

PAPEL DA ACLIMATAÇÃO NA SOBREVIVÊNCIA, REPRODUÇÃO E RESPOSTA
FUNCIONAL DE *Tenuisvalvae notata* (MULSANT) (COLEOPTERA: COCCINELLIDAE)

por

ENGGEL BEATRIZ SILVA DO CARMO

Dissertação apresentada ao Programa de Pós-Graduação em Entomologia, da Universidade Federal Rural de Pernambuco, como parte dos requisitos para obtenção do grau de Mestre em Entomologia.

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(Sob Orientação da Professora Christian Sherley Araújo da Silva Torres - UFRPE)

RESUMO

Para atingir o máximo potencial biótico, os insetos demandam um suprimento adequado de nutrientes e uma faixa ótima de umidade e temperatura. Entretanto, esses animais apresentam a capacidade de aclimação em ambiente fora dessa faixa favorável, o que fornece uma vantagem adaptativa sob condições de estresse. Neste trabalho foram investigadas a capacidade de aclimação, a sobrevivência e a reprodução da joaninha *Tenuisvalvae notata* (Mulsant) (Coleoptera: Coccinellidae) em condições de baixa (12, 14, 16 e 18 °C) e alta (32, 34 e 36 °C) temperaturas. Ainda, foi determinada a taxa de predação e resposta funcional de joaninhas aclimatadas alimentando-se da cochonilha *Ferissia dasyliirii* (Cockrell) (Hemiptera: Pseudococcidae). Por fim, foi investigado o possível efeito da temperatura na esterilização dos adultos. A aclimação favoreceu a sobrevivência das joaninhas, sob as altas e baixas temperaturas. Entretanto, apesar de se manterem vivos após aclimação, houve uma significativa redução da fecundidade abaixo de 18 °C e acima de 34 °C. No geral, não houve efeito da temperatura na capacidade predatória de *T. notata*, mantendo-se com resposta funcional do tipo II. Entretanto, a partir de 32 °C houve uma redução no tempo de manipulação das presas. A fertilidade é reduzida quando as fêmeas ou machos são submetidos à aclimação a altas (32 °C) e

baixas (18 °C) temperaturas, porém sendo mais afetada quando as fêmeas permanecem em baixas temperaturas. A partir da aclimação prévia é possível que *T. notata* seja introduzida em áreas com temperatura fora da faixa ótima de desenvolvimento e mantenha sua capacidade predatória nesse local. Entretanto, pode ser esperado um declínio na população de *T. notata* devido à redução na reprodução dos insetos aclimatados.

PALAVRAS-CHAVE: Controle biológico, joaninha predadora, cochonilhas, bioecologia.

ROLE OF ACCLIMATION IN SURVIVAL, REPRODUCTION AND FUNCTIONAL
RESPONSE OF *Tenuisvalvae notata* (MULSANT) (COLEOPTERA: COCCINELLIDAE)

by

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(Under the Direction of Professor Christian Sherley Araújo da Silva Torres – UFRPE)

ABSTRACT

The maximum biotic potential of insects requires an adequate supply of nutrients and an optimum range of humidity and temperature. However, the insects can acclimate in an environment outside this range, which provides an adaptive performance under stress conditions. In this work, we investigated the acclimation, survival, and reproduction of the ladybeetles *Tenuisvalvae notata* (Mulsant) (Coleoptera: Coccinellidae) under conditions of low (12, 14, 16 and 18°C) and high (32, 34 and 36°C) temperatures. We estimated the rate of predation and functional response of acclimated ladybeetles feeding on mealybug *Ferrisia dasyliirii* (Cockrell) (Hemiptera: Pseudococcidae). Finally, we investigated the possible effect of temperature on adult sterilization. The acclimation favored the survival of the ladybeetles, under the high and low temperatures. However, despite remaining alive after acclimation, there was a significant reduction in fecundity below 18°C and above 34 °C. In general, there was no effect of temperature on the predatory capacity of *T. notata*, maintaining a type II functional response. However, above 32°C there was a reduction in the handling time of the prey. Fertility was reduced when females or males were acclimated to high (32°C) and low (18°C) temperatures, but

females under low temperatures was more affected. From the previous acclimation, it is possible that *T. notata* is introduced in areas with the temperature outside the optimal range of development and maintains its predatory capacity in that location. However, expected it is a decline in the *T. notata* population due to reduced reproduction of acclimated insects.

KEY WORDS: Biological control, bioecology, predatory ladybeetles, mealybugs.

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CAPÍTULO 1

INTRODUÇÃO

Os organismos ectotérmicos, como os insetos, obtêm calor do ambiente tornando sua temperatura corporal semelhante à temperatura ambiente (Holt 1990), respondendo às variações de temperaturas, em especial, à exposição a temperaturas extremas. As respostas podem ser através de alterações fisiológicas e comportamentais, a fim de reduzir a tensão ou aumentar a resistência a mudanças estressantes de fatores climáticos específicos (Bowler 2005). Existe a hipótese de que a aclimação a temperaturas quentes e frias, melhora a sobrevivência dos indivíduos, e essa aclimação térmica dos insetos pode ocorrer a partir da exposição dos mesmos a novas temperaturas no ambiente de criação (Bowler 2005, Weldon *et al.* 2011).

A aclimação é uma mudança rápida e reversível na fisiologia, bioquímica ou anatomia de um organismo em resposta à exposição a uma nova condição ambiental (Leroi *et al.* 1994, Weldon *et al.* 2011), com a característica de favorecer a sobrevivência e permanência deste no ambiente (Weldon *et al.* 2011, Allen *et al.* 2012, Li *et al.* 2018). A capacidade de aclimação térmica é, em geral, mais limitada em organismos de ambientes termicamente estáveis em comparação com aqueles que vivem sob condições mais variáveis (Tomanek 2010, Pallarés *et al.* 2019). Populações tropicais são mais tolerantes ao calor e se recuperam mais rapidamente do que as populações temperadas que, por sua vez, são mais tolerantes ao frio do que as tropicais (David *et al.* 2005). Como as espécies em latitudes mais altas experimentam maior variação de temperatura sazonal, conseqüentemente, prevê-se que suportem variações maiores de temperatura (Sunday *et al.* 2012).

As respostas de aclimação dos insetos são muito variadas e podem ser observadas a partir de alterações nas proteínas e lipídios da membrana (Farkas *et al.* 1994, Watabe *et al.* 1998, Bowler

2005) e em funções, como a locomoção e taxa metabólica (O'Steen & Bennett 2003, Bowler 2005 Lachenicht *et al.* 2010). Estas alterações podem aumentar a tolerância dos insetos ao calor e ao frio quando submetidos a temperaturas letais (Li *et al.* 2011, Allen *et al.* 2012, Hu *et al.* 2014). A sobrevivência dos insetos expostos a temperatura abaixo do ideal depende da duração e da temperatura de exposição, assim quando a temperatura diminui e/ou a duração da exposição aumenta, os insetos podem sofrer danos fisiológicos cumulativos que levam a um alto nível de mortalidade (Colinet *et al.* 2011).

Uma das principais formas dos insetos responderem a variação ambiental, pelo menos em curto prazo, são a plasticidade fenotípica do desempenho fisiológico e tolerância condições abióticas desfavoráveis (Lachenicht *et al.* 2010). Mudanças plásticas abrangem variação reversível e irreversível de fenótipos que ocorrem dentro de uma única geração, e são essencialmente o produto de interação gene e ambiente (DeWitt & Scheiner 2004, Lachenicht *et al.* 2010). Mecanismos usados para lidar com variações de temperatura incluem compensações fisiológicas e bioquímicas, ambas as características relacionadas ao desempenho de como a taxa metabólica responde à aclimação térmica (Lachenicht *et al.* 2010).

A temperatura de criação afeta a tolerância térmica de várias espécies de insetos. Por exemplo, adultos de *Drosophila* spp., ao serem criados a uma temperatura mais baixa tem uma maior probabilidade de sobreviver à exposição a baixas temperaturas (Goto & Kimura 1998, Zeilstra & Fisher 2005, Schou *et al.* 2015). Também, a aclimação a baixa temperatura teve um efeito positivo na capacidade de super-resfriamento de insetos predadores como *Cryptolaemus montrouzieri* Mulsant e *Macrolophus pygmaeus* Rambur, sugerindo que a sua exposição a um curto período de aclimação é suficiente para alcançar um ponto de congelamento a uma temperatura mais baixa do que outras populações mantidas em condições de temperatura normais de criação (Maes *et al.* 2012, 2015). Indivíduos da espécie *Lycaena tityrus* (Poda) criados sob

temperatura de 20 °C têm tempos de recuperação mais curtos ao passar por um choque de frio de -20 °C, comparados a indivíduos criados sob a temperatura de 27 °C (Terblanche & Chown 2006). Assim, estudos confirmam que o ambiente de desenvolvimento do inseto tem um impacto significativo na tolerância ao frio no início da fase adulta.

Além disso, outros estudos sugerem que a aclimação a altas temperaturas subletais pode melhorar significativamente a tolerância ao calor de algumas espécies, em relação a sobrevivência (Muñoz-Valencia *et al.* 2016, Pieterse *et al.* 2017, Li *et al.* 2011, 2018). Entretanto, os graus dessa tolerância ao calor dependem do tempo de exposição e à plasticidade limitada de suas curvas de desempenho (Terblanche & Chown 2006, Weldon *et al.* 2011, Muñoz-Valencia *et al.* 2016), que podem gerar declínios populacionais com temperaturas médias mais quentes (Lachenicht *et al.* 2010).

