EFEITOS SUBLETAIS DE ACARICIDAS UTILIZADOS NO CONTROLE DE *Aceria guerreronis* KEIFER (ACARI: ERIOPHYIDAE) SOBRE *Neoseiulus baraki* (ATHIAS-HENRIOT) (ACARI: PHYTOSEIIDAE)

por

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RESUMO

Aceria guerreronis Keifer é uma das principais pragas do coqueiro no mundo, sendo a aplicação de acaricidas o principal método de controle utilizado contra essa espécie. Contudo, o sucesso do controle químico depende de aplicações corretas e frequentes de acaricidas. Assim, particular atenção tem sido dada a busca de um predador que possa ser eficiente no controle biológico de *A. guerreronis.* Dentre os predadores associados a *A. guerreronis*, *N. baraki* destaca-se como promissor no controle dessa praga. Este estudo teve como objetivo avaliar o efeito subletal de acaricidas utilizados contra *A. guerreronis* em *N. baraki*. Os seguintes parâmetros foram avaliados: atividade global da população, comportamento de acasalamento, resposta funcional, tabela de vida de fertilidade de fêmeas expostas a acaricidas e de sua prole, e comportamento de forrageamento. Efeitos subletais de acaricidas foram observados em todos os parâmetros avaliados. A atividade global e o comportamento de acasalamento do predador foram afetados por azadiractina. O tipo de resposta funcional e tempo de manipulação da presa (*Th*) não foram alterados, mas a taxa de ataque (*a'*) foi reduzida por fenpiroximato e abamectina, esse último também reduziu o pico de consumo. Fêmeas expostas a abamectina (F_0) não produziram

descendentes, fenpiroximato não afetou os parâmetros de tabela de vida das fêmeas expostas (F_0), mas afetou os descendentes produzidos (F_1). Alterações na taxa instantanea de crescimento (r_i) da segunda geração (F_2) não foram observadas. O forrageamento de predadores expostos aos acaricidas foi comprometido, eles não foram capazes de distinguir entre frutos infestados e não infestados. Os acaricidas afetaram pelo menos um parâmetro do predador avaliado indicando que o uso contínuo desses acaricidas pode comprometer a eficiência do controle biológico de *A. guerreronis* por *N. baraki*.

PALAVRAS-CHAVE: Ácaro, predador, controle biológico, controle químico, manejo, seletividade.

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ABSTRACT

Aceria guerreronis Keifer is one of the most important pests of coconut worldwide, and acaricide spraying is the control method most used against this species. However, chemical control success depends on frequent and correct applications of acaricides. Thus, particular attention has been devoted to find a predator that can be effective in *A. guerreronis* biological control. Among the predators associated with *A. guerreronis, N. baraki* stands out as the most promising. This study aimed to evaluated the sublethal effects of acaricides used against *A. guerreronis* on *N. baraki*. The following parameters were evaluated: overall predator activity, mating behaviour, functional response, life table of females exposed to acaricides and their offspring, and forraging behaviour. Sublethal effects of acaricides were observed in all parameters evaluated. The overall activity and the mating behaviour of the predator were affected by azadirachtin. The type of functional response and prey handling time (*Th*) were not altered, but the attack rate (*a*') was reduced by fenpyroximate and abamectin, this last also reduced the consumption peak. Exposed females to abamectin (F_0) did not produce offspring, fenpyroximate did not affect the life table parameters of exposed females (F_0), but affected the offspring (F_1).

Alterations on instantaneous rate of increase (r_i) on the 2nd generation (F_2) were not observed. The foraging of predators exposed to acaricides was impaired, since they were not able to distinguish between infested and uninfested fruits. All acaricides tested affected at least one parameter of the predator, indicating that the frequent use of one of these acaricides might impar the effciency of the biological control of *A. guerreronis* by *N. baraki.*

KEY WORDS: Mites, predator, biological control, chemical control, management, selectivity.

