depending on the subtypes of the products, mode of application, and target species.

The results of this work indicated that only the mixture showed different responses of aphids under the influence of the four different temperatures (Figure 1 - Table 2). The analysis shows that increasing the temperature (28°C) could be enough to cause a significant increase in the toxicity of the mixture (see the values in Table 2) than the other

temperatures (15, 20 and 25°C). This, in turn, increased the mortality of individuals at lower concentrations of the product at the higher temperature. It is already known in the literature that insecticide toxicity can increase or decrease with increasing temperature (Li et al. 2006; Mansoor et al. 2015). A possible hypothesis that explains this increase in toxicity could be that the synergistic effect of the mixture potentiated a greater penetration of thiamethoxam + lambda cyhalothrin in individuals at higher temperatures. Thus, given the need to obtain information on the relationship between temperature and the toxicity of insecticides of different classes, it is of utmost importance that further studies be conducted to have a better understanding of the uptake and elimination of insecticides with temperature change and how these effects affect the biological processes of *M. persicae*.

Agricultural pests are often exposed to low doses/concentrations of insecticides in agroecosystems due to the variable distribution and continuous degradation of the products (Desneux et al. 2004; Duke 2014; Cutler et al. 2022). Uptake of a small amount of insecticides after exposure to these concentrations may contribute to a beneficial stimulatory effect on fecundity, fertility, longevity, intrinsic rate of increase, finite rate of increase, and net reproductive rate of pest aphids (Calabrese and Baldwin 2003; Cutler 2013; Duke 2014; Sial et al. 2018; Ullah et al. 2019b; Cutler et al. 2022; Rix and Cutler 2022).

This effect is termed as hormesis (a biphasic dose-response phenomenon resulting in low-dose stimulation and high-dose inhibition of after insecticide exposure) (Cutler et al. 2022; Guedes et al. 2022), and several authors have already reported hormesis induced by various insecticides on pest aphids, including *M. persicae* (Rix et al. 2016; Wang et al. 2017a; Sial et al. 2018; Tang et al. 2019), *Aphis gossypii* Glover (Chen et al. 2016; Wang et al. 2017b), *Aphis craccivora* Koch (Fouad et al. 2022), *Aphis glycines*

Matsumura (Qu et al. 2015, 2017b). However, little is known about the sublethal effects of insecticide mixtures and their isolated active ingredients combined with temperature variation on pest aphids, especially on *M. persicae*. The results obtained in the present study showed that exposure of sublethal concentrations of these chemicals combined with temperature variation generated significant stimuli on fecundity and survival in females of *M. persicae*, however, and the hormetic responses of each product varied within the thermal regimes (Figure 2, 3 and 4).

The ability of *M. persicae* to cope with different stressors may be achieved by physiological and biochemical mechanisms. The reason for this is that sublethal exposure to the mixture and its isolated ingredients combined with different temperatures may have caused adaptive responses that increased the cellular defenses of the individuals, and consequently increased performance (reproduction and survival) beyond that observed in untreated individuals. In addition, we observed that as the temperature change occurred, the range of sublethal concentrations that caused the hormetic responses in the organisms shifted. It is worth noting that at 20°C the sublethal effects occurred due to chemical stress, as the aphids were kept at this temperature. In this context, it is important that further studies be conducted to understand why hormetic effects at certain temperatures are directly linked at specific sublethal concentration ranges and how the defense mechanisms of individuals are affected by this relationship between the mixture and its isolated actives combined with temperature.

Some studies corroborate the results found here, Zambrano et al. (2021) evaluated the impact of the application of an insecticide based on a mixture of lambda cyhalothrin and thiamethoxam on pest populations and some natural enemies, and showed that populations of *A. gossypii* were higher in plots treated with lambda cyhalothrin + thiamethoxam. Previous investigations have shown that low application doses of

thiamethoxam and lambda cyhalothrin alone also caused induced hormesis effects on pest populations (Wang et al. 2017a; Sial et al. 2018; Li et al. 2019; Ullah et al. 2020; Zhang et al. 2021; Ju et al. 2022).

As previously stated, temperature can affect both the toxicity and efficacy of chemicals (Johnson 1990), and can have effects on development and behavior in insects (Neven 2000). The present study identified that, the combination of chemical (thiamethoxam + lambda-cyhalothrin, thiamethoxam and lambda-cyhalothrin) and thermal stresses of high temperatures (25 and 28°C) imposed a higher physical and physiological fitness cost on *M. persicae* adults, negatively affecting their performance under fecundity and survival of individuals. Therefore, high temperatures trigger physiological damage in aphid populations, negatively impacting their biological fitness (Barlow 1962; Asin and Pons 2001; Davis et al. 2006; Satar et al. 2008). These results can be explained by the fact that thermal and chemical stress together can be detrimental to embryo development (Harrison and Barlow 1973), and therefore, reduced fecundity may occur and population growth may be retarded in subsequent generations (Wang and Shen 2007; Srigiriraju et al. 2010; Etheridge et al. 2019).

In conclusion, the continuous degradation in agroecosystems of neonicotinoid and pyrethroid insecticides alone or in combination with each other, results in frequent exposure of sublethal concentrations under pest insects. The study of hormesis induced by these insecticides in insects becomes of utmost importance due to its potential implication in pest management, as the adaptive mechanism and stress coping abilities of these insects may contribute to the resurgence of pest insects. In addition, temperature may interact positively or negatively with the expression of tolerance of insecticide-exposed individuals. Therefore, all these results contributed to a better understanding of how pesticide-induced hormesis is influenced by temperature variation, and expanded our

knowledge about the side effects of these pesticides used in the agrosystem. This knowledge can contribute to rationalize the application of insecticides and optimize the control of *M. persicae* populations in agricultural areas.

Conclusions

In the present study, we highlight the fact that the mixture of thiamethoxam + lambda-cyhalothrin caused synergistic effect and induced greater efficacy compared to the active ingredients alone. This may be a relevant and efficient strategy for the control of *M. persicae* populations in agricultural areas.

The toxicity of the mixture on female responses was influenced by the different temperature levels. The high temperature (28°C) induced a high toxicity in lower concentrations of thiamethoxam + lambda-cyhalothrin, while in thiamethoxam and lambda-cyhalothrin used alone, the toxicity was mild and without effect in temperature variation. The knowledge of the correlation of temperature with the toxicity of chemicals is of utmost importance for integrated pest management. The absorption and elimination of insecticides with temperature change can affect the biological processes of individuals.

Exposure of sublethal concentrations of the parental generation in both the mixture and isolated products under the temperature regimes markedly increased the longevity and fecundity of adult females of *M. persicae*. The results exemplify the adaptive nature of hormesis induced by these stressors, affecting levels of biological organization in this species.

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Figures captions

Fig 1 (***a***) Toxicity of thiamethoxam + lambda-cyhalothrin, (***b***) Toxicity of thiamethoxam, (***c***) Toxicity of lambda-cyhalothrin on *Myzus persicae* adults at four temperatures (15, 20, 25 and 28°C). Lines denote the estimated lethal concentration (LC) values based on concentration-mortality bioassays using probit analyses. Symbols show the mean mortality for each insecticide concentration applied to each *M. persicae* population. Vertical bars represent the standard error of the mean (SE)

Fig 2 Effects of sublethal exposure to the neonicotinoid + pyrethroid (thiamethoxam + lambda-cyhalothrin) on fecundity (a, c, e, g) and longevity (b, d, f, h) of *Myzus persicae* females to at 15°C (***a***; ***b***), 20°C (***c***; ***d***), 25°C (***e***; ***f***) and 28 ± 2°C (***g***; ***h***).

Fig 3 Effects of sublethal exposure to the neonicotinoid thiamethoxam on fecundity (a, c, e, g) and longevity (b, d, f, h) of *Myzus persicae* females to at 15°C (***a***; ***b***), 20°C (***c***; ***d***), 25°C (***e***; ***f***) and 28 ± 2°C (***g***; ***h***).

Fig 4 Effects of sublethal exposure to the pyrethroid lambda-cyhalothrin on fecundity (a, c, e, g) and longevity (b, d, f, h) of *Myzus persicae* females to at 15°C (***a***; ***b***), 20°C (***c***; ***d***), 25°C (***e***; ***f***) and 28 ± 2°C (***g***; ***h***).

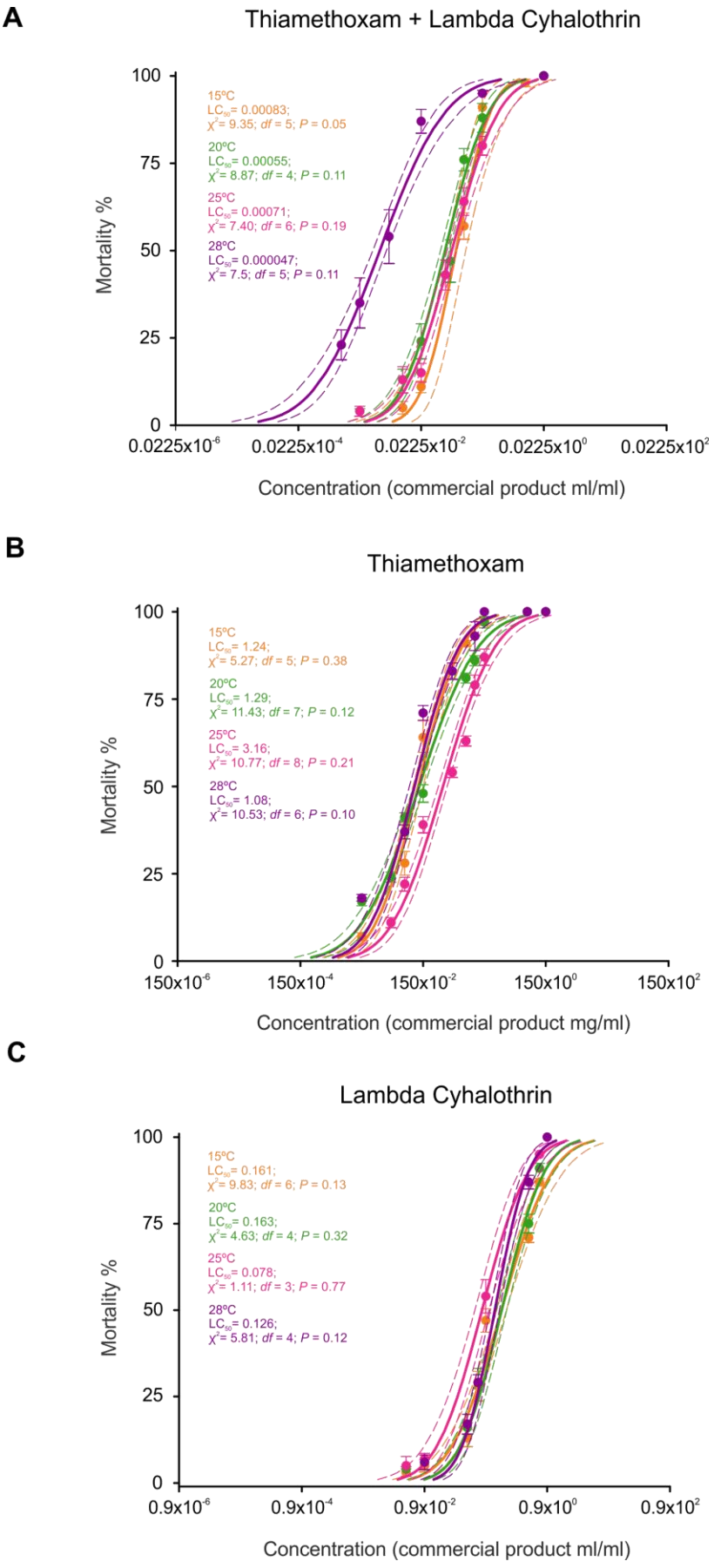
A
Thiamethoxam + Lambda Cyhalothrin
15ºC
LC50= 0.00083;
χ²= 9.35; df = 5; P = 0.05
20ºC
LC50= 0.00055;
χ²= 8.87; df = 4; P = 0.11
25ºC
LC50= 0.00071;
χ²= 7.40; df = 6; P = 0.19
28ºC
LC50= 0.000047;
χ²= 7.5; df = 5; P = 0.11
Mortality %
100
75
50
25
0
0.0225x10-6
0.0225x10-4
0.0225x10-2
0.0225x100
0.0225x102
Concentration (commercial product ml/ml)
B
Thiamethoxam
15ºC
LC50= 1.24;
χ²= 5.27; df = 5; P = 0.38
20ºC
LC50= 1.29;
χ²= 11.43; df = 7; P = 0.12
25ºC
LC50= 3.16;
χ²= 10.77; df = 8; P = 0.21
28ºC
LC50= 1.08;
χ²= 10.53; df = 6; P = 0.10
Mortality %
100
75
50
25
0
150x10-6
150x10-4
150x10-2
150x100
150x102
Concentration (commercial product mg/ml)
C
Lambda Cyhalothrin
15ºC
LC50= 0.161;
χ²= 9.83; df = 6; P = 0.13
20ºC
LC50= 0.163;
χ²= 4.63; df = 4; P = 0.32
25ºC
LC50= 0.078;
χ²= 1.11; df = 3; P = 0.77
28ºC
LC50= 0.126;
χ²= 5.81; df = 4; P = 0.12
Mortality %
100
75
50
25
0
0.9x10-6
0.9x10-4
0.9x10-2
0.9x100
0.9x102
Concentration (commercial product ml/ml)