Em face do aquecimento global, os efeitos induzidos pela aclimação a altas temperaturas subletais devem ser considerados quando se projeta ou se aplica o controle de pragas (Maes *et al.* 2015, Li *et al.* 2018). Isto porque o aumento na tolerância ao frio ou ao calor, após a aclimação, indica o potencial de um inseto para se ajustar gradualmente a temperaturas extremas passíveis de ocorrência no ambiente.

Respostas dos insetos na reprodução ao aumento da temperatura

A temperatura corporal afeta o desempenho dos insetos, ou seja, a taxa de aumento da população é limitada pela termodinâmica (Chown & Nicolson 2004, Dixon *et al.* 2009, Lachenicht *et al.* 2010). Uma série de funções fisiológicas (por exemplo, crescimento, metabolismo, reprodução) ocorre de forma ideal em uma temperatura ótima e, fora desta, o desempenho é reduzido (Martin & Huey 2008, Lachenicht *et al.* 2010).

As altas temperaturas influenciam no funcionamento normal da fisiologia reprodutiva dos organismos (Saxena *et al.* 1992, Janowitz & Fischer 2011, Zhang *et al.* 2014) e a sensibilidade reprodutiva à temperatura já é conhecida em muitas espécies de insetos. Por exemplo, em *Drosophila melanogaster* (Meigen) temperaturas acima de 30 °C causam a esterilidade de 50% dos machos (Rohmer *et al.* 2004). Também, machos de *Sarcophaga crassipalpis* Macquart submetidos a choque térmico de 45 °C, ou pré-tratados a 40 °C, não conseguiram transferir espermatozoides para as fêmeas (Rinehart *et al.* 2000). Similarmente, três efeitos diferentes foram observados em *Anisopteromalus calandrae* (Howard), dependendo da temperatura: i) mortalidade de machos acima de 42 °C, ii) esterilidade de machos emergentes a 40 °C, iii) e subfertilidade de machos a 38°C. Esta sub-fertilidade é caracterizada por uma diminuição de até 7% no fornecimento de espermatozoides em comparação com os machos criados a uma temperatura de 30 °C (Nguyen *et al.* 2013). Em estudo com o besouro *Tribolium castaneum* (Herbst), ondas de calor (5 a 7 °C acima do ideal por 5 dias) reduziram a produção de espermatozoides, viabilidade e a competitividade na fêmea, os espermatozoides armazenados nas fêmeas também foram danificados pelas ondas de calor (Sales *et al.* 2018).

Nas fêmeas, o estresse por calor é evidenciado por uma redução pelo menos temporária na fecundidade, tamanho do ovo e investimento reprodutivo após o estresse e a indução de processos demandantes de energia, que reduzem a alocação de recursos para a reprodução (Krebs & Loeschcke 1994, Dahlhoff & Rank 2007). Lesões diretas podem ser causadas por degeneração do oócito ou desenvolvimento ovariano desordenado (Krebs & Loeschcke 1994), ou por altas temperaturas bloqueando a vitelogênese (Audit-Lamour & Busson 1981, Janowitz & Fischer 2011). A exposição materna ao frio reduz a viabilidade ovo-a-adulto em *D. melanogaster* (Watson & Hoffmann 1996) e em *Drosophila serrata* Malloch (Magiafoglou & Hoffmann 2003), efeitos que provavelmente refletem uma diminuição na taxa de eclosão dos ovos ou uma menor

viabilidade larval. Danos induzidos pelo frio em ovos imaturos poderiam ser amenizados pela aclimação. Além disso, as mudanças no metabolismo materno podem ter alguns efeitos sobre os óvulos maduros presentes nos ovariolos, influenciando a maturação destes (Rako & Hoffmann 2006). A exposição das fêmeas de borboletas *Bicyclus anynana* (Butler) ao calor causou uma redução subsequente na longevidade, fecundidade e tamanho dos ovos. No entanto, as fêmeas que acasalaram com machos sob estresse mostraram, surpreendentemente, uma fecundidade precoce aumentada em vez de diminuída, o que pode indicar um aumento do investimento na reprodução em ambientes estressantes (Janowitz & Fischer 2011).

A temperatura ambiente e a qualidade nutricional dos recursos são fatores decisivos no desempenho reprodutivos de adultos (por exemplo, fecundidade e fertilidade, tamanho e qualidade nutricional do ovo), contribuindo assim para o crescimento diferencial da população (McCaffrey & Horsburgh 1986, Jalali *et al.* 2010, Sebastião *et al.* 2014). Os aspectos fisiológicos e comportamentais para a termorregulação devem, portanto, serem considerados ao estudar as respostas dos insetos à mudança ambiental, que certamente afetará a distribuição geográfica, o forrageamento e a reprodução destes insetos (Woods *et al.* 2015, Blanckenhorn 2018).

Efeito da temperatura no desempenho de inimigos naturais

O aumento da temperatura pode interferir na resposta de ação dos inimigos naturais (Tylanakis *et al.* 2008, Guzmán *et al.* 2016). Entre eles, predadores, parasitoides e patógenos que reduzem as populações de pragas (Driesh & Bellows 1996, Van Lenteren 2012). A resposta de predação de um inseto, também conhecida como curva de resposta funcional varia de acordo com as condições ambientais (Abrams 1982), tais como: a heterogeneidade do habitat, a intensidade da luz (Jalili *et al.* 2010), a planta hospedeira (De Clercq *et al.* 2000, Skirvin & Fenlon 2001), a temperatura (Jalili *et al.* 2010, Li *et al.* 2011, 2018), e o refúgio (Messina & Hanks 1998, Jalili *et*

al. 2010). Portanto, o estudo do impacto dessas variáveis ambientais na biologia de pragas e inimigos naturais é importante para determinar a eficácia do controle biológico.

Sabe-se que a temperatura tem um efeito sobre o desempenho de inimigos naturais. Por exemplo, o ácaro predador *Phytoseiulus persimilis* Athias-Henriot predava um menor número de *Tetranychus urticae* Koch, em temperaturas superiores e inferiores a 25 °C (Skirvin & Fenlon 2003). A joaninha *Adalia bipunctata* L., por sua vez, apresentou maior taxa de ataque e menor tempo de manipulação em temperaturas superiores e inferiores a 23 °C (Jalali *et al.* 2010). Isso pode ser atribuído a mudanças relacionadas à temperatura no metabolismo e na atividade de predadores e presas (McCoull *et al.* 1998).

Coccinellidae é conhecida pela diversidade de espécies (Majerus 2009, Akhavan *et al.* 2013, Honek *et al.* 2017), bem como pelo papel que as larvas e adultos desempenham como predadores de fitófagos (Kundoo & Khan 2017). São predadoras de pulgões, cochonilhas, psilídeos, ácaros, moscas-brancas dentre outros insetos-pragas, e com espécies distribuídas em todo o mundo (Pervez 2005, Kundoo & Khan 2017). É um grupo que possui um hábito alimentar bastante diversificado, com espécies que se alimentam de fungos e pólen, até aqueles que podem se alimentar de plantas, como nos gêneros *Epilachna* e *Henosepilachna*, que neste caso são consideradas pragas (Naz *et al.* 2012).

As joaninhas, ou coccinelídeos, são insetos holometabólicos, e o período de ovo a adulto pode variar entre as espécies e com as condições ambientais e de alimento (Costa Lima 1952, Hagen 1962, Kundoo & Khan 2017). O aquecimento pode afetar o tempo de desenvolvimento, fecundidade e fertilidade das joaninhas e a dinâmica com as espécies de presas, ou resultar em mudanças nos habitats, tornando-os menos favoráveis para algumas joaninhas e mais favoráveis para outras (Honek *et al.* 2017).

***Tenuisvalvae notata* (Mulsant) (Coleoptera: Coccinellidae, Scymninae)**

A joaninha *T. notata* é nativa da América do Sul (Dreyer *et al.* 1997a) e é citada como um potencial agente de controle de cochonilhas Pseudococcidae (Barbosa *et al.* 2014, Peronti *et al.* 2016, Santos *et al.* 2017). Apresenta distribuição nos planaltos colombianos, sendo considerada na região uma espécie predadora preferencial de *Phenacoccus herreni* Cox & Williams (Dreyer *et al.* 1997a), enquanto no Paraguai está associada a *Phenacoccus manihoti* (Matile-Ferrero) (Löhr *et al.* 1990, Dreyer *et al.* 1997a). Sua presença é relatada em vários estados brasileiros, como na região semiárida de Pernambuco, associada à *Phenacoccus solenopsis* Tinsley e *Ferrisia dasyliirii* Cockerell e *Dactylopius opuntiae* (Cockerell) (Barbosa *et al.* 2014, Torres & Giorgi 2018). No Estado de São Paulo, a espécie foi encontrada pela primeira vez predando a cochonilha-rosada-do-hibisco, *Maconellicoccus hirsutus* (Green) (Peronti *et al.* 2016).

A partir de estudo sobre o comportamento de predação de *T. notata*, foi estimado o consumo médio de 158 ninfas neonatas por dia ou 2,2 fêmeas adultas da cochonilha *F. dasyliirii* (Barbosa *et al.* 2014). Quando está sob condições favoráveis ao seu desenvolvimento, com alimento e ambiente sob condições controladas de temperatura 25 ± 2 °C, umidade relativa $60 \pm 10\%$ e fotoperíodo 12:12h (Luz: Escuro), a espécie apresenta período de desenvolvimento completo, de ovo a adulto, em cerca de 36 dias, e mais de 40% das fêmeas conseguem sobreviver a mais de 150 dias (Dreyer *et al.* 1997, Barbosa *et al.* 2014). Estudos recentes demonstraram que *T. notata* pode desenvolver e reproduzir na faixa de temperatura variando entre 20 e 32 °C (Ferreira *et al.* 2020). Porém, anteriormente, Dreyer (1997b) utilizando populações originárias do Brasil e da Colômbia conseguiu desenvolvimento e reprodução de *T. notata* a 18 °C. A temperatura base da espécie foi estimada para 14 °C, temperatura ótima 31 °C e a temperatura máxima para desenvolvimento 36 °C, segundo o modelo não linear Brière-1 (Ferreira *et al.* 2020).