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CAPÍTULO 1

INTRODUÇÃO

O coqueiro (*Cocos nucifera* L.) é uma das Arecaceae mais importantes do mundo (Cintra *et al*. 2009). Diversas são as hipóteses relacionadas a origem desta palmeira, e a mais provável sugere que o centro de origem é o extremo Sudeste da Ásia, nas ilhas do Pacifico de Papua Nova Guiné (Lebrun *et al.* 1998, Gunn *et al.* 2011). A maior produção de coco é registrada no continente Asiático, com 84% da produção mundial, seguido pelo continente Americano com 8,2 %, Oceania 4,2 % e África 3,5 % (FAOSTAT 2013). Dentre os países que mais se destacam na produção de coco a Indonesia ocupa o primeiro lugar seguido por Filipinas, Índia, Brasil e Sri Lanka (FAOSTAT 2013). No Brasil, a região nordeste destaca-se com 80,73 % da produção de coco do país (IBGE 2014).

A produção de coco pode ser afetada por inúmeros fatores, dentre estes, tem-se as pragas. Os ácaros eriofídeos associados ao coqueiro totalizam nove espécies (Navia 2004). Dentre estes, o ácaro-da-necrose-do-coqueiro, *Aceria guerreronis* Keifer (Acari: Eriophyidae), destaca-se como uma das principais pragas desta cultura no mundo (Moore & Howard 1996, Seguni 2002, Fernando *et al*. 2010, Navia *et al.* 2013). *Aceria guerreronis* foi descrito por Keifer em 1965 a partir de espécimes coletadas no Estado de Guerrero no México (Mariau & Julia 1970). Contudo existem diversos relatos anteriores de danos semelhantes aos causados pelo ácaro no continente Americano e na costa da África (Ortega *et al.* 1967, Robbs & Peracchi 1965, Cabral & Carmona 1969, Mariau 1969).

Aceria guerreronis possui corpo alongado com aproximadamente 205-255 μm de comprimento e 36-52 μm de largura, formato vermiforme e apenas dois pares de pernas em todos os estágios pós-embrionários (Keifer 1965). Uma única fêmea pode produzir aproximadamente 66 ovos e o desenvolvimento de ovo a adulto pode ser concluído em 8-10 dias (30 - 35 °C) (Ansaloni & Perring 2004). Ao longo do desenvolvimento a espécie passa pelos estágios de ovo, larva, ninfa e adulto (Ansaloni & Perring 2004). O desenvolvimento e reprodução desse ácaro ocorre na região meristemática do fruto, que compreende a região abaixo das brácteas dos frutos do coqueiro. A colonização desse ácaro é iniciada em frutos com 1-2 meses de idade (tempo após a antese) (Lima *et al.* 2012).

A alimentação do ácaro na região meristemática do fruto provoca injúrias mecânicas que inicialmente são percebidas pelo aparecimento de triângulos cloróticos na epiderme do fruto. Com o crescimento do fruto as manchas tornam-se necróticas. A ocorrência de deformações dos frutos também é freqüente (Moore & Howard 1996). Frutos atacados por esse ácaro tendem a abortar prematuramente quando a infestação é elevada. Embora alguns frutos não abortem prematuramente, estes perdem seu valor comercial devido aos danos provocados pelos ácaros (Mariau 1977, Nair 2002, Rezende 2014). As perdas provocadas devido à alimentação de *A. guerreronis* podem alcançar valores superiores a 60% da produção de coco (Julia & Mariau 1979, Moore *et al.* 1989, Moore 2000, Seguni 2000, Rethinam*et al.* 2003, Wickramananda *et al.* 2007, Rezende 2014). *Aceria guerreronis* é responsável pela redução de 60% do número médio de frutos por planta e de 28% do albúmen líquido em áreas onde não há o controle dessa praga (Rezende 2014).

O principal método de controle de *A. guerreronis* em plantações comerciais tem sido a aplicação de acaricidas (Hernández 1977, Moore & Howard 1996, Ramaraju *et al.* 2002, Rezende

2014). Cinco ingredientes ativos são registrados para o controle desse ácaro junto ao Ministério da Agricultura, Pecuária e Abastecimento (MAPA), sendo eles: espirodiclofeno, fenpiroximato, abamectina, azadiractina e hexitiazoxi (Agrofit 2015). As pulverizações devem ser realizadas direcionando o jato dos acaricidas para os cachos, iniciando em cachos com frutos pouco desenvolvidos (aproximadamente 1 mês de idade) (Oliveira *et al*. 2012). Quando não se realiza seu controle, percebe-se uma diminuição na proporção de frutos destinados a comercialização *in natura* (frutos sem danos) e um aumento na proporção de frutos destinados ao processamento industrial (frutos poucos danificados) e não comercializáveis (frutos danificados e deformados) (Rezende 2014).