FIGURE 1

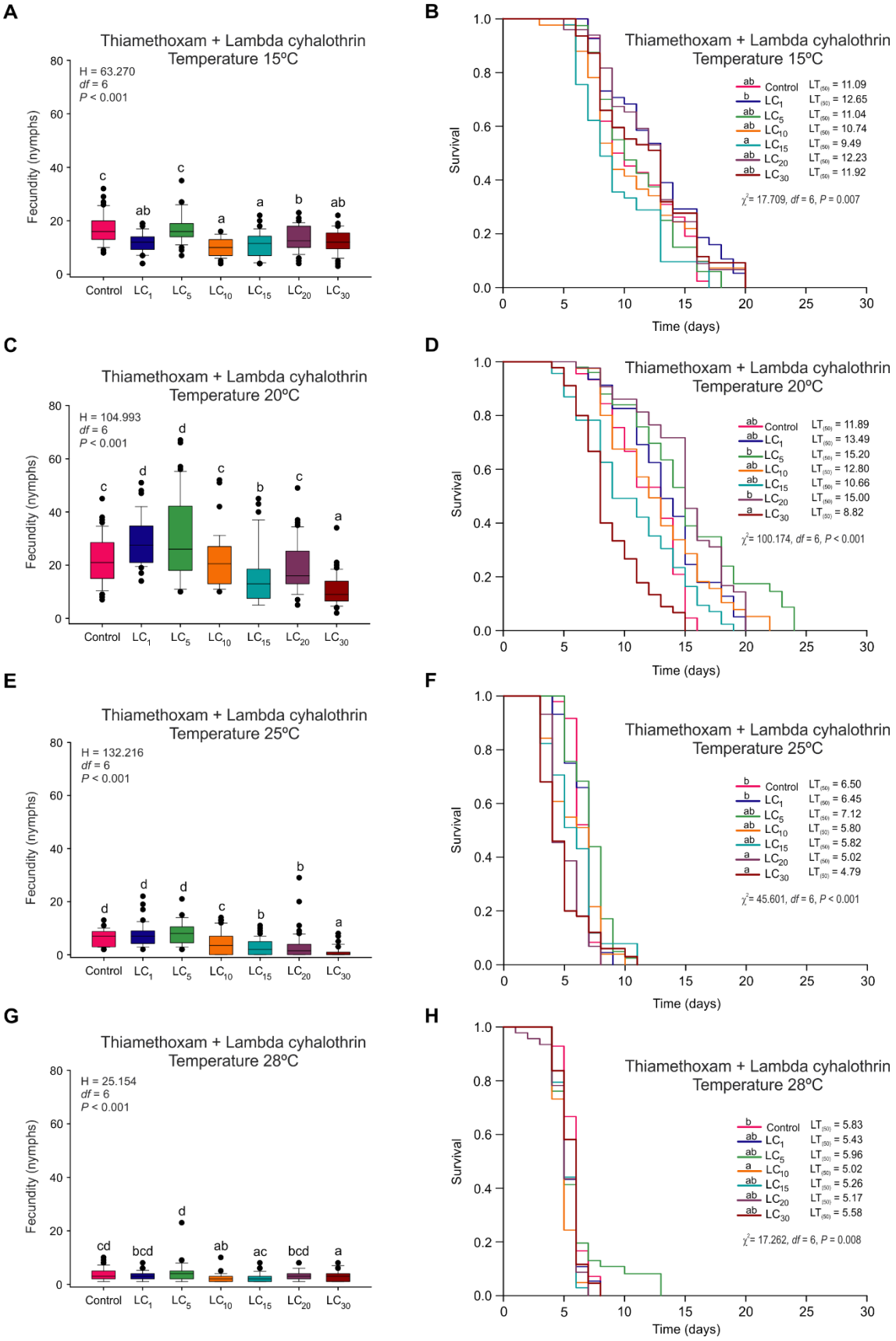
A
Thiamethoxam + Lambda cyhalothrin
Temperature 15ºC
H = 63.270
df = 6
P < 0.001
Fecundity (nymphs)
c
ab
c
a
a
b
ab
Control
LC1
LC5
LC10
LC15
LC20
LC30
B
Thiamethoxam + Lambda cyhalothrin
Temperature 15ºC
Survival
Time (days)
ab Control LT(50) = 11.09
b LC1 LT(50) = 12.65
ab LC5 LT(50) = 11.04
ab LC10 LT(50) = 10.74
a LC15 LT(50) = 9.49
ab LC20 LT(50) = 12.23
ab LC30 LT(50) = 11.92
χ2= 17.709, df = 6, P = 0.007
C
Thiamethoxam + Lambda cyhalothrin
Temperature 20ºC
H = 104.993
df = 6
P < 0.001
Fecundity (nymphs)
c
d
d
c
b
c
a
D
Thiamethoxam + Lambda cyhalothrin
Temperature 20ºC
Survival
Time (days)
ab Control LT(50) = 11.89
ab LC1 LT(50) = 13.49
b LC5 LT(50) = 15.20
ab LC10 LT(50) = 12.80
ab LC15 LT(50) = 10.66
b LC20 LT(50) = 15.00
a LC30 LT(50) = 8.82
χ2= 100.174, df = 6, P < 0.001
E
Thiamethoxam + Lambda cyhalothrin
Temperature 25ºC
H = 132.216
df = 6
P < 0.001
Fecundity (nymphs)
d
d
d
c
b
b
a
F
Thiamethoxam + Lambda cyhalothrin
Temperature 25ºC
Survival
Time (days)
b Control LT(50) = 6.50
b LC1 LT(50) = 6.45
ab LC5 LT(50) = 7.12
ab LC10 LT(50) = 5.80
ab LC15 LT(50) = 5.82
a LC20 LT(50) = 5.02
a LC30 LT(50) = 4.79
χ2= 45.601, df = 6, P < 0.001
G
Thiamethoxam + Lambda cyhalothrin
Temperature 28ºC
H = 25.154
df = 6
P < 0.001
Fecundity (nymphs)
cd
bcd
d
ab
ac
bcd
a
H
Thiamethoxam + Lambda cyhalothrin
Temperature 28ºC
Survival
Time (days)
b Control LT(50) = 5.83
ab LC1 LT(50) = 5.43
ab LC5 LT(50) = 5.96
a LC10 LT(50) = 5.02
ab LC15 LT(50) = 5.26
ab LC20 LT(50) = 5.17
ab LC30 LT(50) = 5.58
χ2= 17.262, df = 6, P = 0.008

FIGURE 2

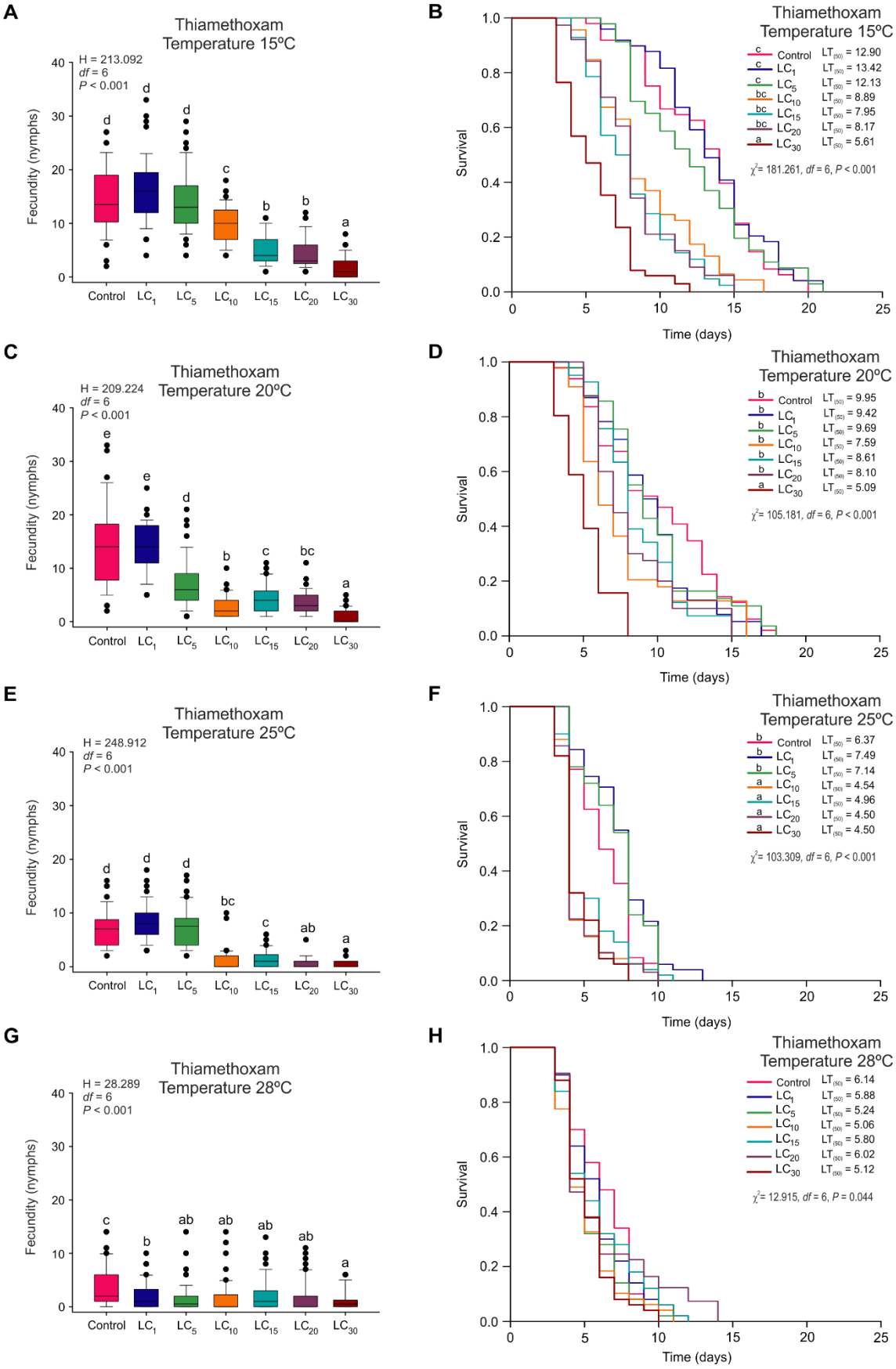
A
Thiamethoxam
Temperature 15ºC
H = 213.092
df = 6
P < 0.001
Fecundity (nymphs)
Control LC1 LC5 LC10 LC15 LC20 LC30
B
Thiamethoxam
Temperature 15ºC
Survival
Time (days)
Control LT(50) = 12.90
LC1 LT(50) = 13.42
LC5 LT(50) = 12.13
LC10 LT(50) = 8.89
LC15 LT(50) = 7.95
LC20 LT(50) = 8.17
LC30 LT(50) = 5.61
χ2= 181.261, df = 6, P < 0.001
C
Thiamethoxam
Temperature 20ºC
H = 209.224
df = 6
P < 0.001
D
Thiamethoxam
Temperature 20ºC
Control LT(50) = 9.95
LC1 LT(50) = 9.42
LC5 LT(50) = 9.69
LC10 LT(50) = 7.59
LC15 LT(50) = 8.61
LC20 LT(50) = 8.10
LC30 LT(50) = 5.09
χ2= 105.181, df = 6, P < 0.001
E
Thiamethoxam
Temperature 25ºC
H = 248.912
df = 6
P < 0.001
F
Thiamethoxam
Temperature 25ºC
Control LT(50) = 6.37
LC1 LT(50) = 7.49
LC5 LT(50) = 7.14
LC10 LT(50) = 4.54
LC15 LT(50) = 4.96
LC20 LT(50) = 4.50
LC30 LT(50) = 4.50
χ2= 103.309, df = 6, P < 0.001
G
Thiamethoxam
Temperature 28ºC
H = 28.289
df = 6
P < 0.001
H
Thiamethoxam
Temperature 28ºC
Control LT(50) = 6.14
LC1 LT(50) = 5.88
LC5 LT(50) = 5.24
LC10 LT(50) = 5.06
LC15 LT(50) = 5.80
LC20 LT(50) = 6.02
LC30 LT(50) = 5.12
χ2= 12.915, df = 6, P = 0.044