Adultos de *T. notata* apresentam um período de pré-cópula de 5 a 10 dias após a emergência (Dreyer *et al.* 1997a, Santos *et al.* 2017), com um número médio de cópulas diárias de 1,17 a 1,91 durante a fotofase (Santos *et al.* 2017). Observou-se também que, após uma única cópula as fêmeas são capazes de ovipositar por 60 dias. A maior fecundidade é observada nos primeiros 10 dias após a cópula e a maior fertilidade dos ovos nos primeiros 20 dias, que decrescem com o passar do tempo (Túler *et al.* 2017). No período de 60 dias, Ferreira *et al.* (2020), contabilizaram fecundidade média de 8 ovos a 20 °C e de 442 ovos à 28 °C.

Diante do exposto, levantamos a hipótese de que é possível que a partir da aclimação de *T. notata* a temperaturas extremas, podemos obter melhor desempenho da espécie para uma faixa de temperatura mais elevada comum para as regiões do Semiárido, bem como condições favoráveis para o crescimento populacional de cochonilhas farinhentas. Assim, neste trabalho temos como objetivo investigar como a aclimação a altas (> 32 °C) e baixas (< 20 °C) temperaturas influenciam a sobrevivência, reprodução e comportamento de predação de *T. notata*.

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CHAPTER 2

TEMPERATURE ACCLIMATION OF THE LADY BEETLE *Tenuisvalvae notata* (MULSANT) (COLEOPTERA: COCCINELLIDAE): SURVIVAL, REPRODUCTION AND PREDATION¹

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¹Carmo, E.B.S., Silva-Torres, C.S.A. & Torres, J.B. Temperature acclimation of the lady beetle *Tenuisvalvae notata* (Mulsant) (Coleoptera: Coccinellidae): Survival, Reproduction and Predation, A ser submetido a Ecological Entomology.

TEMPERATURE ACCLIMATION OF THE LADY BEETLE *Tenuisvalvae notata* (MULSANT)
(COLEOPTERA: COCCINELLIDAE): SURVIVAL, REPRODUCTION AND PREDATION

ABSTRACT – Temperature is the abiotic factor that most affects the biology and fitness of insects, changing their characteristics of life history and behavior. The acclimation of the ladybeetles *Tenuisvalvae notata* (Mulsant) (Coleoptera: Coccinellidae) was studied at low temperatures (12, 14, 16, and 18 °C) and high temperatures (32, 34, and 36 °C). After the emergency, adults (<24h) were submitted to acclimation by increasing or decreasing the temperature, being 1 °C/day, and determined the fertility, fecundity, and survivorship. Furthermore, were estimated the rate of predation and functional response of *T. notata* preying upon the mealybug *Ferrisia dasyliirii* (Cockrell) (Hemiptera: Pseudococcidae) after acclimation. There was a significant reduction in fertility and fecundity at temperatures below 18 °C and above 34 °C, and reduced survival from this last temperature. Predation was not affected in general, maintaining the type II functional response. However, there was a significant reduction in the handling time of the prey at 32 °C. Fertility is reduced when males and females were subjected to high and low acclimation temperatures, but fecundity is lower when females were kept at low temperatures. Therefore, acclimation favors the survival of *T. notata* in temperatures outside the optimum range and does not alter its predation efficiency, but compromises its reproductive capacity, which may cause a population decline in the local after a few generations after release.

KEY WORDS: Temperature, trade-off, sterility, fertility.

ACLIMATAÇÃO A TEMPERATURA DA JOANINHA PREDADORA *Tenuisvalvae notata*
(MULSANT) (COLEOPTERA: COCCINELLIDAE): SOBREVIVÊNCIA, REPRODUÇÃO E
PREDAÇÃO

RESUMO - A temperatura é o fator abiótico que mais afeta a biologia e a aptidão dos insetos, alterando suas características de história de vida e comportamento. A aclimatação da joaninha *Tenuisvalvae notata* (Mulsant) (Coleoptera: Coccinellidae) foi estudada em temperaturas inferiores (12, 14, 16 e 18 °C) e superiores (32, 34 e 36 °C) à temperatura ótima para as espécies (28 °C). Após a emergência na temperatura ótima, adultos (< 24h) foram submetidos à aclimatação por meio do aumento ou redução da temperatura, sendo 1 °C/dia, até a temperatura desejada e determinado a fecundidade, fertilidade e sobrevivência. Ainda, foram determinadas a taxa de predação e resposta funcional de *T. notata* predando a cochonilha *Ferrisia dasyliirii* (Cockrell) (Hemiptera: Pseudococcidae) após aclimatação. Houve redução significativa na fecundidade e fertilidade em temperaturas abaixo de 18 °C e acima de 34 °C, e redução da sobrevivência a partir desta temperatura. A predação não foi afetada no geral, mantendo a resposta funcional do tipo II. Porém, houve redução significativa no tempo de manipulação da presa a 32 °C. A fecundidade é reduzida quando machos e fêmeas são submetidos a altas e baixas temperaturas de aclimatação, mas a fertilidade é menor quando as fêmeas são mantidas sob baixas temperaturas. Portanto, a aclimatação favorece a sobrevivência de *T. notata* em temperaturas fora da faixa ótima e não altera sua eficiência de predação, mas compromete sua capacidade reprodutiva, o que poderá causar um declínio populacional no local após poucas gerações pós liberação.

PALAVRAS-CHAVE: Temperatura, trade-off, esterilidade, fertilidade.

Introduction

Temperature affects the physiological and biochemical processes in exothermic animals, such as insects. It is a major factor determining developmental times, survival, and distribution of the species (Cossins & Bowler 1987). The environmental changes implicate insect adaptations triggering metabolic costs (Régnière *et al.* 2012). The insect responses have been characterized as trade-offs for energy allocation, where two or more physiological functions may demand the source (Boggs 2009). When insects are found under optimal temperature conditions, which usually varies around 20 °C (Dixon *et al.* 2009), the nutrients taken are transformed by metabolism and allocated as energy directed to maintenance, reproduction, and survival (Boggs 2009). Otherwise, when exposed to extreme temperature conditions, they may prioritize a specific function, or even die when temperatures are below or above their survival limits (Blanckenhorn 2018).

Insect acclimation has been a growing research theme, due to global environmental changes characterized mostly by local temperature fluctuations (Somero 2010, Maes *et al.* 2012, Sørensen *et al.* 2013). Moreover, body acclimation is characterized by the gradual long-term response of an organism to habitat changes through physiological responses (Kingsolver & Huey 1998, Somero 2010). Thus, acclimation helps insects to deal with habitat temperature fluctuation with direct effects on their fitness (Milosavljević *et al.* 2019). For large production in insectaries such as natural enemies, colonies are reared under optimal physical environmental conditions to produce insects on a large scale in a short time (Chown & Nicolson 2004, Ikemoto 2005, Ortiz *et al.* 2016). Therefore, acclimation in the rearing facility before the release of natural enemies can increase their survival under different thermal conditions in the target habitat, as well as conserve their reproductive potential (Kristensen *et al.* 2008, Angilletta 2009, Sørensen *et al.* 2013).

Ladybeetles (Coleoptera: Coccinellidae) represent one of the most diverse group of natural enemies of insect pest species (Dixon & Dixon 2000, Majerus 2009). Research on this group have shown the impact of temperature on fecundity and fertility (Sørensen *et al.* 2013, Zhang *et al.* 2014, Ferreira *et al.* 2020), body size (Sørensen *et al.* 2013, Maes *et al.* 2015), developmental times and survival (Sebastião *et al.* 2014, Maes *et al.* 2015, Ferreira *et al.* 2020), and the number of generations accomplished. Also, other studies have shown that temperature may affect predatory behavior and establish the limits of their biological activities (Pervez 2005). For instance, the coccinellids *Adalia bipunctata* L. and *Cryptolaemus montrouzieri* Mulsant increase their attack rate, and decrease prey manipulation time under higher temperatures (Jalali *et al.* 2010, Sørensen *et al.* 2013, Maes *et al.* 2015). These characterize potential responses of the natural enemies in new habitats, as well as their potential to control the target pest (Obrycki & Kring 1998, Milosavljević *et al.* 2019).

Many ladybeetles species are used in the biological control of aphids, psyllids, and mealybugs worldwide (Dixon & Dixon 2000, Majerus 2009, Sørensen *et al.* 2013). Moreover, the ladybeetle *Tenuisvalvae notata* (Mulsant), which is from South America has contributed to the management of mealybugs (Hemiptera: Pseudococcidae) in Brazil and other countries (Dreyer *et al.* 1997ab, Barbosa *et al.* 2014, Peronti *et al.* 2016). It is distributed in tropical and subtropical regions, with an optimal temperature range varying from 25 to 28 °C, and maximum and minimum temperature thresholds at 35 and 14 °C, respectively (Dreyer *et al.* 1997ab, Ferreira *et al.* 2020). Even though the estimated maximum and minimum temperature thresholds for *T. notata* is quite large, previous study by Ferreira *et al.* (2020) has shown that *T. notata* can survive under the tested temperatures of 20 °C and 36 °C, but with lack of reproduction when exposed to temperatures greater than 32 °C, and these authors hypothesized that this could be related to male sterilization due to high temperatures.