A toxicidade de acaricidas a *A. guerreronis* foi avaliada em duas populações (Itamaracá-PE e Petrolina-PE) (Monteiro *et al.* 2012). Neste estudo, os autores observaram que fenpiroximato foi o produto mais eficaz no controle de *A. guerreronis* e que o continuo uso de abamectina pode selecionar populações para a resistência (Monteiro *et al.* 2012). Contudo, diferentes pesquisadores relataram a ineficiência do controle químico contra *A. guerreronis* sob o perianto dos frutos (Mariau & Julia 1970, Moore & Alexander 1987, Moore *et al.* 1989, Silva *et al.* 2013). Esse fato deve-se, provavelmente, ao habitat confinado da praga, dificultando seu controle uma vez que as brácteas dos frutos servem como barreira física, impedindo a ação direta dos acaricidas sobre as colônias de *A. guerreronis* abaixo do perianto (Mariau & Tchibozo 1973, Hernández 1977, Melo *et al.* 2012, Monteiro *et al.* 2012, Silva *et al.* 2013). Desta forma, o contato com os acaricidas ocorre apenas quando esses ácaros estão em processo de dispersão (Silva *et al.* 2013). Assim, acaricidas só são eficientes se aplicados frequentemente, iniciando em frutos ainda em desenvolvimento (Moore & Howard 1996, Ramaraju *et al.* 2002, Melo *et al.* 2012, Silva *et al.* 2013). A necessidade de aplicações frequentes em um curto intervalo de tempo pode dificultar a

utilização desse método de controle para produtores com baixa produtividade, devido ao alto custo de controle (Mariau & Tchibozo 1973, Hernández 1977, Ramaraju *et al.* 2002, Melo *et al.* 2012).

Muita atenção tem sido dada a busca de inimigos naturais que possam ser eficientemente utilizados no controle biológico de *A. guerreronis*. Esse fato deve-se a importância econômica da cultura de coqueiro para países tropicais, sobretudo as perdas provocadas pelo ataque de *A. guerreronis* e as limitações do uso do controle químico contra essa praga (Aratchige *et al.* 2007, Lawson-Balagbo *et al.* 2008, Reis *et al.* 2008, Negloh *et al.* 2011, Lima *et al.* 2012). Levantamentos realizados no Brasil revelaram que *Neoseiulus baraki* (Athias-Henriot), *Neoseiulus paspalivorus* (De Leon), *Proctolaelaps bickleyi* Bram e *Proctolaelaps bulbosus* Moraes, Reis & Gondim Jr. são as espécies mais freqüentes em associação com *A. guerreronis* no perianto dos frutos (Lawson-Balagbo *et al.* 2008, Reis *et al.* 2008). Resultados semelhantes têm sido encontrados na Florida (Howard *et al*. 1990) e em Cuba (Cabrera *et al*. 1992). Na África, *Neoseiulus neobaraki* Zannou, Moraes & Oliveira, *N. baraki* e *N. paspalivorus* são os predadores mais freqüentes associados a *A. guerreronis* (Negloh *et al.* 2011), enquanto na Ásia, *N. baraki* e *N. paspalivorus* são as espécies mais frequentes (Moraes *et al.* 2004).