FIGURE 3

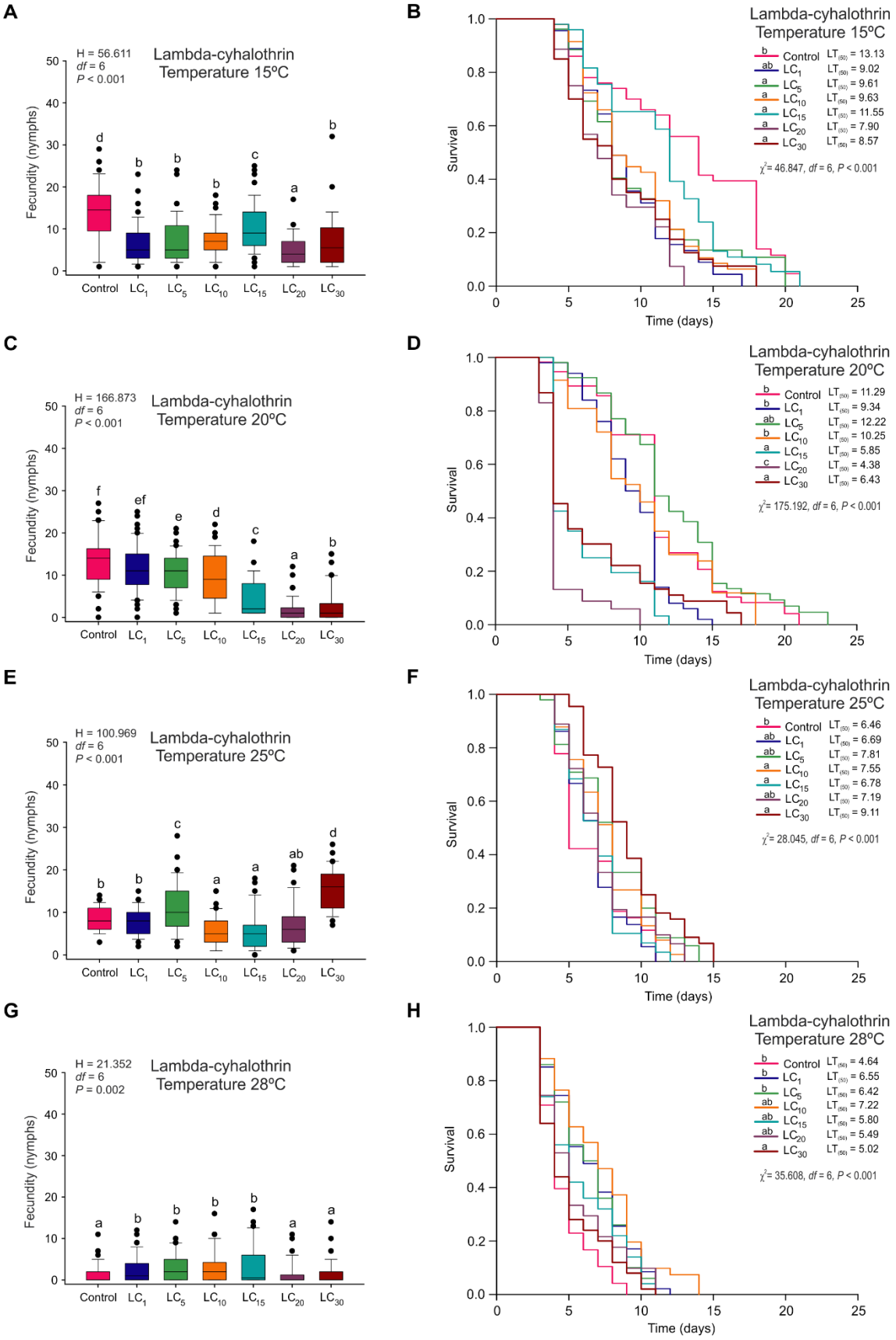
A
H = 56.611
df = 6
P < 0.001
Lambda-cyhalothrin
Temperature 15ºC
Fecundity (nymphs)
Control
LC1
LC5
LC10
LC15
LC20
LC30
B
Lambda-cyhalothrin
Temperature 15ºC
Control LT(50) = 13.13
LC1 LT(50) = 9.02
LC5 LT(50) = 9.61
LC10 LT(50) = 9.63
LC15 LT(50) = 11.55
LC20 LT(50) = 7.90
LC30 LT(50) = 8.57
χ2 = 46.847, df = 6, P < 0.001
Survival
Time (days)
C
H = 166.873
df = 6
P < 0.001
Lambda-cyhalothrin
Temperature 20ºC
D
Lambda-cyhalothrin
Temperature 20ºC
Control LT(50) = 11.29
LC1 LT(50) = 9.34
LC5 LT(50) = 12.22
LC10 LT(50) = 10.25
LC15 LT(50) = 5.85
LC20 LT(50) = 4.38
LC30 LT(50) = 6.43
χ2 = 175.192, df = 6, P < 0.001
E
H = 100.969
df = 6
P < 0.001
Lambda-cyhalothrin
Temperature 25ºC
F
Lambda-cyhalothrin
Temperature 25ºC
Control LT(50) = 6.46
LC1 LT(50) = 6.69
LC5 LT(50) = 7.81
LC10 LT(50) = 7.55
LC15 LT(50) = 6.78
LC20 LT(50) = 7.19
LC30 LT(50) = 9.11
χ2 = 28.045, df = 6, P < 0.001
G
H = 21.352
df = 6
P = 0.002
Lambda-cyhalothrin
Temperature 28ºC
H
Lambda-cyhalothrin
Temperature 28ºC
Control LT(50) = 4.64
LC1 LT(50) = 6.55
LC5 LT(50) = 6.42
LC10 LT(50) = 7.22
LC15 LT(50) = 5.80
LC20 LT(50) = 5.49
LC30 LT(50) = 5.02
χ2 = 35.608, df = 6, P < 0.001

FIGURE 4

Table 1. Sub-lethal concentrations of Lambda-cyalothrin, Thiametoxam and Thiametoxam + Lambda-cyalothrin used under different temperatures (15; 20; 25 and 28 °C)

Thiametoxam + Lambda-cyalothrin 15°C		**Thiametoxam 15°C**		**Lambda-cyalothrin 15°C**	
Lethal concentration (LC)	**Commercial product ml/ml**	**Lethal concentration (LC)**	**Commercial product ml/ml**	**Lethal concentration (LC)**	**Commercial product ml/ml**
LC_1	0.0000778	LC_1	0.062	LC_1	0.005
LC_5	0.0001559	LC_5	0.149	LC_5	0.013
LC_{10}	0.0002259	LC_{10}	0.238	LC_{10}	0.023
LC_{15}	0.0002900	LC_{15}	0.327	LC_{15}	0.034
LC_{20}	0.0003537	LC_{20}	0.421	LC_{20}	0.046
LC_{30}	0.0004887	LC_{30}	0.633	LC_{30}	0.074
Thiametoxam + Lambda-cyalothrin 20°C		**Thiametoxam 20°C**		**Lambda-cyalothrin 20°C**	
Lethal concentration (LC)	**Commercial product ml/ml**	**Lethal concentration (LC)**	**Commercial product ml/ml**	**Lethal concentration (LC)**	**Commercial product ml/ml**
LC_1	0.00002700	LC_1	0.022	LC_1	0.008
LC_5	0.00006547	LC_5	0.073	LC_5	0.020
LC_{10}	0.00010507	LC_{10}	0.139	LC_{10}	0.032
LC_{15}	0.00014445	LC_{15}	0.213	LC_{15}	0.044
LC_{20}	0.00018585	LC_{20}	0.298	LC_{20}	0.056
LC_{30}	0.00028057	LC_{30}	0.519	LC_{30}	0.084
Thiametoxam + Lambda-cyalothrin 25°C		**Thiametoxam 25°C**		**Lambda-cyalothrin 25°C**	
Lethal concentration (LC)	**Commercial product ml/ml**	**Lethal concentration (LC)**	**Commercial product ml/ml**	**Lethal concentration (LC)**	**Commercial product ml/ml**
LC_1	0.00002857	LC_1	0.090	LC_1	0.003
LC_5	0.00007312	LC_5	0.255	LC_5	0.008
LC_{10}	0.00012105	LC_{10}	0.445	LC_{10}	0.013
LC_{15}	0.00016987	LC_{15}	0.648	LC_{15}	0.019
LC_{20}	0.00022252	LC_{20}	0.874	LC_{20}	0.024

LC_{30}	0.00034537	LC_{30}	1.419	LC_{30}	0.038
Thiametoxam + Lambda-cyalothrin 28°C		**Thiametoxam 28°C**		**Lambda-cyalothrin 28°C**	
Lethal concentration (LC)	**Commercial product ml/ml**	**Lethal concentration (LC)**	**Commercial product ml/ml**	**Lethal concentration (LC)**	**Commercial product ml/ml**
LC_1	0.0000005	LC_1	0.051	LC_1	0.012
LC_5	0.0000019	LC_5	0.124	LC_5	0.024
LC_{10}	0.0000038	LC_{10}	0.201	LC_{10}	0.035
LC_{15}	0.0000062	LC_{15}	0.277	LC_{15}	0.045
LC_{20}	0.0000092	LC_{20}	0.358	LC_{20}	0.055
LC_{30}	0.0000170	LC_{30}	0.543	LC_{30}	0.075

Table 2. Relative toxicity of neonicotinoid and pyrethroid (i.e., thiametoxam + lambda-cyhalothrin, thiametoxam and lambda-cyhalothrin) to individuals of *Myzus persicae*.

Insecticides	**Temperatures**	**N**	**LC_{50} (95% CI) ml /ml**	**χ^2**	***P***	***TR***
Thiametoxam	15°C	100	0.00083 (0.00055– 0.0012) a	9.35	0.05	176.59
+ Lambda-	20°C	100	0.00055 (0.00046 – 0.00065) a	8.87	0.11	117.02
cyhalothrin	25°C	100	0.00071 (0.00060 – 0.00085) a	7.50	0.19	151.06
	28°C	100	0.0000047 (0.000036 – 0.000061) b	7.5	0.11	-
Insecticides	**Temperatures**	**N**	**LC_{50} (95% CI) ml/ml**	**χ^2**	***P***	***TR***
Thiametoxam	15°C	100	1.24 (1.02– 1.49)	5.27	0.38	1.14
	20°C	100	1.29 (1.06 – 1.56)	11.43	0.12	1.19
	25°C	100	3.16 (2.67 – 3.71)	10.77	0.21	2.92
	28°C	100	1.08 (0.89 – 1.28)	10.53	0.10	-
Insecticides	**Temperatures**	**N**	**LC_{50} (95% CI) ml/ml**	**χ^2**	***P***	***TR***
Lambda-	15°C	100	0.161 (0.135 – 0.195)	9.83	0.13	2.06
cyhalothrin	20°C	100	0.163 (0.134 – 0.197)	4.63	0.32	2.08
	25°C	100	0.078 (0.059 – 0.101)	1.11	0.77	-
	28°C	100	0.126 (0.106 – 0.150)	5.81	0.12	1.61

N: number of individuals tested.
LC_{50} (95%): lethal concentration to cause mortality in 50% of individuals
CI: confidence intervals.
$\chi 2$: Chi-square for lack-of-fit to the probit model.
P: Probability associated with the chi-square statistic.
TR= calculated by dividing the LC_{50}s of the different temperatures by the smallest LC_{50}

Table 3. Temperature coefficients of thiamethoxam + lambda-cyhalothrin, thiamethoxam and lambda-cyhalothrin on *Myzus persicae* adults.