According to the beneficial acclimation hypothesis (Leroi *et al.* 1994, Wilson & Franklin 2002), an insect that is exposed to a gradual increasing (or decreasing) of temperatures enhances the physiological performance, fitness, or survival at extreme temperatures compared to those suddenly exposed to new conditions. Thus, it would be expected that if *T. notata* was subjected to temperatures out of its optimal range after adult emergence, the adults might survive, reproduce and maintain their predatory potential under conditions of thermal stress. Furthermore, the goals of this study were to investigate how acclimation to high (>32 °C) and low (<20 °C) temperatures impact the survival, reproduction, and predatory behavior of *T. notata*. In this context, our hypotheses were: i) if there is a trade-off regarding energetic costs in acclimated *T. notata* adults, female survival would be favored to detriment of fecundity and fertility; ii) *T. notata* prey consumption would increase as temperature increases to compensate for energy expenditure under these conditions; and iii) if temperature out of the optimal range affects *T. notata* reproduction due to male sterility, then females coupled with acclimated males would suffer a decline in fertility. Our research will be useful to analyze the physiological plasticity and performance of a predator, regarding its adaptation and establishment under different temperatures conditions.

Material and Methods

Insects. This study was carried out at the Laboratory of Insect Behavior of the Universidade Federal Rural de Pernambuco (UFRPE), Recife, Brazil. The mealybug *Ferrisia dasyliirii* (Cockrell) was collected from cotton plants in the experimental area of the UFRPE (Recife, Pernambuco, Brazil 8.017070°S, 34.944362°W) identified by Vitor Cezar Pacheco da Silva and the ladybeetle *T. notata* was collected from cotton plants infested with the cotton mealybug *Phenacoccus solenopsis* Tinsley (Surubim, Pernambuco, Brazil, 7.833056°S, 35.754722°W) identified by José Adriano Giorgi. The colonies were maintained under laboratory conditions (28

± 2 °C; $65 \pm 5\%$ R.H.; 12 L: 12D), previously described as ideal for reproduction and maintenance of those insect species (Ferreira *et al.* 2020).

The mealybug colony was reared on pumpkins (var. Jacarezinho), following the methodology established by Sanches & Carvalho (2010) and adapted to our conditions (Oliveira *et al.* 2014). Briefly, the pumpkins were infested by gravid mealybug females and spent about 30 days to be fully covered with the mealybug colony. Mealybug infested pumpkins were offered to *T. notata* as prey, in transparent Plexiglass™ cages (40 × 25 × 20 cm), wherein the ladybeetle completed its development. For the experiments, pupae of *T. notata* were collected and after adult emergence, they were separated by sex by morphological differences, females have two black spots on the superior portion of the head between the eyes and are usually larger than males (Barbosa *et al.* 2014) and kept individually on plastic Petri dishes (5 cm diam) before submitting them to acclimation treatments.

Survival and reproduction of *Tenuisvalvae notata* under Acclimation Conditions. The aim of this experiment was to evaluate the reproduction and survival of *T. notata* adults submitted to thermal acclimation at high and low temperatures. The temperatures were chosen based on the study of Ferreira *et al.* (2020) and Dreyer (1997a) that showed a limit of development and reproduction of the species under temperatures below 18 °C and above 36 °C.

The adults (<24h old) were subject to thermal acclimation by increasing or reducing the temperature at the rate of 1°C per day, into growth chambers (BOD Incubator), which reached the maximum/minimum temperature treatment as follow. Low temperature treatments were 12, 14, 16 and 18 °C, whereas high temperature treatments were 32, 34, 35 and 36 °C, respectively. When the temperature (low or high) was reached, more than 20 couples were formed and allowed to mate for 24h. Females were isolated from males and reared under the same temperature condition

after acclimation. Daily oviposition and egg viability were recorded for 60 days from 20 females that had oviposition. Female survival was also monitored in each treatment condition.

Predatory Capacity of *Tenuisvalva notata* after Acclimation. The aim of this experiment was to assess the effects of acclimation on the predatory behavior of *T. notata* upon *F. dasyliirii*. Thus, adult females (5-10 days old) subjected to acclimation process as previously described to 34, 32, 18 and 16 °C and under the control (rearing) temperature of 28°C, were isolated and placed in plastic Petri dishes (5 cm diam). The predators were starved for 24h prior the experiment set up to standardize their hunger level. According to Barbosa *et al.* (2014), *T. notata* adults consume an average 2.2 *F. dasyliirii* adult females, or 3.6 3rd-instar nymphs per day. Therefore, in this bioassay, adult *T. notata* females were provided 1, 2, 4, 6, and 8 *F. dasyliirii* adult females per day with 20 replicates per prey density. The consumption was recorded after 24h.

Impact of Low and High Temperature on *Tenuisvalvae notata* Egg Viability. Virgin *T. notata* adults (male and females) 5-10 days old were subjected to acclimation temperatures of 16, 18, 32 and 34 °C, respectively. Acclimation was obtained as previously described, starting from the optimal rearing temperature of 28 °C for *T. notata* (Ferreira *et al.* 2020). In order to understand whether temperature would cause male or female sterilization, couples were formed according to the following treatments: i) females *T. notata* from 28 °C (n = 20) rearing condition vs. acclimated males of each low (16 °C, n = 16, and 18 °C, n = 16) and high temperatures (32 °C, n = 12, and 34 °C, n = 13) (to evaluate male); ii) males *T. notata* from 28°C rearing condition vs. acclimated females of each low (16 °C, n = 20, and 18 °C, n = 13) and high temperatures (32 °C, n = 12, and 34 °C, n = 18) (to evaluate females). The established pairs of male and female were observed until the first mating and, after accomplishing a mating, males were removed. The females were placed under their acclimation or rearing temperature conditions, and fecundity and egg hatching rate were recorded for 60 days.

Egg viability of *Tenuisvalvae notata* under Low Temperatures. This experiment evaluated the effects of low-temperature acclimation and male presence on fertility (evaluated as egg hatching ability). Thus, after adult emergence, they were subjected to acclimation up to 16 °C, as previously described. Pairs were formed and allowed to mate in a Petri dish (5 cm diam) and reared under the acclimation temperature. These male and female pairs were assigned to the following treatments: i) pairs were separated after 24h; ii) pairs maintained together for 20 days. Each treatment had 20 replicates. Female oviposition was tallied daily, and 50% of eggs were subjected to the optimal temperature of 28 °C (control) and 50% of eggs subjected to 16 °C. Egg hatching was recorded daily according to the female condition (alone or paired) and temperature (control or acclimation) for ascertain about fertility.

Statistical Analyses. Data of female survival and time of oviposition were subjected to the Weibull frequency distribution through the survival package in the software R (R Development Core Team 2020). Differences between treatments were evaluated by aggregating factor levels until the consequential change in deviance was significant at ($P < 0.05$).

Data of female fecundity and egg hatching rate were subjected to the Shapiro-Wilk normality test. As data were not normally distributed, they were subjected to analysis of deviance (ANODEV) through the generalized linear model (GLM) with Poisson distribution, with dispersion corrected by the quasi-Poisson function, followed by an analysis of residuals to verify the adequacy of error distribution, and model construction. The data of egg viability were subjected to analysis of deviance (ANODEV) through general linear models GLM with Binomial distribution. The difference among treatments was evaluated through the analysis of contrast ($P < 0.05$), as described by Crawley (2007).

The functional response of acclimated *T. notata* preying upon *F. dasyliirii* adult females was estimated according to Barbosa *et al.* (2014). The functional response curve is based on a logistic

regression of the number of mealybugs consumed per density, determined using the CATMOD procedure in SAS (SAS Institute 2002), the equation obtained was used to determine the type of functional response, as described by Juliano (1993). The determining handling time (T_h) and attack rate (a') parameters were estimated using nonlinear least square regression based on the proportion of prey eaten (N_a/N_0) as a function of initial prey densities (N_0) (PROC NLIN, SAS Institute 2002). Comparisons of functional response parameters T_h and a' determined for different temperature acclimation were performed using the 95% confidence interval mean overlapping procedure (Di Stefano 2005).

Results

Reproduction and Survival of *Tenuisvalvae notata* under Acclimation Conditions. Ladybeetles subjected to acclimation to high (above 28 °C) and low (below 20 °C) temperatures exhibited significant reduction in fecundity. There was a significant reduction in the number of eggs laid when females were subjected to temperatures below ($F_{4,95} = 179.93$, $P < 0.001$) and above 28 °C ($F_{4,95} = 65.49$, $P < 0.001$) (Figs. 1A and C). Moreover, egg hatching rate was also affected by higher ($F_{4,95} = 139.44$, $P < 0.001$) and lower temperatures ($F_{4,95} = 414.86$, $P < 0.001$) (Figs. 1B and D). When lady beetles were acclimated to higher temperatures, egg hatching was reduced. In addition, very few eggs hatched at 35 °C and no eggs hatched at 36 °C. Similarly, when lady beetles were acclimated to lower temperatures, the effect on egg hatching was even greater, there was a decrease on hatched eggs at 18 °C, and no hatching was observed under lower temperatures.

In addition, the oviposition period was significantly reduced with the increase in temperature from 28 to 36 °C ($\chi^2 = 155.36$, $df = 4$, $P < 0.001$) (Fig. 2A). The average oviposition period for females reared at 28 °C was $49.3.7 \pm 3.4$ days, and decreased to 3.65 ± 0.6 days at 36 °C (Fig. 2A). In contrast, oviposition period was significantly increased when females were acclimated at 18 °C

(57.3 ± 0.9 days) and $16\text{ }^{\circ}\text{C}$ (52.6 ± 1.4 days). However, a sharp reduction is observed, on average, of 13.8 ± 1.1 , and 11.4 ± 1.4 days, when females were acclimated to $14\text{ }^{\circ}\text{C}$ and $12\text{ }^{\circ}\text{C}$, respectively ($\chi^2 = 144.28$, $df = 4$, $P < 0.001$) (Fig. 2B).