Dentre os predadores associados a *A. guerreronis*, *N. baraki* é o predador mais encontrado com mais frequência em alguns Estados do Nordeste (Lima *et al*. 2012). Este predador apresenta o corpo achatado e setas dorsais curtas (Moraes *et al.* 2004). Estas características possibilitam-no colonizar os espaços situados entre as brácteas e a epiderme do fruto (perianto), local não acessível a outros predadores (Lima *et al.* 2012, Melo *et al.* 2015). *Neoseiulus baraki* destaca-se também por conseguir localizar, por apresentar alta taxa predatória para esta presa e preferir *A. guerreronis* como presa a outras fontes de alimento, (Domingos *et al.* 2010, Melo *et al*. 2011,

Lima *et al.* 2012). Esse predador apresenta um desenvolvimento de ovo a adulto de 5,7 dias e a longevidade da fêmea é de aproximadamente 20 dias quando alimentado com *A. guerreronis* (Domindos *et al.* 2010). Fernando *et al.* (2010) observaram que a liberação inundativa de *N. baraki* em campo ocasionou uma diminuição significativa de 30% na população de *A. guerreronis*. Embora a utilização de ácaros predadores seja uma alternativa promissora para o controle de *A. guerreronis*, apenas a população natural de *N. baraki* nos cultivos de coqueiro não tem sido suficiente para diminuir as perdas provocadas pela praga em níveis aceitáveis (não representando perdas aos produtores). Além disso, o necessário uso intensivo de acaricidas pode comprometer a ação desses predadores, diminuindo a população em campo (Lima *et al.* 2013a,b, 2015a,b), além de poder levar a seleção de populações da praga resistentes a acaricidas (Monteiro *et al.* 2012).

A combinação de métodos de controle, em especial do controle químico e controle biológico pode ser usado no Manejo Integrado de Pragas (MIP), visto que este tem como objetivo reduzir a população de pragas com custos econômicos relativamente mais baixos (Croft 1990). No sistema de produção de coco, a utilização de ambos os métodos de controle poderia aumentar a eficiência de controle de *A. guerreronis* uma vez que os acaricidas atuariam quando a praga estivesse em processo de dispersão (fora do perianto do fruto), enquanto que o predador atuaria sob o perianto dos frutos. Para tornar esta tática do MIP possível, acaricidas devem ser prioritariamente compatíveis com o agente de controle biológico, visto que os predadores comumente entram em contato com os acaricidas através da pulverização direta, do caminhamento sobre a superfície pulverizada ou ao se alimentar de presas contaminadas.

Três características dos pesticidas devem ser consideradas para tornar o controle químico compatível com o biológico, são eles: toxicológico, momento da aplicação e direcionamento

espacial da aplicação (Hassan & Van de Veire 2004, Roubos *et al.* 2014). A característica toxicológica leva em consideração as propriedades inerentes aos pesticidas e está relacionada com a seletividade fisiológica, baseando-se nas diferenças fisiológicas entre a praga e inimigo natural (Ripper *et al.* 1951). O momento da aplicação deve ser ajustado para que se evite a exposição do pesticida ao inimigo natural ou para que a pulverização ocorra quando os inimigos naturais estiverem no estágio menos susceptível ao pesticida (Hassan & Van de Veire 2004). O direcionamento espacial da aplicação pode reduzir a quantidade de ingrediente ativo utilizado uma vez que a pulverização de pesticidas ocorrerá apenas em áreas com alta densidade populacional da praga ou em partes da planta preferida pela praga (National Research Council 2000).

Acaricidas registrados e/ou utilizados no controle de *A. guerreronis* foram testados quanti a toxicidade sobre o predador *N. baraki*, a avaliação foi realizada através das comparações das CL_{50} encontradas para a praga e para o predador. Segundo Lima *et al.* (2013a) fenpiroximato e clorfenapir são compatíveis com *N. baraki*. Adicionalmente, esses autores sugerem que esse predador apresenta elevada detoxificação de fenpiroximato por cytocromo P450 dependente de monoxigenases, tornando-o tolerante a este acaricida. Contudo, os acaricidas abamectina, carbosulfano e clorpirifós foram incompatíveis com o predador (Lima *et al.* 2013a).

Indivíduos que sobrevivem à exposição direta de pesticidas podem sofrer efeitos subletais, que são definidas como indução não aparente da mortalidade (Deusneux *et al.* 2007), uma vez que a degradação natural dos inseticidas/acaricidas ao longo do tempo resulta na exposição a doses subletais (Badji *et al.* 2007; Desneux *et al.* 2005; Guedes *et al.* 2016). Efeitos subletais são resultados de alterações na fisiologia ou comportamento do organismo, o qual sobrevive a exposição a pesticidas (Deusneux *et al.* 2007).