Thiametoxam + Lambda-cyhalothrin		
Temperatures	**Temperature coefficients**	
15 – 20°C	1.50	No effect
15 – 25°C	1.16	No effect
15 – 28°C	17.49	Strong
20 – 25°C	1.28	No effect
20 – 28°C	11.60	Strong
25 – 28°C	14.96	Strong
Thiametoxam		
Temperatures	**Temperature coefficients**	
15 – 20°C	1.04	No effect
15 – 25°C	2.54	Slight
15 – 28°C	1.14	No effect
20 – 25°C	2.44	Slight
20 – 28°C	1.19	No effect
25 – 28°C	2.92	Slight
Lambda-cyhalothrin		
Temperatures	**Temperature coefficients**	
15 – 20°C	1.01	No effect
15 – 25°C	2.07	Slight
15 – 28°C	1.28	No effect
20 – 25°C	2.09	Slight
20 – 28°C	1.29	No effect
25 – 28°C	1.61	No effect

The effects of temperature coefficients were "no effect" = < 2, "slight" = (2-5), and "strong" = > 5

Supplementary Information (SI)

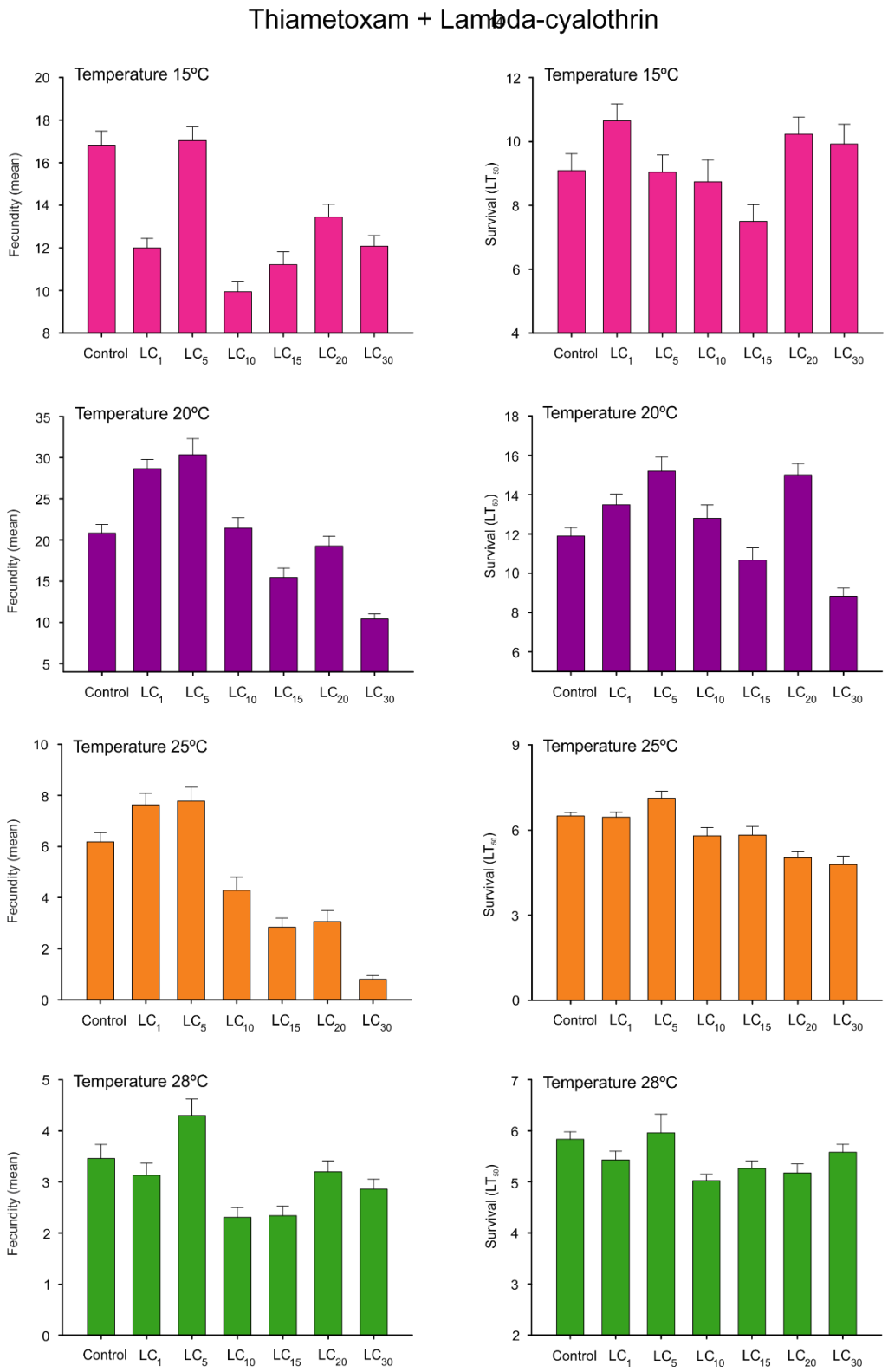

Figure 1. Mean fecundity and survival (LT_{50}) of *Myzus persicae* females under varying temperatures after intoxication with thiametoxam + lambda-cyhalothrin.

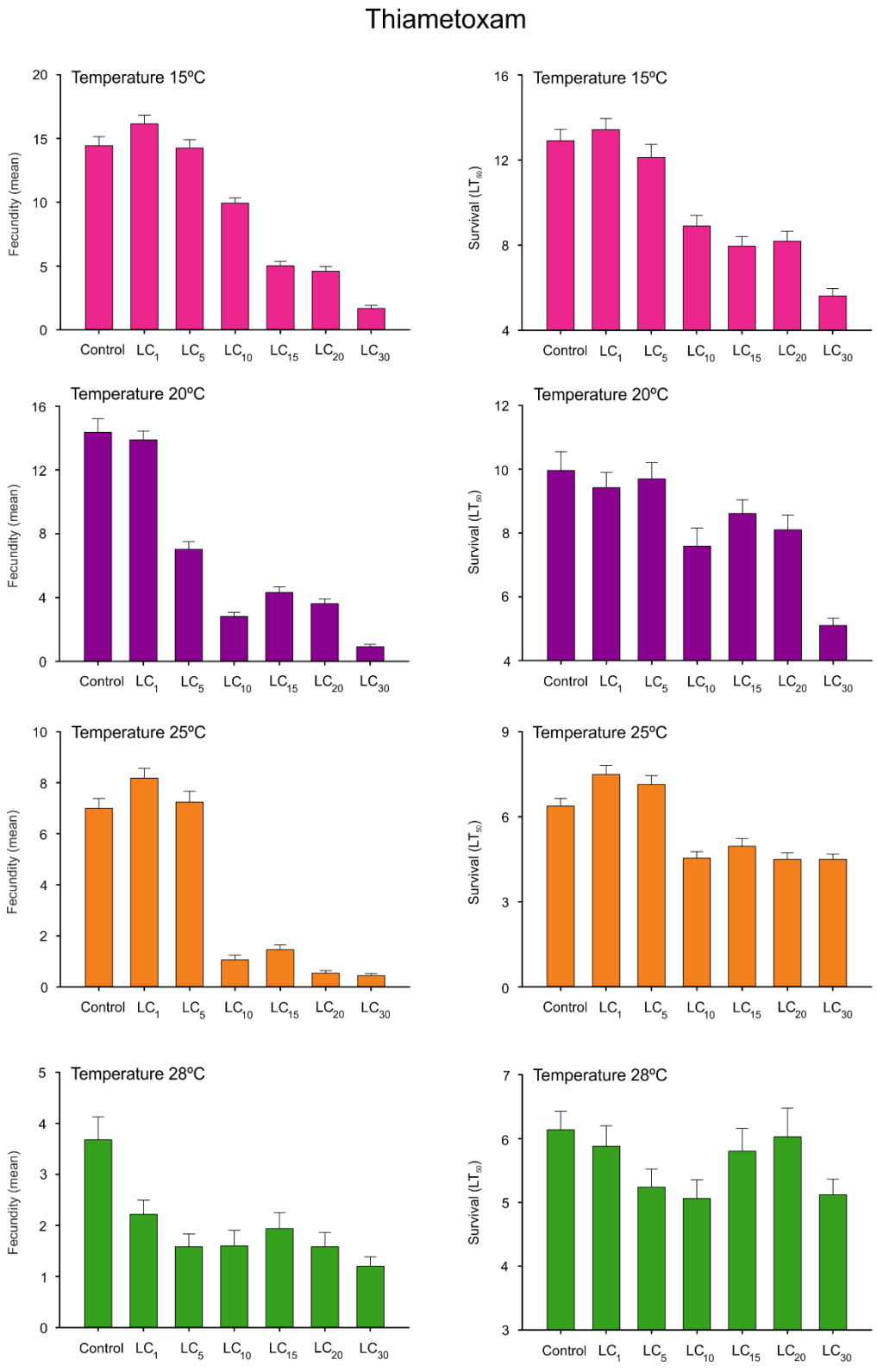

Figure 2. Mean fecundity and survival (LT_{50}) of *Myzus persicae* females under varying temperatures after intoxication with thiametoxam.

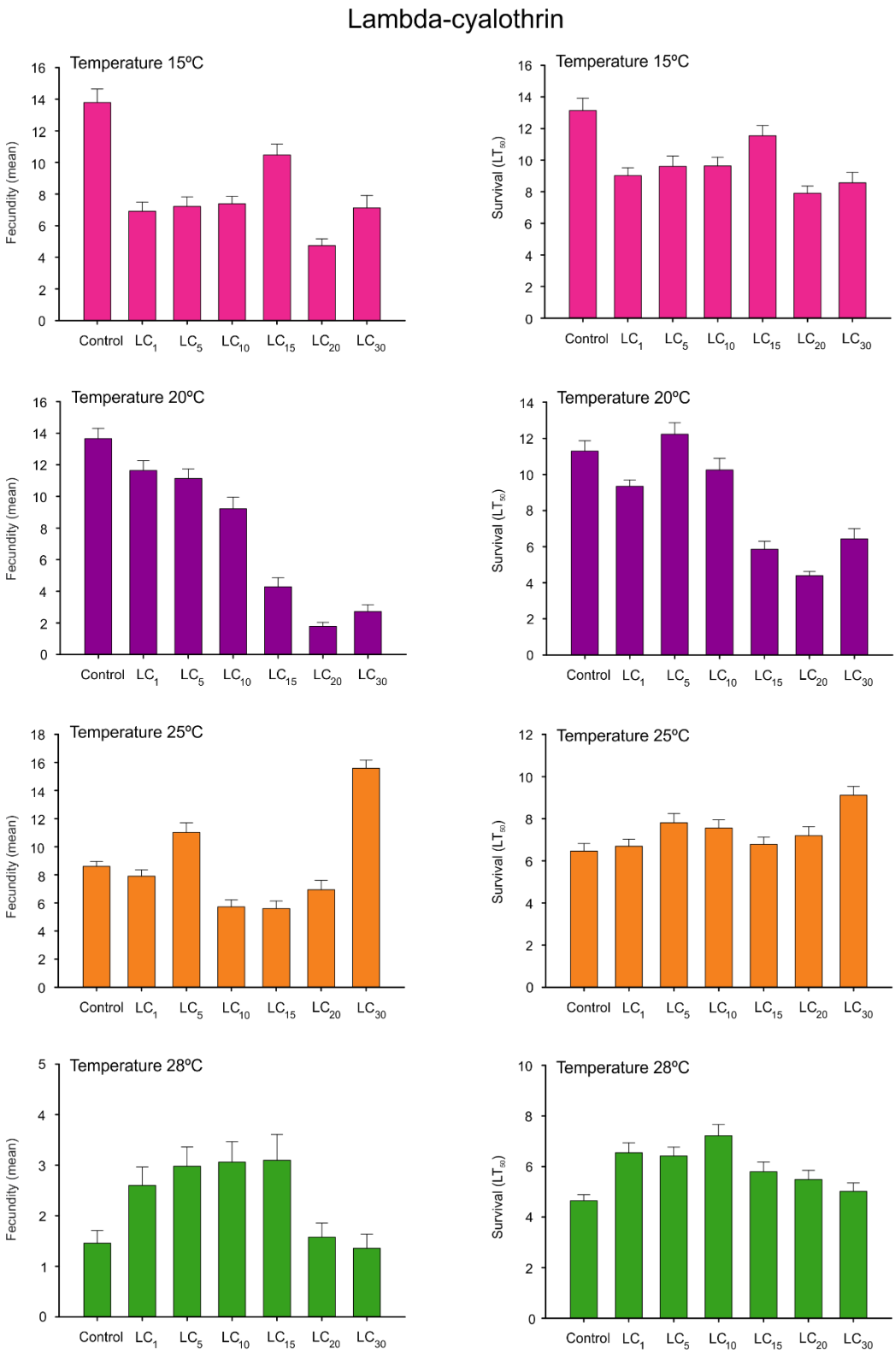

Figure 3. Mean fecundity and survival (LT_{50}) of *Myzus persicae* females under varying temperatures after intoxication with lambda-cyhalothrin.

ARTICLE IV

Lethal, sublethal effects and oxidative stress of imidacloprid on the biological traits of *Myzus persicae* (Sulzer) (Hemiptera, Aphididae) under varying temperatures

Version prepared according to *Science of the Total Environment*.

Abstract

Imidacloprid is a neonicotinoid insecticide widely used to control insect pest aphids, and given the fact that aphids are often exposed to low concentrations of insecticides in agroecosystems, the aim of the work was to investigate the lethal and sublethal (LC_1, LC_5, LC_{10}, LC_{15}, LC_{20} and LC_{30}) exposure of neonicotinoid (imidacloprid) under temperature variation (15, 20, 25 and 28°C), and evaluate the oxidative stress responses to the sublethal concentration of imidacloprid (LC_1) under temperature variation and at two-time intervals (12 and 48 hours), through the content of malondialdehyde (MDA) and H_2O_2, on the biological characteristics of *Myzus persicae* Sulzer (1776) (Hemiptera: Aphididae). The results showed that high temperatures (25 and 28°C) induced higher toxicities at lower concentrations of imidacloprid. Stimulatory effects on reproduction and survival duration of the parental generation were observed in of individuals exposed to low concentrations of imidacloprid, inferring that hormesis in individuals was induced by these stressors. In addition, the accumulation of H_2O_2 and MDA evidenced the occurrence of oxidative stress in adults of this species. Given that the variable distribution and degradation of insecticides in the field result in a range of concentrations over time and space, hormetic responses may have significant implications for the design of control strategies and pest resistance management practices in agricultural areas. Therefore, the findings found in this study contribute to a better understanding of the response mechanism of *M. persicae* under two stressors (temperature and insecticide).