Ladybeetle survival was not affected by acclimation at low temperatures. In lower temperatures, all female lived for the whole evaluation period of 60 days ($\chi^2 = 4.3$, $df = 4$, $P = 0.37$). In contrast, survival was affected when the lady beetles were acclimated to high temperatures. Survival decreased significantly to less than 30 days at 35 and $36\text{ }^{\circ}\text{C}$ ($\chi^2 = 132.06$, $df = 4$, $P < 0.001$, Fig. 3).

Predatory Capacity of *Tenuisvalva notata* after Acclimation. Regardless of temperature acclimation, *T. notata* females preying upon *F. dasyliirii* exhibited a type II functional response (Table 1). Females *T. notata* consumed an average of 0.126 ± 0.051 mealybug per day, with a higher attack rate at $34\text{ }^{\circ}\text{C}$, not statistically different from other temperatures. For most temperatures, the handling time was not affected, except for $32\text{ }^{\circ}\text{C}$, when the handling time was significantly reduced in comparison to $28\text{ }^{\circ}\text{C}$ (Table 2). The ratio between the exposure and handling time represents the potential number of prey consumed per day (T/Th ; Table 2). Therefore, *T. notata* females may consume up to 3.48 females of *F. dasyliirii* at $32\text{ }^{\circ}\text{C}$, per day.

Impact of Low and High Temperature on *Tenuisvalvae notata* Egg Viability. Regardless of whether females were acclimated to low ($F_{2,50} = 128.4$, $P < 0.001$) or high ($F_{2,47} = 29.56$, $P < 0.001$) temperatures, they laid significantly fewer eggs compared to those reared under $28\text{ }^{\circ}\text{C}$ (Fig. 4A). Similarly, when only males were subjected to acclimation there was a significant reduction in female fecundity at high ($F_{2,49} = 65.51$, $P < 0.001$) and low temperatures ($F_{2,42} = 21.625$, $P < 0.001$) (Fig. 4C).

Regarding the egg hatching, there was a significant reduction when females were subjected to acclimation at low temperatures, and eggs did not hatch at $16\text{ }^{\circ}\text{C}$ ($df = 2,50$; Deviance = 32.52; P

< 0.001) (Fig. 4B). In contrast, there was no significant effect on fertility when only males were acclimated at low temperatures (df = 2, 42, Deviance = 1.18, P = 0.556) (Fig. 4D). Similarly, there was no effect of high temperatures on fertility for acclimated females (df = 2, 47, deviance = 0.43, P = 0.805) and males (df = 2, 49, Deviance = 2.13, P = 0.345).

Overall, there was a significant reduction in the average number of oviposition days when female and males were subjected to acclimation at high and low temperatures, in comparison to those maintained under 28 °C (Fig. 5), except for females mated with males acclimated at 18 °C, which did not differ from those kept at 28 °C.

Whereas females kept at 28 °C oviposited for an average (49.3 ± 3.4) of 60 days, females acclimated to low temperatures oviposited for 41.8 ± 4.6 days at 18 °C and for 42.0 ± 1.9 days at 16 °C ($\chi^2 = 7.23$, df = 2,48, P = 0.027). Moreover, females acclimated at high temperatures oviposited for 45.2 ± 3.3 days at 32 °C and for 38.0 ± 2.0 days at 34 °C differing from females reared under optimal temperatures ($\chi^2 = 9.66$, df = 2, 47, P < 0.0001) (Fig. 5). In addition, females paired to males acclimated at 16 °C oviposited for 38.6 ± 3.9 days ($\chi^2 = 7.28$, df = 2, 42, P = 0.026), for 23.3 ± 2.8 days, and 16.4 ± 2.5 days when mated with males acclimated at 32 °C and 34 °C, respectively ($\chi^2 = 45.86$, df = 2, 49, P < 0.0001) (Fig. 5).

Egg viability of *Tenuisvalvae notata* at Low Temperatures. Temperature at which eggs were subjected after oviposition significantly affected egg viability (df = 1, 137, Deviance = 73.98, P < 0.001), as well as temperature of adult acclimation after emergence (df = 1, 136, Deviance = 8.035, P = 0.0045). However, there was no effect of the presence of males on female fertility (df = 1, 135, Deviance = 0.196, P = 0.66), nor the interaction of those factors (df = 1, 134, Deviance = 0.238, P = 0.63).

Finally, when the parents were subjected to the optimal temperature of 28 °C and the eggs were submitted at the same temperature, egg hatching was about 80%. In contrast, when those eggs were

incubated at 16 °C, there was a significant reduction in egg viability to almost no eggs hatching (Fig. 6). Moreover, when parents were subjected to low temperature of 16 °C and eggs at 28 °C, egg hatching was also significantly reduced in comparison to those kept under optimal conditions, and eggs did not hatch at 16 °C.

Discussion

Abiotic factors, in special, temperature variation can alter the insects' population growth (David *et al.* 2005). Besides, the behavior, development and reproduction of insects are favored within an optimal temperature range between 15 and 30 °C, which varies with the species (Chown & Nicolson 2004). In this context, insect species considered as tropical or subtropical are favored by warmer temperatures, whereas species that occur in temperate regions are favored by a mild climate (David *et al.* 2005). However, due to a series of factors, such as i) high dispersal capacity of insects; ii) introduction of exotic species as a result of globalization; and iii) climate changes due to natural phenomenon, the insects need to adapt to new environmental conditions to guarantee survival and reproduction. Therefore, insect acclimation to new temperature conditions will promote an adaptive physiological response to favor the insect survival in such places, and this adaptation will be reflected in its population density.

In the present study, we found that the lady beetle *T. notata* can acclimate to changes in temperature, above or below 28 °C, its optimal temperature (Ferreira *et al.* 2020). After acclimation, *T. notata* adults survived on a temperature range from 12 °C to 36 °C fitting to the hypothesis of trade-offs of guaranteeing energy for survival in expenses of reproduction under temperature stresses. Moreover, both fecundity and fertility of *T. notata* were affected by extreme low and high temperatures. Thus, it is expected that after acclimation *T. notata* can survive in places where the temperature is outside its optimal range, but it will have a decline in its

population over time if the temperature does not return to the range from 20 to 32 °C (Ferreira *et al.* 2020).

Individuals that spend more energy on reproduction usually have a reduction in longevity in comparison to those that do not mate and reproduce (Dixon & Dixon 2000, Mishra 2005, Mirhosseini *et al.* 2014). For *T. notata*, acclimated insects responded with a reduction in fecundity and fertility. Similarly, Ferreira *et al.* (2020) found that 36 °C is the maximum threshold temperature for *T. notata* survival. Nevertheless, acclimated adults were able to survive in temperatures up to 36 °C, and females were able to lay eggs for about 10 days, but lack egg hatching. Likely, the long exposition of the eggs in temperatures above 32 °C may impose difficulties to the embryo development due to protein denaturation, less amount of glycogen, loss of water, and lower oxygen level beyond the physiological limits that would allow embryo survival and development (Geister *et al.* 2009, Zhang *et al.* 2014, Koch 2015). In addition, studies have shown that temperatures above 35 °C can reduce the eclosion and survival of larvae of different ladybeetles species, including *C. montrouzieri*, *Nephus includens* Kirsch, *Rodolia cardinalis* (Mulsant), and *Oenopia conglobata* L. (Canhilar *et al.* 2001, Grafton-Cardwell *et al.* 2005, Solangi *et al.* 2013, Jalili *et al.* 2018).

The developmental rate of insects follows the degree-days model, and the lower threshold temperature induces development metabolism (Campbell *et al.* 1974). According to Ferreira *et al.* (2020), 14 °C is the lower temperature threshold for *T. notata* development. The current study showed that at low temperatures (< 18 °C), acclimated *T. notata* females were able to survive, but had a gradual reduction in the total oviposition period, fecundity, and fertility. Since temperatures of 12 °C and 16 °C allowed *T. notata* survival, but not its reproduction, it is clear that there exist a trade-off. Therefore, it's possible that the environmental conditions under lower temperatures in this study have not supplied enough heat energy for embryo development and egg eclosion.

Because *T. notata* eggs were not viable under temperatures below 16 °C, it is expected that through time this would cause a decline in population numbers of this species if temperature conditions do not increase above 20 °C. Also, previous studies have shown that lady beetle eggs are more sensitive to changes in temperature, whereas adults and larvae can adjust themselves in the environment via behavioral responses (i.e. move to areas of microclimate more favorable) (Chown & Nicolson 2004, Chen *et al.* 2019).