Estudos vêm sendo conduzidos com intuito de avaliar o efeito subletal de acaricidas em ácaros predadores (Ibrahim & Yee 2000, Bowie *et al.* 2001, Kim & Yoo 2002, Poletti *et al.* 2007, Teodoro *et al.* 2009, Hamedi *et al.* 2010, Lima *et al.* 2013a,b). Nesses estudos, o efeito subletal observado foi manifestado através da redução da sobrevivência de fêmeas, aumento do período de pré-oviposição, diminuição do período de oviposição, diminuição da fecundidade média, mudanças nos parâmetros da tabela de vida (Ibrahim & Yee 2000; Hamedi *et al.* 2010; Lima *et al.* 2013b), alteração na resposta funcional do predador (Poletti *et al.* 2007), no comportamento de forrageamento (Teodoro *et al.* 2009) e no caminhamento (Lima *et al.* 2013a). Desta forma, o uso contínuo de acaricidas pode alterar a dinâmica populacional do predador. Assim, antes de integrar algum acaricida e/ou inseticida em um sistema de produção é importante coletar informações da sua compatibilidade com seus inimigos naturais. Logo, o objetivo do presente trabalho foi estudar, sob condições de laboratório, o efeito subletal de acaricidas utilizados no controle de *A. guerreronis* em *N. baraki*, avaliando os seguintes aspectos: atividade global da população, acasalamento e fecundidade. Diante dos resultados encontrados para tais parâmetros foram verificados os efeitos dos acaricidas na resposta funcional do predador. Em seguida, foi avaliada a tabela de vida de fertilidade de fêmeas contaminadas e de sua prole (primeira e segunda geração), como também o forrageamento do predador.

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CAPÍTULO 2

BIOINSECTICIDE-PREDATOR INTERACTIONS: AZADIRACHTIN BEHAVIORAL AND REPRODUCTIVE IMPAIRMENT OF THE COCONUT MITE PREDATOR *Neoseiulus baraki*

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RESUMO – O uso de pesticidas sintéticos tem sido a forma dominante de controle de pragas desde a década de 1940. No entanto, biopesticidas estão surgindo como alternativas sustentáveis de controle de pragas, com o uso predominante em sistemas de produção agrícolas oragânica. Em primeiro lugar dentre os biopesticidas botânicos está o limonóide azadiractina, cuja segurança ambiental tem sido objeto de debate e de pesquisas minuciosas nos últimos anos. Produção de coco, particularmente a produção orgânica de coco, é um dos sistemas agrícolas em que a azadiractina é usada como um dos principais métodos de controle de pragas para o manejo do ácaro do coqueiro, *Aceria guerreronis* Keifer (Acari: Eriophyidae). O manejo desta espécie de ácaro também beneficia muito a predação por *Neoseiulus baraki* (Athias-Henriot) (Acari: Phytoseiidae). Aqui, nós avaliamos os potenciais impactos comportamentais de azadiractina no predador do ácaro do coqueiro, *N. baraki*. Nós exploramos os efeitos deste biopesticida na atividade global do predador, tempo de procura a fêmeas, e comportamento de acasalamento e fecundidade. Azadiractina comprometeu a atividade global do predador, reduzindo-a para cerca de metade. No entanto, a busca a fêmea não foi afetada. Em contraste, o comportamento de acasalamento foi comprometido por exposição azadiractina, particularmente quando machos predadores foram expostos ao biopesticida. Consequentemente, a fecundidade do predador foi também comprometida por azadiractina, promovendo dúvidas sobre sua segurança ambiental e seletividade a agentes de controle biológico.

PALAVRAS-CHAVE: biopesticida, pesticidas biorational, efeitos subletais, fitoseídeo predador, controle biológico, produção orgânica.