Key words: aphids, hormesis, MDA and H_2O_2, toxicity.

Highlights

- The variation of thermal regimes influenced the toxicity of imidacloprid.
- Exposure of *M. persicae* to mild insecticide stress can result in stimulatory (hormetic) effects.
- Sublethal concentrations of imidacloprid combined with temperature variation induced stimulation in female reproduction and survival.
- Low concentration of imidacloprid (LC_1) combined with variation in temperature regimes accumulated MDA and H_2O_2, causing oxidative stress in individuals of *M. persicae.*

1. Introduction

The green peach aphid, *Myzus persicae* (Sulzer), is an agricultural pest that exhibits wide distribution worldwide, feeding on more than 400 plant species (Blackman and Eastop, 2000; Van Emden and Harrington, 2017). Through its direct feeding, *honeydew* production, and transmission of more than 100 plant viruses, this species generates significant yield losses in economically important crops (Bass et al., 2014; de Little et al., 2016).

In agricultural areas, aphids can be exposed to a wide variety of stressors, from temperature and nutritional stress (Kansman et al., 2020; Soh et al., 2018), to agrochemicals (Tang et al., 2019). Temperature is a key abiotic factor that regulates population dynamics, development rates, and seasonal occurrence of these individuals (ectothermic organisms) (Alford et al., 2012; Baral et al., 2022; Cocu et al., 2005). In addition, temperature is also important in the activity and performance of insecticides used in pest insect management programs (Horn, 2019), it affects insecticide properties such as differences in volatility, product stability, and insect metabolism (Johnson, 1990).

This relationship can be seen in the study by Swelam et al. (2022), which reported that increased temperature caused greater toxicity of fipronil against the larvae of *Spodoptera littoralis* (Boisd.).

Currently, aphid control methods basically focus on the use of agrochemicals, which includes organophosphates, carbamates, pyrethroids, and neonicotinoids (Bass et al., 2014; Devonshire et al., 1998). However, insecticides in the field can be degraded over time by several factors, such as their variable distribution due to misapplication, drift, and degradation of the formulation over time (Cutler et al., 2022; Guedes et al., 2010; Müller, 2018; Rix and Cutler, 2022). Individuals that are exposed to sublethal doses of pesticides can manisfest various effects on their population dynamics, whether physiological or behavioral (Deng et al., 2016; Desneux et al., 2007; He et al., 2013; Ullah et al., 2019). These effects characterized as hormesis, can cause an improvement in the biological fitness (longevity, fecundity, immune capacity, and/or sex ratio) of individuals (Lu et al., 2016; Qu et al., 2015; P. Wang et al., 2017).

Neonicotinoids, are modulators of nicotinic acetylcholine receptors (nAChRs), and effectively control *M. persicae* (Wang et al., 2016; Watson et al., 2011). After its introduction into the field, imidacloprid has become the main product used to control sucking insect pests, and is the most popular insecticide in the world (Bass et al., 2015; Cui et al., 2016). Several previous studies have investigated the effects of sublethal doses of imidacloprid on life traits of *M. persicae* (Christopher Cutler et al., 2009; Janmaat et al., 2011; Rix et al., 2016; Yu et al., 2010). Zeng et al. (2016) indicated in their study, that the nymphal period, female longevity, TPOP and mean generation time (T) of *M. persicae* were significantly prolonged, when early adults were exposed to LC_{30} of imidacloprid. In this view, hormesis is relevant in agricultural systems, because insecticide-induced

hormetic effects may favor species increase, resurgence and/or secondary outbreaks of pests (Cutler, 2013; Cutler et al., 2022; Guedes et al., 2010).

All aerobic organisms are subjected to the production of reactive oxygen species (ROS) as metabolic products, and in general, ROS production and antioxidant extraction processes are in balance (Boardman et al., 2012). However, heat stress and insecticide exposure can upset this balance, generating oxidative stress and causing accumulation of high levels of ROS, resulting in lipid peroxidation (LPO) through destruction of cellular lipids (Dong et al., 2022; Khurshid et al., 2021; Lalouette et al., 2011; Zhang et al., 2022). The most common ROS include superoxide ($O2^{-}$), hydrogen peroxide (H2O2), and hydroxyl radical (·OH) (Jones and Sies, 2007; Sharma et al., 2012). Hence, controlled modulation of ROS levels in organisms is extremely important, as oxidative stress responses are implicated in modulating hormetic responses. Khurshid et al. (2021) reported that temperature changes affect the activities of antioxidant enzymes, high temperature stress in *M. persicae* resulted in increased lipid damage by ROS.

In the context of all this, the knowledge of the combination of temperature on the toxicity of insecticides against aphids is of utmost importance in making pest management decisions, therefore, there is a need for more precise studies to provide functional evidence that may be crucial to define the magnitude and mutual influence of different stressors (thermal and chemical) on the defense mechanisms used by *M. persicae*. Therefore, the aim of the work was to investigate how lethal and sublethal exposure of imidacloprid together with temperature variation affect the behavior of *M. persicae*. In addition, our goal was also to provide a clearer understanding of the patterns and manifestation of oxidative stress in the hormetic responses of these individuals under two stressors (thermal and chemical).

2. Material and Methods

2.1.Insect, insecticide and temperature variation

The experiment was carried out in the Laboratory of Molecular Biology and Ecotoxicology (M.E.E.T) at the Entomology Department of UFLA.

The *M. persicae* colony was established from an already established laboratory rearing. Aphid age standardization was performed with about 100 newly hatched nymphs, which were placed on leaf discs (12 cm diameter) of *Nicandra physalodes* plants under 6% hydrogel, and held for about 8 days. This method was used to ensure that all aphids are the same age (and growth stage) at the beginning of each bioassay. The colony was kept in a climate-controlled chamber (BOD, ELETRO*lab*), with temperature maintained at 20 ± 2°C, relative humidity at 70 ± 10%, and photophase of 16 hours.

The class of insecticide evaluated was the neonicotinoid imidacloprid (Evidence 700 WG). Serial dilutions were prepared using distilled water containing 0.01% (v/v) Tween 20, and were used immediately after preparation in order to minimize any chemical decomposition.

To evaluate the effects of temperature variation on responses to insecticide exposure, four temperatures were selected: 15ºC, 20ºC, 25ºC, and 28ºC. All bioassays were maintained in a climate-controlled chamber with relative humidity at 70 ± 10% and and photophase of 16 hours.

Temperature coefficients were calculated as the ratio of the highest to lowest LC_{50} values, and were considered positive if toxicity increases with increasing temperature, negative if toxicity decreases with increasing temperature, and no effect if unaffected by increasing temperature (Sparks et al., 1982). The effects of temperature coefficients were "no effect" = < 2, "slight" = (2-5), and "strong" = > 5, following the methodology of (Liu et al. 2016).

2.2. Exposure to insecticide by foliar immersion

The evaluation of insecticide toxicity was based on the dose response curve and evaluated by the foliar immersion method proposed by the Insecticide Resistance Action Committee (IRAC, 2009).

Initial preliminary tests were conducted to determine the experimental dose range for insecticide. Next, at least seven concentrations were used to establish dose-response curves with a target mortality ranging from 0% to 100%. Concentrations of the active ingredient ranged from 2.8e-^{4} to 2.8 mg/ml for imidacloprid. The insecticide was diluted with distilled water, and for the control only distilled water was used.

Leaf discs (5.6 cm in diameter) of *Brassica oleraceae* var. *acephala* were cut and individually dipped for about 6 seconds in the insecticide and control solutions, and then were placed at room temperature to dry, for about 2 hours. The leaf discs were placed with the abaxial surface downward in Petri dishes (5.6 cm diameter) under 10% agar, and sealed with plastic film, with several small holes made to allow gas exchange and humidity stabilization.

Five replicates were made for each bioassay, and each replicate was inoculated with 20 adult aphids aged up to 48 hours, totaling 100 adults for each treatment. Aphid mortality was assessed under a magnifying glass (Zeiss Stemi 2000C – Stereo Microscope 1.5x) after 48 hours of the exposure period. Aphids that did not move their legs when touched with a fine brush were considered dead.

2.3. Sublethal exposure bioassay of the parental generation

For sublethal exposure, the concentrations LC_1, LC_5, LC_{10}, LC_{15}, LC_{20} and LC_{30} of the insecticides were selected (Table 1). Leaf discs were dipped into the insecticide solutions

and the control solution, and were placed in Petri dishes as described above. 100 adult females were casually distributed on the treated leaves.

After 48 hours, 50 females were removed from the Petri dishes and individualized in a Petri dish with a new leaf disc not treated with insecticide. The dishes were filled with 10% agar and sealed with plastic wrap. The leaf discs not treated with insecticide were replaced every 5 days during the experiment, and kept in climate-controlled chambers.

The experiment had fifty repetitions for each treatment. The initial fecundity rates of the aphid during the pre-treatments with the sublethal doses of the insecticide were not recorded, because the nymphs were not removed during this period. After exposure, fecundity and longevity of the adults were checked daily during their life. The newly hatched nymphs were counted and removed daily.

2.4. Oxidative stress

2.4.1. Sample Preparation

About 800 adult females up to 48 hours old were placed on leaf discs (12 cm diameter) of *B. oleraceae* plants under 10% agar. The leaf discs were dipped in the insecticide solutions and the control solution, and were placed in Petri dishes as described in subitem 2.4. The sublethal concentration LC_1 of the insecticide Evidence 700 WG was selected and evaluated for each temperature, thus, $3.00x10^{-3}$; $3.70x10^{-3}$; $3.36x10^{-3}$; $1.13x10^{-4}$ and $1.58x10^{-4}$ a.i. mg/ml was used for 15°C, 20°C, 25°C Eq (Average of LC_1 of 15° C and 20°C), 25°C and 28°C respectively.

After 12 and 48 hours of exposure to the insecticide along with temperature, 400 mg of adult aphids were weighed and macerated in liquid N with PVPP and homogenized in 1500 µL of 0.1% Trichloroacetic Acid (TCA). Samples were centrifuged at 12000 g for 15 min at 4°C. The supernatants were collected and reserved at -20°C to start the

biochemical assays. The assays were performed with three replicates for each treatment, containing 0.4 g of adult females per microtube (1.5 ml).

2.4.2. Measurement of malondialdehyde (MDA) and hydrogen peroxide (H_2O_2) content

Malondialdehyde (MDA) content was determined by quantification of lipid peroxidation by the TBARS method, according to the method described by (Buege and Aust 1978). The supernatant (125 µl) was mixed with 250 µl of the mixture of 0.5% (w/v) thiobarbituric acid (TBA) and 10% trichloroacetic acid (TCA) and used for the MDA assay. The absorbance of each sample was measured at 535 nm and corrected for non-specific turbidity by subtracting the absorbance at 600 nm. The values were expressed as ηmol of MDA.g^{-1} fresh material (MF).

Following the protocol of (Velikova et al. 2000) hydrogen peroxide (H_2O_2) was measured. The supernatant (45µl) was collected and reacted with 45µl of 10mM potassium phosphate buffer (pH 7.0) and 90µl of 1M potassium iodide. Absorbance measurements were performed at 390 nm. The level of H_2O_2 production was calculated from a standard curve, using H_2O_2 250 µM, at concentrations from 0 to 45 µmol. The values were expressed as ηmol of H_2O_2.g^{-1}MF.

All experiments were performed in duplicates.

2.5. Statistics

The mortality rate of adults was corrected for the natural mortality observed in controls (i.e., cabbages treated with distilled water) prior to analysis. Dose-mortality curves were estimated by probit analysis using the PROC PROBIT procedure (SAS Institute, Cary, NC, USA), with a Probit regression method analysis, to obtain 95% confidence intervals.

Sublethal concentrations between LC_1 and LC_{30} were calculated using SAS - Statistical Analysis Systems. Fecundity data were subjected to one-way analysis of variance (ANOVA), and survival results were subjected to survival analysis, which was performed using Kaplan-Meier estimators (log-rank method) with SigmaPlot 12.0 (Systat Software, San Jose, CA, USA). The overall similarity between survival times and median survival times (LT_{50} values) was tested using the $\chi 2$ log-rank test, and pairwise comparisons between curves were performed using the Holm-Sidak test ($P < 0.05$).

Analysis of variance (ANOVA) of biochemical results was performed in SigmaPlot 12.0 (Systat Software, San Jose, CA, USA), considering a P value < 0.05 as significant.

3. Results

3.1. Exposure to the insecticide by leaf dipping

The toxicity of imidacloprid to adults of *M. persicae* was investigated 48 hours after exposure to leaf immersion. The mortality of aphids showed significant differences in the LC_{50} of the temperatures. The effects of treatments on aphid responses to increasing concentrations of imidacloprid depended on the different temperatures. In general, higher temperatures induced higher toxicities at lower concentrations. The dose-response curves represented in Figure 1 showed the different responses found for each temperature evaluated.