Another hypothesis regarding lower temperature effects on *T. notata* reproduction is some kind of reproductive diapause, or physiological adaptation to allow the population persistence in some areas through time of adverse conditions. Some lady beetle species from temperate regions enter diapause or quiescence as adults (Sakaki *et al.* 2019), induced by a reduction in the photophase (i.e. number of light hours in 24h). For instance, a reduction in the number of light hours increase developmental times and induce a reproductive diapause in *Coelophora saucia* (Mulsant), *Harmonia axyridis* (Pallas), *Hippodamia convergens* Guérin-Méneville, and *Hippodamia variegata* (Goeze) (Omkar & Pathak 2006, Reznik & Vaghina 2013, Obrycki 2020), in response to the limitation in food availability and quality as a function of environmental conditions found by the insects (Michaud & Qureshi 2006). Lady beetle species capable of avoiding reproduction during time of unsuitable environmental conditions can benefit from a reduction in energy demand when prey is also scarce (Hodek 1986, Iperti 1999, Michaud & Qureshi 2005). Besides, different genotypes or populations of the same species can differ in their tendency to enter diapause, according to environmental conditions (Hodek 1986, Iperti 1999, Obrycki 2020). As reported, in Paraguay *T. notata* was found in areas of freezing (0 °C) temperatures and short days during the winter (Dreyer *et al.* 1997b). In contrast, other lady beetle species such as *Lindorus lophantae* Blaisdell, *C. montrouzieri*, and *R. cardinalis*, do not enter diapause, but their larvae are capable of resisting climate changes through a slower developmental

time, and the adult increases pre-oviposition period in the winter (Özgökçe *et al.* 2006, Sakaki *et al.* 2019). Further studies are needed to clarify whether or not *T. notata* is capable of entering reproductive diapause or have other adaptations to sustain its population in an area under lower temperatures for longer periods.

On the other hand, *T. notata* response to higher temperatures may indicate whether the species will be able to adapt to global warming. Global warming can affect the period of development and distribution of insects induce a difference of presence in time and space that coccinellid and prey species could coexist (Honek *et al.* 2017). Prey related to *T. notata*, such as *Maconellicoccus hirsutus* and *P. solenopsis*, develop at temperatures between 15 and 35 °C (Chong *et al.* 2008, Barbosa *et al.* 2014, Peronti *et al.* 2016) and benefit from the increase in temperature, with a shorter life cycle and increasing reproductive rate, like other species of Pseudococcidae (Oliveira *et al.* 2014, Bertin *et al.* 2018). This predator is reported in places of high temperatures in the semiarid region of Pernambuco (Barbosa *et al.* 2014, Torres & Giorgi 2018), which suggests that the species can present behavioral and physiological strategies to survive these climatic conditions.

Regarding predation rate, it was expected that with the increase in temperature the lady beetles would respond with an increase in mealybug predation, due to a higher metabolism and energetic cost of predators under this condition (McCoull *et al.* 1998), until reaching a metabolic threshold activity (Brown *et al.* 2004, Sentis *et al.* 2012). In this study, *T. notata* showed an increase in predation rate when subjected to 32 °C, as a consequence of a reduction in handling time as expected in warmer conditions (Sentis *et al.* 2012). However, there was a decline in predation rate at temperatures ≥ 34 °C, suggesting that predation rate increases with temperature up to an optimal threshold (Jalali *et al.* 2010, Sentis *et al.* 2012). The daily consumption rate of immature and adult coccinellids increases with temperature within the range that favors their

survival and development (Ives 1981, Dixon *et al.* 2009). According to Barbosa *et al.* (2014), *T. notata* shows a decrease in the proportion of prey consumed as prey density increases at 25 °C, reflecting a type II functional response. This decrease in predation rate can be a result of the wax covering the body of adult mealybugs, as adult females were provided as prey, affecting prey-handling time and attack rate (Barbosa *et al.* 2014), as these variables are a result of the behavioral interactions between predator-prey, including searching, camouflage, attack, and escape (Englund *et al.* 2011). In addition, a type II functional response was found to the lady beetles *C. montrouzieri* (Qin *et al.* 2019) and *Hyperaspis polita* Weise (Seyfollahi *et al.* 2019) that prey on adult mealybugs as well.

Previous studies have suggested that a reduction in insect reproduction can be related to effects of temperature variation on spermatogenesis, through direct injuries on testicles, and seminal vesicles being emptied, or with a reduced amount of sperm in Diptera, Coleoptera, and Hymenoptera insects (David *et al.* 2005, Nguyen *et al.* 2013, Zhang *et al.* 2014). Therefore, we hypothesized that females mated with males under high and low temperatures would have a reduction in fertility, due to male sterility. In contrast, we found that the temperature reduced fecundity and fertility of *T. notata* when both sexes were subjected to acclimation. Especially, when *T. notata* females were exposed to temperatures below 18 °C, they had a significant reduction in fitness, even when egg laid were subjected to the temperature of 28 °C after oviposition. These findings suggest that to have a good reproductive performance, both males and females *T. notata* need favorable temperatures for their own development, oviposition, and F1 embryo development. Other coccinellids such as *Cheilomenes sexmaculata* F. when subjected to temperatures above 35 °C also had a reduction in fertility and fecundity (Wang *et al.* 2013, Mirhosseini *et al.* 2014). In this context, some studies have found that high temperatures may cause thermal shock, and injuries on oocytes and ovarian development of insects (Krebs &

Loeschcke 1994, Rinehart *et al.* 2000, Zhang *et al.* 2014). However, the effects of thermal stress on insect reproduction can not be analyzed separately by gender, when many studies have shown that each insect species can express its vulnerability to thermal stress differently in males and females (Janowitz & Fisher 2011, Porcelli *et al.* 2017). Besides, from our results, we cannot assure yet that *T. notata* males suffer thermal sterilization, as both males and females when acclimated impacted *T. notata* reproduction. Therefore, further studies regarding the direct observation and dissection of *T. notata* gonads (testicles and ovaries) after acclimation are necessary to better understand the impact of temperature fluctuation on its reproduction.

In conclusion, the acclimation of *T. notata* adults to temperatures between 12 to 18 °C and 32 °C to 36 °C, can favor its survival in areas where the temperature is limiting to its development. Also, if food is available under these temperature conditions, *T. notata* adults do not change their daily predation rate, with only a small change in prey handling time, and remain with a type II functional response, which is common to insect predators (Jalali *et al.* 2010, Sentis *et al.* 2012). Moreover, this is the first report of acclimation improving survival of *T. notata* that might improve survival of predators after release under similar conditions, or help to maintain insect adults alive in rearing facilities according to demand for field releases. Nonetheless, it is evident that even with the acclimation of adults under temperatures out of the optimal range *T. notata* suffers impact on its fitness, with difficulties in the establishment of viable progeny under conditions of thermal stress, showing a possible progressive decline in population numbers after few generations. It is important to say that biological and behavioral studies under more natural settings with temperature fluctuation throughout the day and seasons, and its effects on developmental time, oviposition sites, offspring production distribution on plant canopy, and foraging time, are necessary.

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Table1. Estimated parameters of the proportions of *Ferrisia dasyliirii* adults consumed by *Tenuisvalvae notata* females under different temperatures.

Temperatures	Parameter	Value (\pm SE)	χ^2	P
16°C	Intercept	0.3907 \pm 0.2858	1.87	0.1716
	Linear	-0.2373 \pm 0.0493	23.19	<0.0001
	Quadratic	ns ¹	-	-
	Cubic	ns ¹	-	-
18°C	Intercept	0.4876 \pm 0.2815	3	0.0832
	Linear	-0.2317 \pm 0.0481	23.21	<0.0001
	Quadratic	ns ¹	-	-
	Cubic	ns ¹	-	-
28°C	Intercept	2.4771 \pm 0.6228	15.82	<0.0001
	Linear	-0.8612 \pm 0.2591	11.05	0.0009
	Quadratic	0.0552 \pm 0.0245	5.09	0.0241
	Cubic	ns ¹	-	-
32°C	Intercept	2.6897 \pm 0.3642	54.55	<0.0001
	Linear	-0.3898 \pm 0.0559	48.7	<0.0001
	Quadratic	ns ¹	-	-
	Cubic	ns ¹	-	-
34°C	Intercept	8.0870 \pm 2.1952	13.57	0.0002
	Linear	-4.3383 \pm 1.4665	8.75	0.0031
	Quadratic	0.7502 \pm 0.3025	6.15	0.0131
	Cubic	ns ¹	-	-

¹ns, stand for parameters lacking statistical significance requiring reduction to lower level equation models.

Table 2. Means (\pm 95% confidence intervals) of attack rate, handling time, and estimated number of adult *Ferrisia dasyliirii* killed per day by *Tenuisvalvae notata* females.

Temperatures (°C)	Attack rate [a (h ⁻¹)]	95% CI	Handling time [T _h (h)]	95% CI	Estimated no. of killed prey.day ⁻¹
16	0.043a	0.026-0.061	15.2a	16.3-14.1	1.58
18	0.048a	0.028-0.068	14.1a	15.1-13.1	1.7
28	0.074a	0.049-0.099	10.8a	11.3-10.2	2.22
32	0.11a	0.171-0.039	6.9b	7.4-6.4	3.48
34	0.13a	0.176-0.075	9.5b	9.9-9.0	2.54

¹Means within a column followed by the same letter are not significantly different (pairwise comparisons through 95% confidence intervals).