BIOINSECTICIDE-PREDATOR INTERACTIONS: AZADIRACHTIN BEHAVIORAL AND REPRODUCTIVE IMPAIRMENT OF THE COCONUT MITE PREDATOR *Neoseiulus baraki*

ABSTRACT – Synthetic pesticide use has been the dominant form of pest control since the 1940s. However, biopesticides are emerging as sustainable pest control alternatives, with prevailing use in organic agricultural production systems. Foremost among botanical biopesticides is the limonoid azadirachtin, whose perceived environmental safety has come under debate and scrutiny in recent years. Coconut production, particularly organic coconut production, is one of the agricultural systems in which azadirachtin is used as a primary method of pest control for the management of the invasive coconut mite, *Aceria guerreronis* Keifer (Acari: Eriophyidae). The management of this mite species also greatly benefits from predation by *Neoseiulus baraki* (Athias-Henriot) (Acari: Phytoseiidae). Here, we assessed the potential behavioral impacts of azadirachtin on the coconut mite predator, *N. baraki.* We explored the effects of this biopesticide on overall predator activity, female searching time, and mating behavior and fecundity. Azadirachtin impairs the overall activity of the predator, reducing it to nearly half; however, female searching was not affected. In contrast, mating behavior was compromised by azadirachtin exposure particularly when male predators were exposed to the biopesticide. Consequently, predator fecundity was also compromised by azadirachtin, furthering doubts about its environmental safety and selectivity towards biological control agents.

KEYWORDS: biopesticide, biorational pesticides, sublethal effects, phytoseiid predator, biological control, organic production.

Introduction

The use of synthetic pesticides has been the dominant method of agricultural pest control since the early 1940s (Metcalf 1980, Cooper & Dobson 2007). However, the continuing shift in society's attitudes and behaviors towards crop protection products has led to drastic changes in the development of new pesticides, where emphasis is placed on improved human and environmental safety profiles (Matsumura 2004, Manuweera 2008, Manuweera *et al.* 2008, Casida & Durkin 2013). The science behind the negative perception of synthetic pesticides, which is deeply ingrained among the general public, is debatable as it is largely based on insecticides such as organochlorines that have been banned for over 40 years (Metcalf 1980, Matsumura 2004, Krämer et al. 2012, Casida & Durkin 2013). Curiously, more than 70% of the current groups of synthetic insecticides have natural analogs (Gerwick & Sparks 2014). This fact, together with the perceived general (and invalid) notion that natural compounds are safer than their synthetic counterparts (Coats 1990, Kidd 2000), explains the allure of natural pesticides, or biopesticides, and the drastic reemergence of interest in these compounds, particularly compounds that are plant-derived, also referred to as botanical pesticides (Isman 2006, Regnault-Roger *et al.* 2012, Isman & Grieneisen 2014).

The current burgeoning of scientific interest in biopesticides in general, and in botanical pesticides in particular, has only led to a limited amount of credible information and to a small increase in their practical use as crop protection agents (Amoabeng *et al.* 2014, Isman & Grieneisen 2014). Slow action, brief persistence, relatively high cost for large-scale production, and legislative limitations are the main reasons for the limited expansion of biopesticide use in agriculture (Isman 2006, Amoabeng *et al.* 2014, Villaverde 2014). The 1960s Western discovery of the insecticidal activity of the limonoid triterpene azadirachtin, extracted from the seeds of the

Indian neem tree (*Azadirachta indica* A. Juss (Meliaceae)), is one of the likely catalysts of the latest growth in interest and spurt in academic research on botanical insecticides, as well as the subsequent commercialization of plant essential oils as insecticides (Regnault-Roger *et al.* 2012, Isman & Grieneisen 2014). It is also interesting that azadirachtin remains the most successful botanical pesticide in agricultural use worldwide (Mordue (Luntz) *et al.* 2010, Isman & Grieneisen 2014).

Azadirachtin arguably stands out as the most widely used botanical pesticide since the onset of synthetic pesticides for pest control, which is well established in organic agriculture, public health, home and garden, and selected agricultural settings (Mordue (Luntz) *et al.* 2010, MAPA 2014). This biopesticide has unique features and can act as an arthropod anti-feedant, growth regulator and sterilant, while its safety to vertebrates is broadly recognized (Mordue (Luntz) *et al.* 2010, Regnault-Roger *et al.* 2012). However, the earlier perception of azadirachtin's safety towards non-target arthropods has been questioned (Qi *et al.* 2001, Medina *et al.* 2004, Cordeiro *et al.* 2010). Such a change in perception is the likely consequence of a shifting in focus, from reliance on acute lethal effects, to sublethal effects of insecticidal compounds (Stark & Banks 2003, Desneux *et al*. 2007, Guedes & Cutler 2014).