For the temperature of 15ºC imidacloprid presented a LC_{50}= 0.21 mg a.i /ml, for 20ºC it presented a LC_{50}= 0.08 mg a.i /ml, for 25°C it obtained a LC_{50}= 0.008 mg a.i /ml, and for 28°C it obtained a LC_{50}= 0.01 mg a.i /ml (Figure 1 – Table 2).

Table 3 shows the temperature coefficients between the ranges of 15-20°C, 15-25°C, 15-28°C, 20-25°C, 20-28°C and 25-28°C in adult females of *M. persicae* exposed to imidacloprid. The ranges between 15-25°C, 15-28°C, 20-25°C and 20-28°C showed a

strong temperature coefficient, and the 15-20°C showed a slight temperature coefficient and 25-28°C had no effect.

3.2. Parental generation sublethal exposure bioassay

Exposure within 48 hours in *M. persicae* adults to imidacloprid LCs had a significant effect on the longevity and fecundity of exposed individuals (F0 generation) (Figure 2). Initial aphid fecundity rates during pretreatment with the sublethal doses of the insecticide were not recorded because no nymphs were removed during this period. After exposure, fecundity was assessed daily.

The fecundity of females with the sublethal doses of imidacloprid at 15°C temperature (Figure 2A) was significantly reduced only after exposure to LC_{10}, LC_{15}, LC_{20} and LC_{30}, while LC_1 and LC_5 showed no significant differences compared to the control ($H = 172.507$; $df = 6$; $P < 0.001$). At 20°C temperature (Figure 2C), fecundity was significantly reduced at LC_{20} and LC_{30}, on the other hand, when females were exposed to LC_1 fecundity was significantly increased when compared to the control ($H = 151.891$; $df = 6$; $P < 0.001$). At 25°C (Figure 2E), the reduction in fecundity occurred when the aphids were exposed to LC_{30} ($H = 42.049$; $df = 6$; $P < 0.001$). At 28°C (Figure 2G), fecundity increased significantly at LC_5, LC_{10}, LC_{15} and LC_{20} ($H = 28.987$; $df = 6$; $P < 0.001$).

Compared to the control group (13.06 days) at 15°C temperature (Figure 2B), adult longevity of F0 was not significantly different at concentrations LC_1 and LC_5 (14.15 and 11.85 days), but was significantly reduced when adults were exposed to LC_{10}, LC_{15}, LC_{20} and LC_{30} (7.59; 7.30; 6.81; 5.75 days) ($\chi^2 = 182.098$; $df = 6$; $P < 0.001$). At 20°C temperature (Figure 2D), longevity was significantly reduced at LC_{30} (2.64 days) when compared to the control (6.34 days), on the other hand, when females were exposed to LC_1 and LC_5 longevity was significantly increased compared to the control ($\chi^2 = 143.289$;

df = 6; *P* < 0.001). At 25°C temperature (Figure 2F), female longevity when exposed to LC_{10} was significantly higher (9.08 days) relative to the control (8.36 days), and was significantly reduced at concentrations LC_1 and LC_{30} (7.25 and 7.36 days) (χ^2 = 23.303; *df* = 6; *P* < 0.001). However, no significant difference was found in the longevity of females at the 28°C temperature (χ^2 = 6.564; *df* = 6; *P* < 0.001) (Figure 2H).

3.3. Measurement of malondialdehyde (MDA) and hydrogen peroxide (H_2O_2) contents

The influence of low concentration of imidacloprid at different temperatures (15, 20, 25 Eq, 25 and 28°C) and exposure time (12 and 48h) on malondialdehyde (MDA) and hydrogen peroxide (H_2O_2) contents in *M. persicae* is presented in Figure 3. The results showed that treatment with LC_1 at 15 and 28°C ($2.9x10^{-3}$ and $1.5x10^{-4}$ a.i. mg/ml) led to a higher accumulation of hydrogen peroxide (H_2O_2) (Figure 3A) within 12 hours (1.033 and 1.234 µmol $H_2O_2g^{-1}$MF), whereas in the case of malondialdehyde (MDA) (Figure 3B) the highest accumulation occurred in LC_1 ($1.5x10^{-4}$ a.i. mg/ml) at 28°C (1.00 µmol $MDAg^{-1}$MF).

The hydrogen peroxide (H_2O_2) content (Figure 3C) at the 48 hours exposure showed significant differences, and the highest production occurred in CL_1 ($3.6x10^{-3}$ a.i. mg/ml) at 20°C (0.778 µmol $H_2O_2g^{-1}$MF). The elevation of malondialdehyde (MDA) (Figure 3D) by the insecticide remained strongly evident even in LC_1 at 25°C Eq ($3.2x10^{-3}$ a.i. mg/ml) when exposed to 48h (1.815 µmol $MDAg^{-1}$MF).

4. Discussion

Aphids can develop adaptive mechanisms to survive and reproduce in stressful environments (Kennedy and Stroyan, 1959; Van Emden and Harrington, 2017), but can

undergo behavioral and/or physiological changes by the interaction of various stressors (chemical, physical, or biological) (Hooper et al., 2013; Rix and Cutler, 2022; Sokolova, 2013). The toxicity and sublethal effects of *M. persicae* exposed to imidacloprid is already well established in the literature, however, most of these studies evaluated the effects on the survival, growth and fecundity of this species without taking into account the influence of temperature variation. In this study, we show the effect of toxicity and sublethal concentrations of imidacloprid under temperature variation on the biological traits of *M. persicae* individuals. In addition, we gain a better understanding of how oxidative stress acts in response to heat stress along with the agrochemical in individuals of this species.

It is known that the toxicity of insecticides can vary due to several temperature-dependent factors affecting their physical and chemical properties, such as stability, vaporization, penetration, activity, degradation, absorption, and translocation (Johnson, 1990). The results of the present work showed that the different responses of aphids under imidacloprid toxicity were due to the influence of the four different temperature levels. According to the LC_{50} of the insecticide, high temperatures (25 and 28°C) induced a high toxicity at lower concentrations of the product (Figure 1). The analysis shows that increased temperature (25 and 28°C) may be sufficient to cause a significant increase in the toxicity of imidacloprid (see values in Table 2) than milder temperatures (15 and 20°C). This, in turn, will increase the mortality of individuals at lower concentrations of the product at higher temperatures. The reason for this is that, the neonicotinoid imidacloprid is a systemic, agonist insecticide that activates the nicotinic acetylcholine receptor (nAChR), neurotoxins that can cause a variety of behavioral/physiological effects (Buckingham et al., 1997; Liu and Casida, 1993; Matsuda et al., 2001), and generally exhibit positive temperature coefficient, i.e. the temperature coefficient is

positive when the toxicity of the insecticide increases with increasing temperature (Srigiriraju et al., 2010).

The effect of temperature on the toxicity of various insecticides has already been documented for different insect species (Ma et al., 2012; Rao et al., 2021). Swelam et al. (2022) reported that the toxicity of fipronil to *Spodoptera littoralis* (Boisd.) (Lepidoptera: Noctuidae) was influenced by temperature, i.e., the toxicity of the insecticide was higher at higher temperatures. However, there is little information available on the influence of temperature on the toxicity of imidacloprid to pest aphids, for example in the study by Srigiriraju et al. (2010), the authors evaluated the effects of post-treatment temperature on the efficacy of four types of insecticides against *M. persicae*, and reported that the toxicity of imidacloprid increased with increasing post-exposure temperature, LC_{50} values at 15°C were significantly higher than at temperatures of 20 and 25°C.

Although the relationship between the insecticides and temperature tested in this study was clearly shown, the exact mechanism has not been fully investigated. In this sense, it is of utmost importance that further studies be conducted to have a better understanding of how these effects affect the processes of imidacloprid uptake and elimination with the change of temperature under *M. persicae* individuals.

In agroecosystems, agricultural pests are often exposed to low doses of chemicals due to their variable distribution and continuous degradation (Cutler et al., 2022; Desneux et al., 2004; Duke, 2014). Consequently, this exposure causes various sublethal effects to organisms affecting their biological traits (physical or chemical). This phenomenon can be defined as hormesis, which is characterized by inhibition at high doses and stimulation at low doses following pesticide exposure (Cutler, 2013; Duke, 2014; Rix and Cutler, 2022).

When stimulation occurs at low doses, especially of agrochemicals under insects, the impacts can result in protective effects that can lead to improved performance in the biological fitness of the organism (Berry III and López-Martínez, 2020; Calabrese and Baldwin, 2003; Cutler, 2013; Mattson, 2008; Rix and Cutler, 2022). The most common stimulatory effects observed in insects are in relation to fecundity, fertility, longevity, intrinsic rate of increase, finite rate of increase, and net reproductive rate of individuals (Calabrese and Baldwin, 2003; Shang et al., 2021; Sial et al., 2018; Ullah et al., 2019). Due to this reason, the study of pesticide-induced hormetic responses in insects has become of utmost importance due to its potential in implication in pest management.

Insecticide-induced hormesis on pest aphids, including *M. persicae* (Rix et al., 2016; Sial et al., 2018; Tang et al., 2019; P. Wang et al., 2017), *Aphis gossypii* Glover (Chen et al., 2016; S. Wang et al., 2017), *Aphis craccivora* Koch (Fouad et al., 2022), *Aphis glycines* Matsumura (Qu et al., 2017, 2015) is already well established in the literature. However, little is known about how the sublethal effects of imidacloprid in conjunction with temperature variation interferes with the biological aspects of *M. persicae*. The results obtained in the present study showed that significant changes occurred in fecundity and survival in females of *M. persicae* due to the two combined stressors (thermal and chemical), and the hormetic responses of individuals under the product varied within the thermal regimes (Figure 2).

The ability of *M. persicae* to cope with diverse stressors may be achieved by physiological and biochemical mechanisms. The reason for this is that sublethal exposure to the neonicotinoid at all four temperature levels may have caused adaptive responses that increased the cellular defenses of the individuals, and consequently increased performance (reproduction and survival) beyond that observed in untreated individuals. In addition to this fact, we observed that as the temperature changed, the range of

sublethal concentrations that caused the hormetic responses in the organisms shifted. It is worth noting that at 20°C the sublethal effects occurred due to chemical stress, as the aphids were reared at this temperature. In this scenario, it is important that further studies be conducted to understand why hormetic effects at certain temperatures are directly linked at specific sublethal concentration ranges of the product and how the defense mechanisms of individuals are affected by this relationship between stressors.

The toxicity of chemicals and the biological fitness of insects can be strongly affected by changes in temperature (Johnson, 1990; Neven, 2000). When physiological injuries occur under different stressors, individuals exhibit impacts on their biological characteristics (physical or chemical) during their lifetime (González-Chang et al., 2016; Neven, 2000). In this study, the combination of chemical stress (imidacloprid) and high temperatures (25 and 28°C) imposed a higher physical and physiological fitness cost on this species, decreasing its tolerance and performance. Therefore, high temperatures trigger physiological lesions in aphid populations, negatively impacting their development (Asin and Pons, 2001; Barlow, 1962; Davis et al., 2006; Satar et al., 2008).

As mentioned earlier, aphids are exposed to a wide range of stressors (biotic and abiotic), and among them we can include oxidative stress which is characterized by increased production of reactive oxygen species (ROS), leading to protein entanglement and aggregation within cells and cell death (Kodrík et al., 2015; Mao et al., 2020; Mittler, 2017; Sies, 2017). To prevent damage to DNA, proteins, and lipids caused by ROS, antioxidant enzymes are involved in the response to oxidative damage, such as, superoxide dismutase (SOD), catalase (CAT), and peroxidase (POD) (Berry III and López-Martínez, 2020; Cutler et al., 2022; Garcia-Caparros et al., 2021; Rix and Cutler, 2022). In addition to these enzymes, heat shock proteins (HSP) also play a key role in cellular protection against various stressors (Chen et al., 2018; King and MacRae, 2015).

To test whether imidacloprid exposure together with temperature induces oxidative stress in *M. persicae*, we evaluated hydrogen peroxide (H_2O_2) and malondialdehyde (MDA) levels. Although our study did not include enzymatic tests, we report that the performance of oxidative stress in *M. persicae* individuals is confirmed by the accumulation of hydrogen peroxide (H_2O_2) and malondialdehyde (MDA). Hydrogen peroxide (H_2O_2) is one of the most abundant ROS in cells (Sies, 2017), its presence causes severe oxidative stress, affecting the structure of lipids, proteins, and DNA (Zhang et al., 2019). Hydrogen peroxide (H_2O_2) concentrations increased significantly when 12-hour individuals were exposed to low-dose imidacloprid combined with temperatures considered out of their optimal range (15 and 28°C), indicating a clear effect of the insecticide with temperature through biochemical processes. On the other hand, hydrogen peroxide (H_2O_2) accumulation was significantly higher at 20°C temperature at the 48-hour exposure. Malondialdehyde (MDA) is a byproduct of lipid hydroperoxides, which is widely used as an indicator of oxidative damage to cells (del Rio et al., 2005), we can observe in our work that as the temperature and exposure time increase, a significant increase in lipid peroxidation is observed. Thus, we can state that the stressors (insecticide and temperature) caused oxidative stress in the individuals.