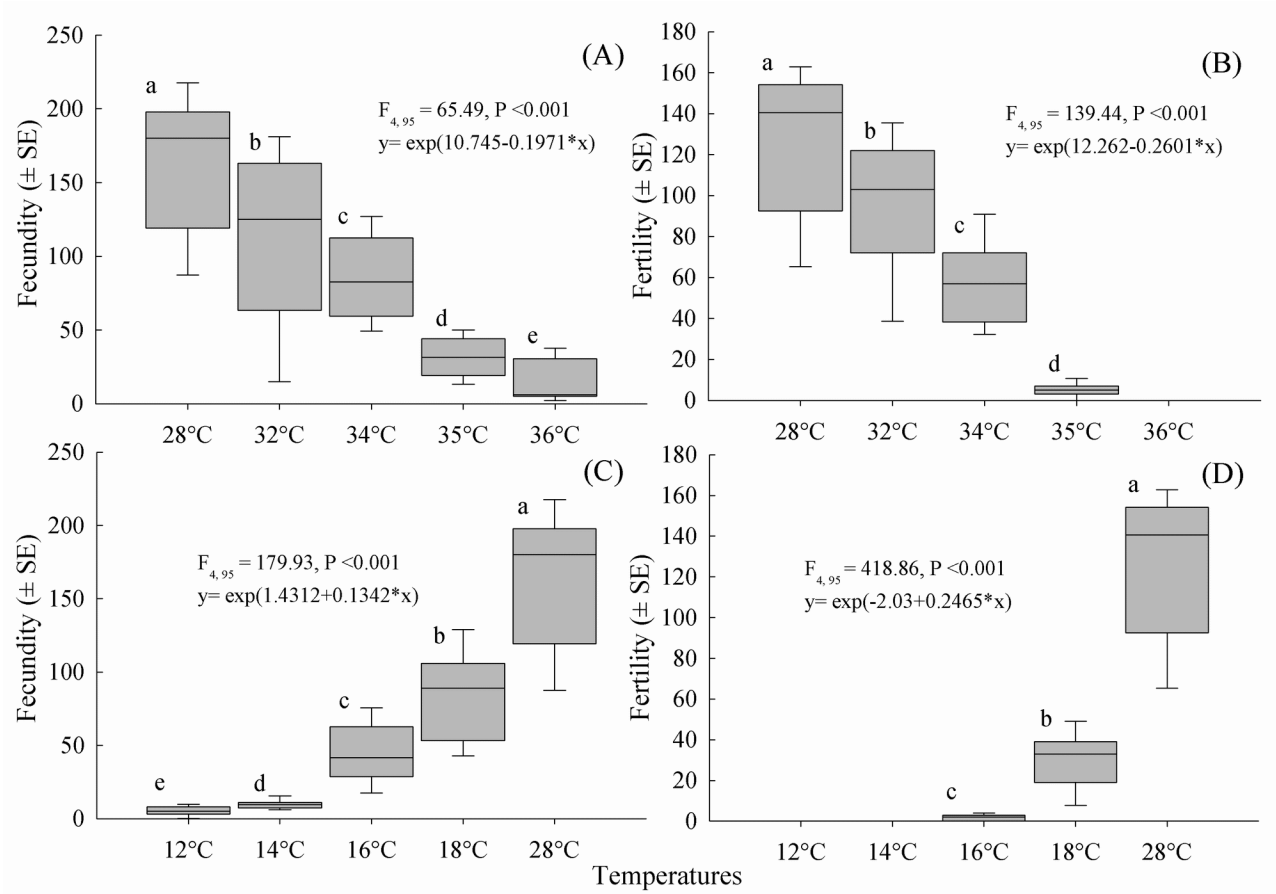


Figure 1. Average (± SE) number of eggs laid (fecundity) by *Tenuisvalvae notata* and egg hatching (fertility) after acclimation to high (A, B) and to low (C, D) temperatures during 60 days observation period.

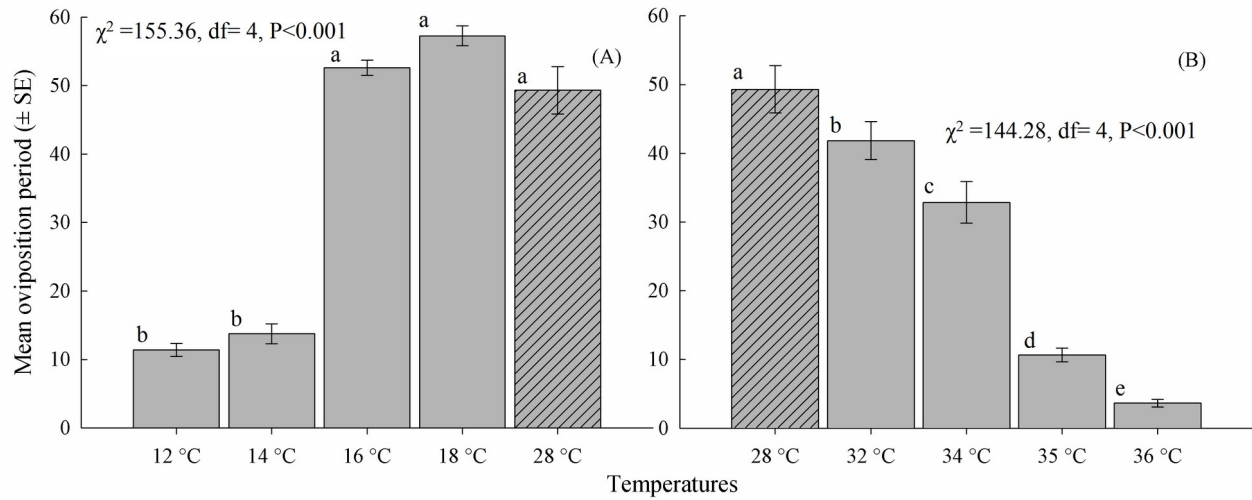


Figure 2. Average (\pm SE) oviposition days of *Tenuisvalvae notata* after acclimation to low temperatures (A) and to high temperatures (B), during 60 days observation period.

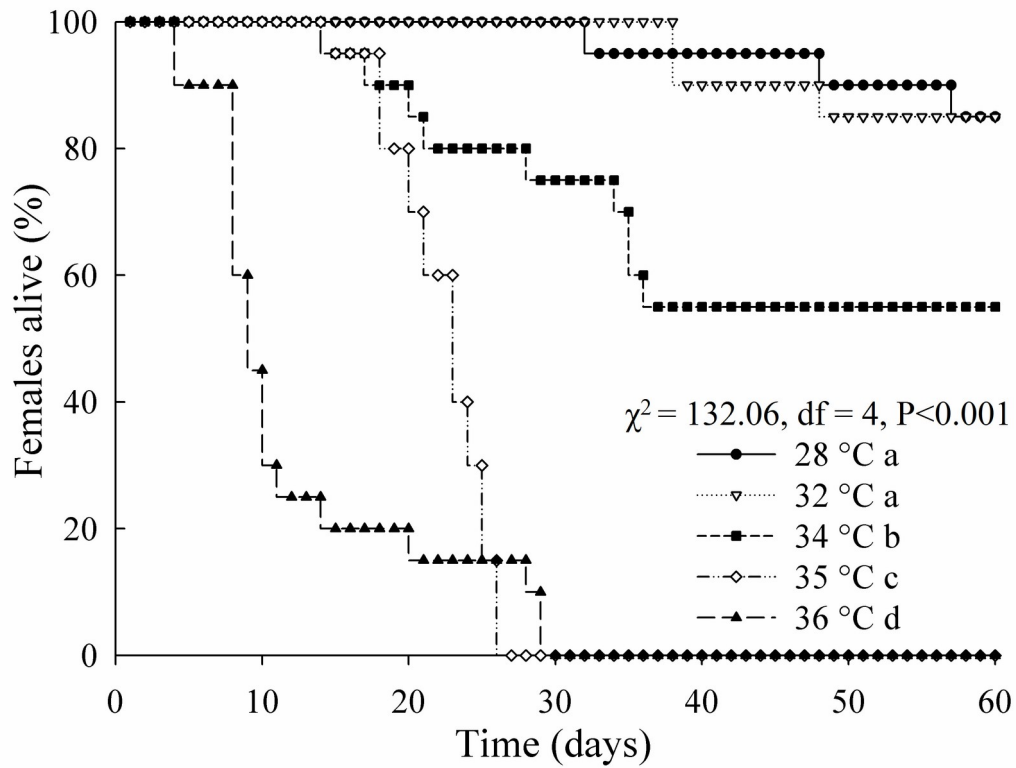


Figure 3. Survival (%) of *Tenuisvalvae notata* females under different acclimation temperature during 60 days observation period (censored Weibull model). Shape parameter taken as $\alpha = 2.29$.