Phytophagous mites and their predators are a focus of attention not only regarding the sublethal impact of crop protection compounds, but also regarding the effect that azadirachtin has on these species (Stark *et al.* 1997, Stark & Banken 1999, Cordeiro *et al.* 2013). The coconut production system, particularly organic production, represents one of the agricultural systems where azadirachtin use is important for controlling the coconut mite, *Aceria guerreronis* Keifer (Acari: Eriophyidae). The management of *A. guerreronis* also benefits from the predatory mite species *Neoseiulus baraki* (Athias-Henriot) (Acari: Phytoseiidae) (Aratchige *et al.* 2007, Melo *et*

al. 2011, Lima *et al*. 2012, Lima *et al.* 2013a). The lethal effect of acaricides in the predatory mite *N. baraki* has been a subject of attention. Azadirachtin was recognized as exhibiting low acute toxicity to *N. baraki*, but was shown to spark behavioral avoidance on this predator, potentially limiting its foraging behavior (Lima *et al.* 2013a, Lima *et al.* 2013b). Here, we assessed the potential sublethal behavioral effects of azadirachtin, at its label rate for controlling the coconut mite, and the potential consequences in the overall activity, mating and fecundity of the coconut mite predator *N. baraki*.

Material and methods

Ethics Statement. This study did not involve any endangered or protected species. The species studied is a species of predatory mite from a colony maintained in laboratory, where the experiments were performed and no specific permission was required.

Predatory mites and azadirachtin. Specimens of the mite predator *N. baraki* were field-collected from coconut fruits infested with the coconut mite, *A. guerreronis*, on Itamaracá Island (07°46'S, 34°52'W; Pernambuco, Brazil). Predator colonies were established from 100 females, which were obtained from, and maintained on, coconut perianth. *Aceria guerreronis* was provided as prey every other day. The mites were maintained under laboratory conditions at 27.5 ± 0.5°C, 70 ± 10% RH, and 12:12 (LD) photoperiod.

Acaricides. Azadirachtin was the insecticide/acaricide used in the experiments. The compound was used in its commercial formulation (AzaMax, 1.2 g a.i./L, emulsifiable concentrate, DAV Agro, Ituverava, SP, Brazil) at the label rate registered and recommended for the coconut mite, *A. guerreronis,* in Brazil (i.e., 30 mg a.i./L) (MAPA 2014). No predatory mite mortality takes place at this insecticide concentration, which is sublethal to *N. baraki* based on previous determinations

(Lima *et al.* 2013a), preventing any confounding effect of mortality on the sublethal experiments performed. Indeed, no azadirachtin mortality was observed in the experiments here performed, as expected.

Overall mite group activity. Rather than assessing individual mite activity, bioassays of the overall group activity were performed with unsexed adult predatory mites in congruence with the aggregate distribution of the species observed on coconut fruits (Zhang & Sanderson 1997, Fernando *et al.* 2003, Reis *et al.* 2008). The methods used in this study were adapted from Lima *et al.* (2013a) as follows: individual discs of black polyvinyl chloride (PVC; 1.2 cm diameter) were immersed for 5 s in azadirachtin solution (30 mg a.i./L) and allowed to air-dry for 2 h before being glued to a piece of wood (1 cm thick) and placed in the center of a Petri dish (6 cm diameter) containing water (0.5 cm deep). This set up allowed the PVC disc to float on the surface of the water, preventing mite escape. Each disc received 10 adult couples of the predatory mite (8 days old) and eight disc arenas were used for each treatment (i.e., azadirachtin-treated discs as well as untreated control discs, were only water was used). The overall mite group activity in each disc arena was digitally recorded for 10 min by an automated video tracking system per unit of time (ViewPoint LifeSciences, Montreal, Quebec, Canada). The overall activity was digitally determined by the change in captured pixels per fraction of time (Δ pixels/s x 10^{-2}) corresponding to summation of any change in position and posture of the individuals within the arena. The length of time that the mites spent inactive (variation lower than 4 pixels'/s x 10^{-2}), under slow (variation between 4 and 8 pixels'/s x 10^{-2}) or fast activity (variation over 8 pixels'/s x 10^{-2}) was also recorded, as was the rate of change in activity within each of these three categories. The bioassays were performed under 27 ± 2°C.