Dong et al. (2022) evaluated the effect of lambda-cyhalothrin on oxidative stress *in M. persicae*, and reported that hydrogen peroxide (H_2O_2) and malondialdehyde (MDA) concentrations increased significantly at all time points after lambda-cyhalothrin treatment, indicating that excess ROS and oxidative stress are caused by the insecticide. Furthermore, they indicated that the induction of a cDNA sequence (MpHsp70) encoding a member of the HSP70 family, showed an important role in aphid defense mechanisms against stressors.

Thus, it is possible that the treatments that resulted in the low levels of hydrogen peroxide (H_2O_2) and malondialdehyde (MDA) found in this study may be due to increased expression or activity of antioxidants and HSP. Increased these activities may protect insects from cellular damage under stressful conditions, including low-dose agrochemicals that are associated with increased longevity, reproduction, and stress tolerance (Calabrese and Baldwin, 2003; Cutler et al., 2022; King and MacRae, 2015; Rix and Cutler, 2022). However, it is of paramount importance to investigate whether there is actually actuation of these protective proteins that maintain homeostasis and cellular function against imidacloprid-induced oxidative stress at the different temperatures. With these results, we will be able to understand the tolerance and adaptability of this species in stressful environments.

In conclusion, the continuous degradation of pesticides in agricultural areas, especially neonicotinoids, results in frequent exposure of sublethal concentrations under pest insects. The study of this insecticide-induced hormesis in insects becomes extremely important due to its potential implication in pest management, since the adaptive mechanism and stress coping abilities of these insects may contribute to the resurgence of pest insects, especially when temperature may interact positively or negatively with the expression of tolerance of insecticide-exposed individuals. Furthermore, we evidenced the occurrence of oxidative stress by the combined effect of imidacloprid and the temperature variation, which consequently caused the unbalance of the biological activities of the individuals. Therefore, all these results contributed to a better understanding of how pesticide-induced hormesis can be influenced by temperature variation. This knowledge can contribute to rationalize the application of insecticides and optimize the control of *M. persicae* populations in agricultural areas.

5. Conclusions

Temperature changes influenced the toxicity of imidacloprid on *M. persicae* individuals, showing that this agrochemical has a positive temperature coefficient. It is very important to know the temperature coefficient of a chemical, as this will allow the selection of more effective products under certain environmental conditions.

Due to the variable distribution and degradation of pesticides in the field, aphids are exposed to low concentrations of the product. The increased survival and fecundity of *M. persicae* subjected to insecticide/temperature stress exemplifies the adaptive nature of hormesis induced by these stressors.

Furthermore, in the present study, we present the first comprehensive evaluation of oxidative stress in this species under two stressors. Imidacloprid together with temperature variation caused accumulation of H_2O_2 and MDA disturbing equilibrium in the individuals, which provides us with strong evidence for the occurrence of oxidative stress.

6. References

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Figures captions

Figure 1. Toxicity of Imidacloprid on *Myzus persicae* adults at four temperatures (15, 20, 25, and 28°C). Lines denote the estimated lethal concentration (LC) values based on concentration-mortality bioassays using probit analyses. Symbols show the mean mortality for each insecticide concentration applied to each *M. persicae* population. Vertical bars represent the standard error of the mean (SE).

Figure 2. Effects of sublethal exposure to the neonicotinoid imidacloprid on fecundity (a, c, e, g) and longevity (b, d, f, h) of *Myzus persicae* females to at 15°C (***a***; ***b***), 20°C (***c***; ***d***), 25°C (***e***; ***f***) and 28 ± 2°C (***g***; ***h***).

Figure 3. The effects of low concentration of imidacloprid on malondialdehyde (MDA) and hydrogen peroxide (H_2O_2) content in *M. persicae* females. 12 h (***A***) and 48 h (***C***) exposure on H_2O_2 quantification. Exposure of 12 h (**B**) and 48 h (***D***) in MDA quantification.

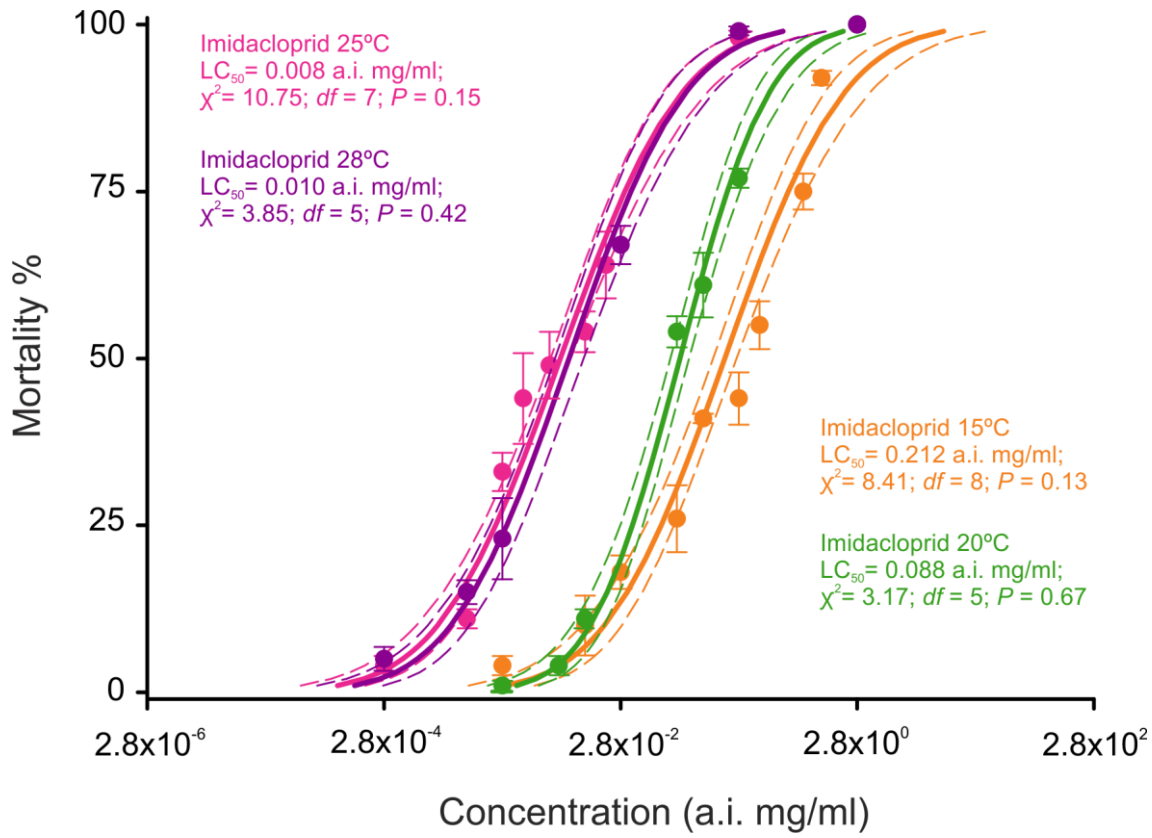
Imidacloprid 25ºC
LC50= 0.008 a.i. mg/ml;
χ2= 10.75; df = 7; P = 0.15
Imidacloprid 28ºC
LC50= 0.010 a.i. mg/ml;
χ2= 3.85; df = 5; P = 0.42
Imidacloprid 15ºC
LC50= 0.212 a.i. mg/ml;
χ2= 8.41; df = 8; P = 0.13
Imidacloprid 20ºC
LC50= 0.088 a.i. mg/ml;
χ2= 3.17; df = 5; P = 0.67
Mortality %
100
75
50
25
0
2.8x10-6
2.8x10-4
2.8x10-2
2.8x100
2.8x102
Concentration (a.i. mg/ml)

FIGURE 1

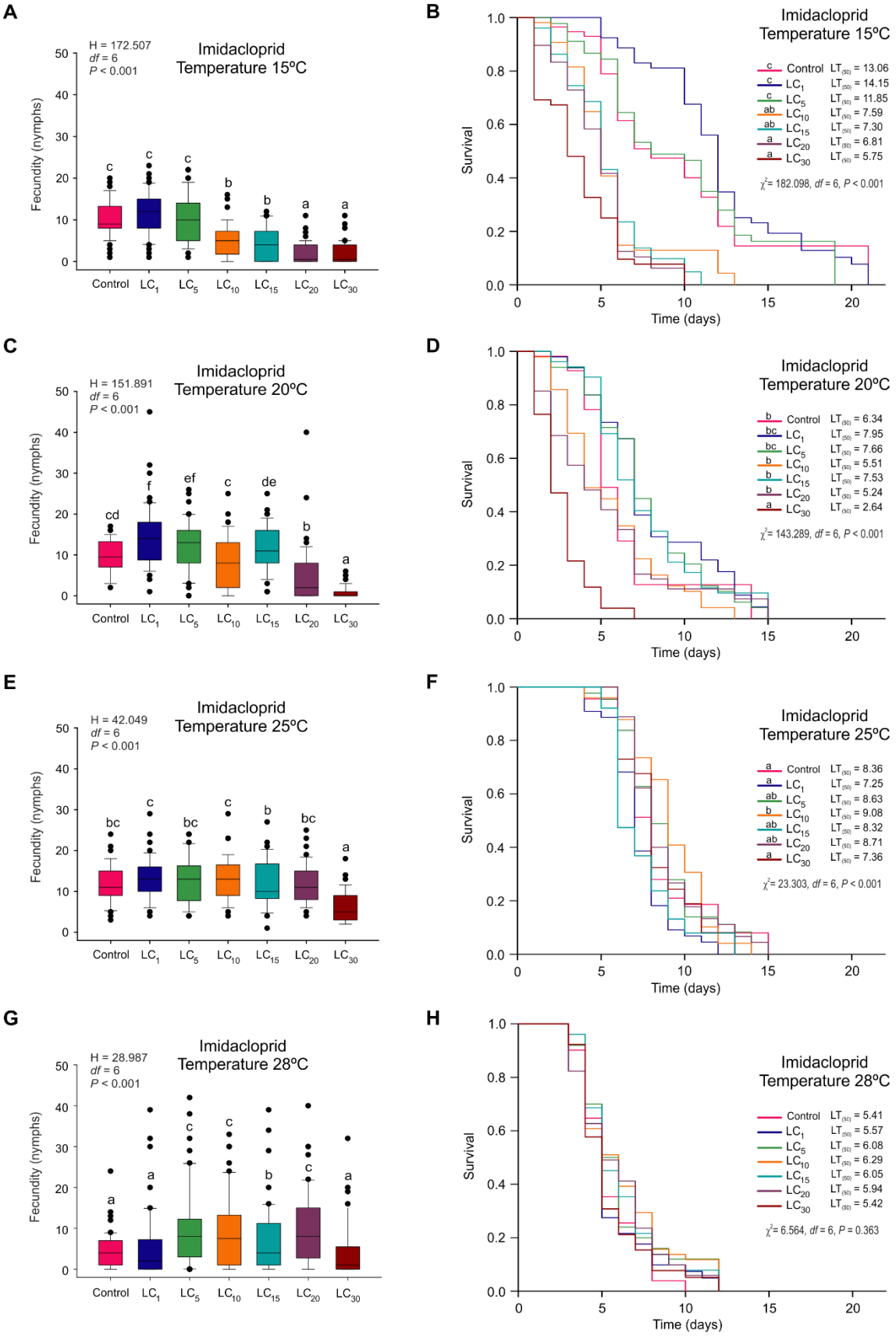
A
H = 172.507
df = 6
P < 0.001
Imidacloprid
Temperature 15ºC
Fecundity (nymphs)
Control LC1 LC5 LC10 LC15 LC20 LC30
B
Imidacloprid
Temperature 15ºC
Survival
Time (days)
Control LT(50) = 13.06
LC1 LT(50) = 14.15
LC5 LT(50) = 11.85
LC10 LT(50) = 7.59
LC15 LT(50) = 7.30
LC20 LT(50) = 6.81
LC30 LT(50) = 5.75
χ2= 182.098, df = 6, P < 0.001
C
H = 151.891
df = 6
P < 0.001
Imidacloprid
Temperature 20ºC
D
Imidacloprid
Temperature 20ºC
Control LT(50) = 6.34
LC1 LT(50) = 7.95
LC5 LT(50) = 7.66
LC10 LT(50) = 5.51
LC15 LT(50) = 7.53
LC20 LT(50) = 5.24
LC30 LT(50) = 2.64
χ2= 143.289, df = 6, P < 0.001
E
H = 42.049
df = 6
P < 0.001
Imidacloprid
Temperature 25ºC
F
Imidacloprid
Temperature 25ºC
Control LT(50) = 8.36
LC1 LT(50) = 7.25
LC5 LT(50) = 8.63
LC10 LT(50) = 9.08
LC15 LT(50) = 8.32
LC20 LT(50) = 8.71
LC30 LT(50) = 7.36
χ2= 23.303, df = 6, P < 0.001
G
H = 28.987
df = 6
P < 0.001
Imidacloprid
Temperature 28ºC
H
Imidacloprid
Temperature 28ºC
Control LT(50) = 5.41
LC1 LT(50) = 5.57
LC5 LT(50) = 6.08
LC10 LT(50) = 6.29
LC15 LT(50) = 6.05
LC20 LT(50) = 5.94
LC30 LT(50) = 5.42
χ2= 6.564, df = 6, P = 0.363