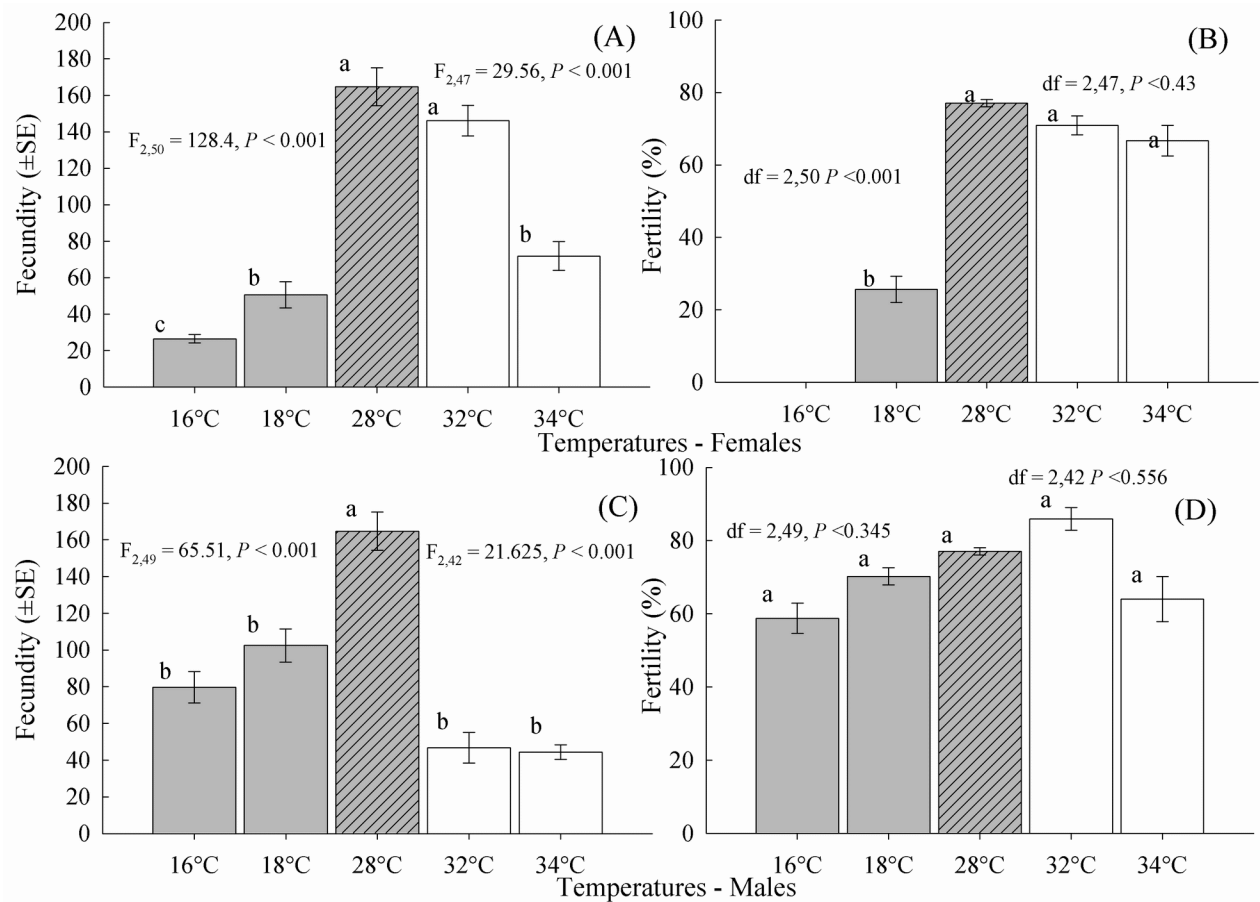


Figure 4. Average (\pm SE) fecundity and fertility of *Tenuisvalvae notata* subjected to low (16 °C and 18 °C) - gray bars and to high (32 and 34 °C) - empty bars, after acclimation to temperatures and paired to a partner (male or female) from the control temperature 28 °C (dashed bars). Note: In A and B, females were acclimated, whereas in C and D males were acclimated prior to mating.

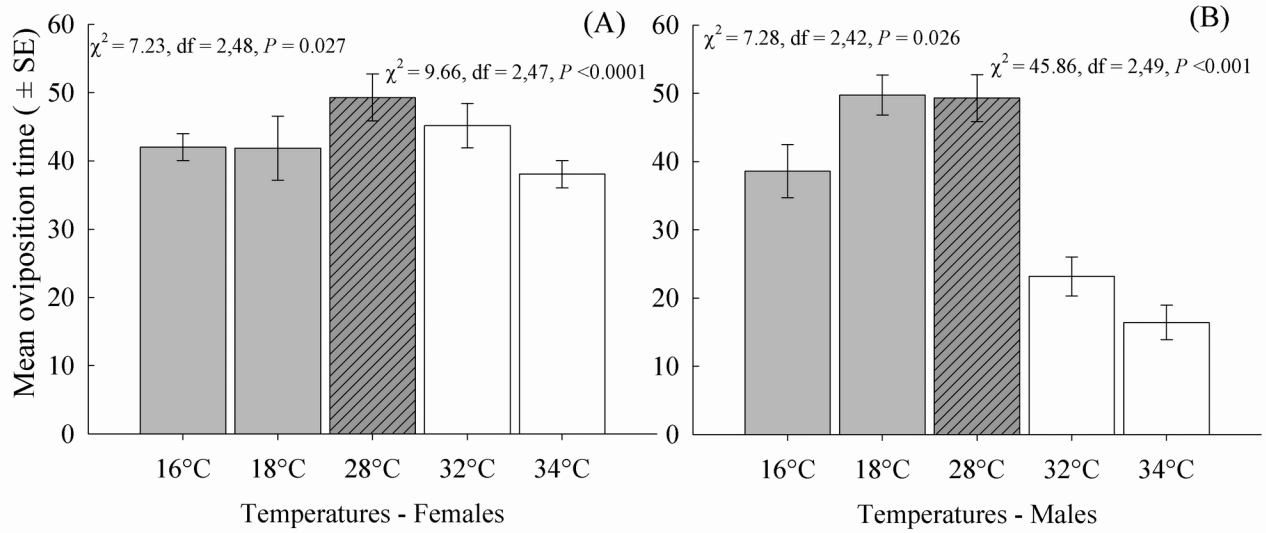


Figure 5. Average (\pm SE) of reproductive days of *Tenuisvalvae notata* subjected to low (16 °C and 18 °C) - gray bars, and to high (32 and 34 °C) - empty bars, acclimation temperatures and paired to a partner (male or female) from the control temperature 28 °C – dashed bars. Note: In A, females were acclimated, whereas B males were acclimated prior to mating.

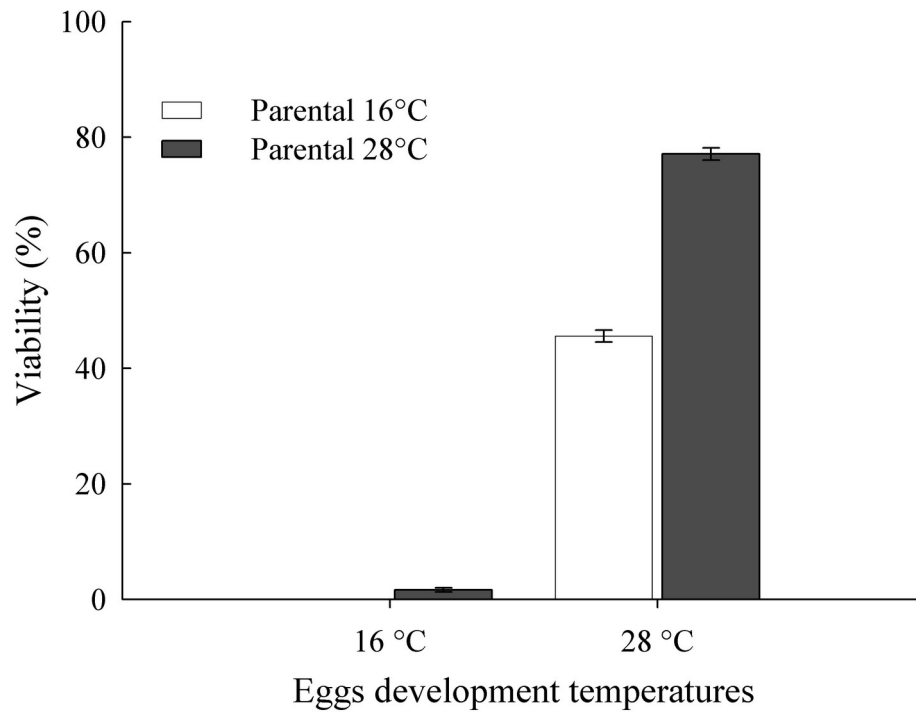


Figure 6. Egg fertility (egg hatching \pm SE) when these eggs were subjected to two different temperatures (28° or 16 °C) of parental development. Means are significantly different ($P < 0.001$).

CONSIDERAÇÕES FINAIS

A temperatura é um dos principais fatores abióticos com efeito limitante para o estabelecimento e desenvolvimento das espécies, e uma das principais características observadas em uma área ao se ter a intenção de liberar um inimigo natural em campo. Nesse contexto, a aclimatação é uma técnica utilizada em várias práticas, com o objetivo de conseguir uma melhor resposta de um organismo quando exposto a um ambiente diferente ao qual está habituado, induzindo este a uma breve adaptação. A joaninha *Tenuisvalvae notata* é um importante agente de controle biológico de cochonilhas-farinhentas. Portanto, é importante investigar o possível o impacto que esta espécie sofrerá ao ser criada de forma massal em condições ideais, para posteriormente ser liberada em campo. Assim, este estudo demonstrou o potencial de aclimatação da espécie em uma faixa de temperatura mais ampla e seus efeitos na predação, desenvolvimento, reprodução e sobrevivência, sob condições de temperatura similares àquelas relatadas nas áreas de ocorrência natural bem como de estimativas de distribuição global a partir de modelos bioclimáticos.

Este estudo também buscou entender, as causas de uma redução ou ausência de fertilidade quando o inseto é inserido num ambiente com temperaturas desfavoráveis. A partir dos resultados, sugere-se que a redução na reprodução não se dá apenas pelo efeito de esterilização de um dos sexos, visto que tanto os machos como a fêmea apresentaram redução no desempenho reprodutivo após aclimatação. É possível que outros fatores possam estar relacionados, tais como alterações comportamentais e fisiológicas, desde o comportamento de cópula até a falta de fornecimento de energia para o desenvolvimento do embrião.

Em relação à capacidade predatória, observamos que *T. notata* após aclimação não altera no geral sua eficiência de predação, se mantendo com reposta funcional do tipo II, com o aumento da densidade de presas e variação da temperatura fora da faixa ideal, mesmo que tenham apresentado alterações no tempo de manipulação de presas em temperaturas mais elevadas.

A espécie *T. notata* se desenvolve numa faixa de temperatura de 20-32 °C, na qual consegue se reproduzir, e responder bem a aclimação em relação a sua sobrevivência. Logo, seria uma espécie viável para ser introduzida em diferentes áreas, em relação à amplitude térmica. Entretanto, devido, ao efeito da temperatura sobre a reprodução que impedem o desenvolvimento da prole, seriam necessárias liberações em locais de temperatura fora da faixa ótima.