FIGURE 2

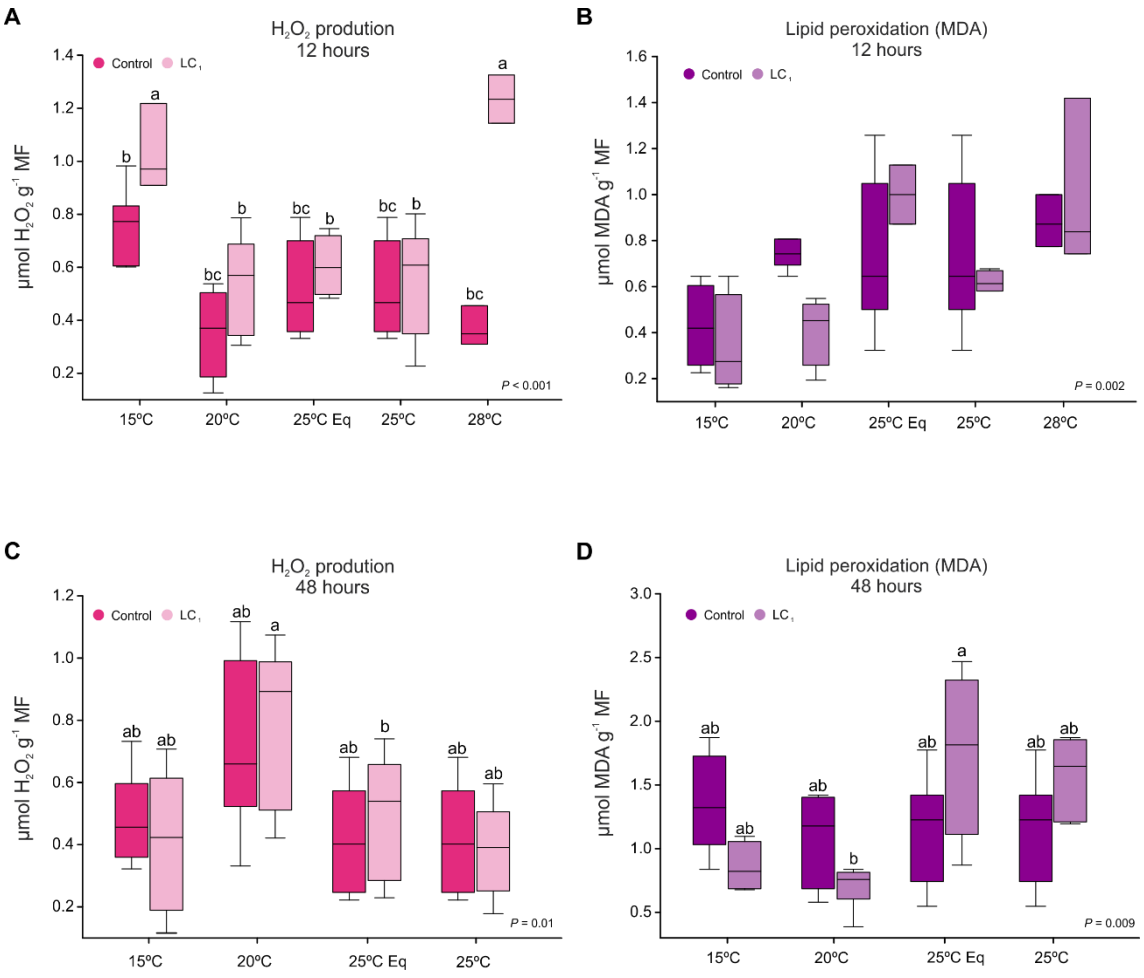
A
H2O2 prodution
12 hours
Control
LC1
µmol H2O2 g-1 MF
P < 0.001
15ºC
20ºC
25ºC Eq
25ºC
28ºC
B
Lipid peroxidation (MDA)
12 hours
µmol MDA g-1 MF
P = 0.002
C
H2O2 prodution
48 hours
P = 0.01
D
Lipid peroxidation (MDA)
48 hours
P = 0.009

FIGURE 3

Table 1. Lethal concentration of imidacloprid in active ingredient (a.i)

Imidacloprid 15°C	
LCs	**Concentrations mg (a.i)/ml**
LC_1	0.0029
LC_5	0.0104
LC_{10}	0.0203
LC_{15}	0.0318
LC_{20}	0.0455
LC_{30}	0.0814
Imidacloprid 20°C	
LCs	**Concentrations mg(a.i)//ml**
LC_1	0.0036
LC_5	0.0093
LC_{10}	0.0154
LC_{15}	0.0215
LC_{20}	0.0281
LC_{30}	0.0434
Imidacloprid 25°C	
LCs	**Concentrations mg(a.i)//ml**
LC_1	0.0001
LC_5	0.0004
LC_{10}	0.0007
LC_{15}	0.0012
LC_{20}	0.0018
LC_{30}	0.0032
Imidacloprid 28°C	
LCs	**Concentrations mg(a.i)//ml**
LC_1	0.00015
LC_5	0.00053
LC_{10}	0.00102
LC_{15}	0.00159
LC_{20}	0.00226
LC_{30}	0.00400

Table 2. Relative toxicity of neonicotinoid (i.e., imidacloprid) to individuals of *Myzus persicae*.

Insecticide	Temperatures	N	LC_{50} (95% CI) mg (a.i.)/ml	χ^2	*P*	*TR*
Imidacloprid	15°C	100	0.212 (0.170– 0.268) a	8.41	0.13	26.5
	20°C	100	0.088 (0.074 – 0.106) a	3.17	0.67	11
	25°C	100	0.008 (0.007 – 0.010) b	10.75	0.15	-
	28°C	100	0.010 (0.007 – 0.013) b	3.85	0.42	1.25

N: number of individuals tested.
LC_{50} (95%): lethal concentration to cause mortality in 50% of individuals
CI: confidence intervals.
a.i.: active ingredient
$\chi 2$: Chi-square for lack-of-fit to the probit model.
P: Probability associated with the chi-square statistic.
TR= calculated by dividing the LC_{50}s of the different temperatures by the smallest LC_{50}

Table 3. Temperature coefficients of imidacloprid on *Myzus persicae* adults.

Imidacloprid		
Temperatures	**Temperature coefficients**	
15 – 20°C	2.39	Slight
15 – 25°C	25.16	Strong
15 – 28°C	21.30	Strong
20 – 25°C	10.27	Strong
20 – 28°C	8.69	Strong
25 – 28°C	1.18	No effect

The effects of temperature coefficients were "no effect" = < 2, "slight" = (2-5), and "strong" = > 5

Appendix – supplementary material

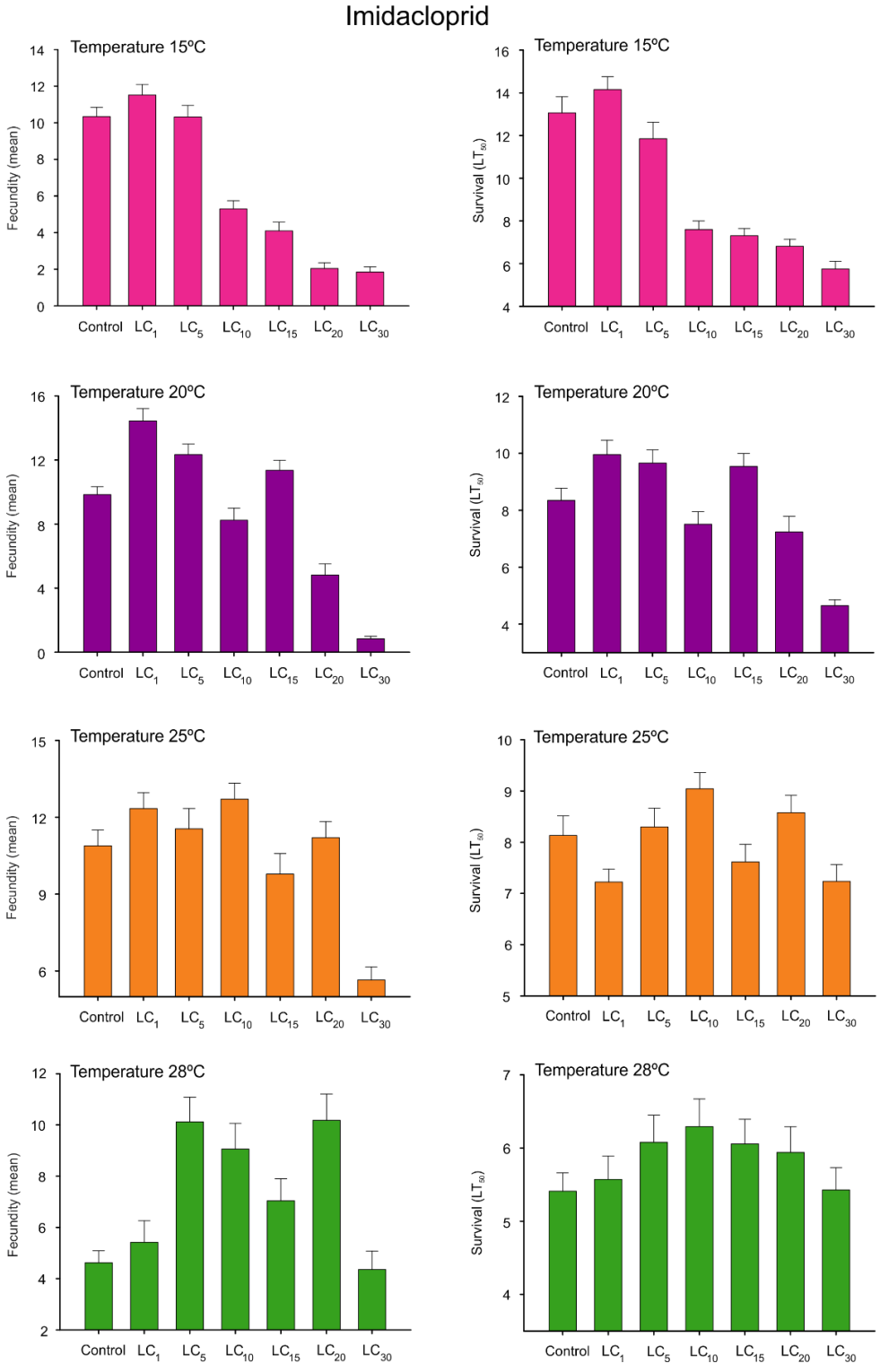

Figure A1. Mean fecundity and survival (LT_{50}) of *Myzus persicae* females under varying temperatures after intoxication with imidacloprid.

Concluding considerations

Myzus persicae Sulzer (1776) (Hemiptera: Aphididae) is considered one of the major agricultural pests with the potential to generate significant yield losses in several crops worldwide. Studies on this pest species are important to provide potential tools and find bases for efficient pest management measures. To increase this knowledge, it is necessary to use appropriate methodologies for laboratory rearing, as performed in this study. The refinement of these techniques and the addition of new, cheaper technologies may enable the maintenance and availability of these individuals in the laboratory.

In the present study, the use of *Nicandra physalodes* combined with hydrogel as rearing substrate, demonstrated the ability for *M. persicae* to produce offspring under controlled conditions, and great potential for mass rearing, thus providing an innovative proposal to adapt and validate the way of aphid rearing under laboratory conditions.

The use of agrochemicals remains one of the main management strategies for *M. persicae*, in the present study, we highlight the fact that the increase/decrease of temperature may induce higher/lower efficacy of chemicals on individuals of this species. Climate change may have the potential to alter the benefits/costs balance of pesticide use in the agricultural context, and with these results, relevant and efficient strategies for controlling *M. persicae* populations in agricultural areas can be made.

Furthermore, several recent studies are showing that exposure to low doses of a contaminant can induce a hormetic response in these individuals, which can lead to an increase in population growth at a higher rate than would be observed without the application of the products. In the present study, we also show how the result of the correlation of temperature with chemical toxicity can induce a hormetic response in the

biological characteristics of this species, and that it contributed to a beneficial stimulatory effect on the fecundity and longevity of individuals.

Efficient and economical pest control is only possible by planning and implementing an integrated management system, and the results obtained in this study provided important information about the adaptive coping mechanisms of these organisms in challenging environments, which will be of paramount importance for designing management strategies for *M.persicae* in agroecosystems.