Male mate-searching behavior. Pieces of coconut perianth (0.5 cm^3) were placed in individual wells of bioassay trays with an adhesive cover (128 cells; Bio-Serv, Frenchtown, NJ, USA) and subsequently immersed for 5 s in either azadirachtin solution (30 mg a.i./L) or water (control), and allowed to dry for 2 h. Individual virgin male and female mites (8 days old) were released in each well containing a treated piece of coconut perianth (0.5 cm^2) and were confined for 16 hours of exposure. Approximately 200 coconut mites (*A. guerreronis*) were also transferred to each well to serve as a food source for the predators. After insecticide exposure, each virgin male mite was released at the edge of a PVC disc arena (1.9 cm diameter), which was surrounded by a layer of glycerin to prevent escape. The opposite margin of each PVC disc contained an opening (0.5 cm diameter) covered with voile, under which 10 virgin females were contained within the cut bottom of an Eppendorf tube (1 cm diameter). The mite walking pattern when in search of the virgin females was recorded using the ViewPoint video tracking system. This system recorded the length of time it took each male to find the contained (virgin) females, and lasted for up to 10 min. The following treatments were used, each with 20 replicates: untreated male with untreated females, azadirachtin-treated male with untreated females, untreated male with azadirachtin-treated females, and azadirachtin-treated male with azadirachtin-treated females.

Mating behavior and associated fecundity. The mating behavior of the predatory mite, *N. baraki*, was recorded and assessed by building ethograms and analyzing the first order sequential behavioral transitions and time budgets observed, as well as the lifetime fecundity of each couple. Each virgin mite couple was exposed to azadirachtin, or not, as previously described (subsection "*Male mate-searching behavior"*). Male and female were placed at opposite sides on the surface of a PVC disc arena (0.25 cm). The treatments employed were the same described in the subsection "*Male mate-searching behavior"*, namely: untreated male with untreated females,

azadirachtin-treated male with untreated females, untreated male with azadirachtin-treated females, and azadirachtin-treated male with azadirachtin-treated females. Twenty replicates (i.e., couples) were used for each treatment. The mating behavior of each couple was recorded following the protocol of Pappas *et al.* (2005). Briefly, the initial approach between male and female were characterized by contact using their anterior (gnathosoma to gnathosoma), lateral (male's gnathosoma to female's lateral part of idiosoma) or posterior portions (male's gnathosoma to female's posterior part of idiosoma). The male subsequently climbed on the female, moved into the mating position (venter-to-venter) and finally copulated (Pappas *et al.* 2005). The recording continued until the end of the first mating, when the couple separates and the experiment was interrupted. The behavioral traits assessed included: walking, male and female meeting, mounting, and copulating. At the end of mating, the females were retrieved and individualized in untreated pieces of perianth (0.5 cm^3) within bioassay trays (128 cells) and provided with *A. guerreronis* as a food source. The piece of perianth was replaced every other day, and egg-laying was recorded daily until female death. This bioassay was performed under the same controlled environmental conditions of mite rearing.

Statistical analyses. The assumptions of normality and homoscedasticity were checked (PROC UNIVARIATE; SAS v. 9) (SAS 2008), and $\log_{10} x$ transformation was necessary to stabilize the variance for male mate-searching time, male walking time until female mounting, and duration of mounting. Data from overall group activity (Δ pixels/s x 10^{-2}) and total female fecundity (no. eggs laid/female) were subjected to analysis of variance (PROC GLM; SAS v. 9) (SAS 2008), as were the data from male mate-searching behavior (min), where treatment differences were subsequently subjected to Tukey's HSD test ($P< 0.05$; SAS v.9) (SAS 2008). Ethograms depicting the sequence and frequency of events were manually constructed for each mating treatment based on first order

behavioral transitions. The sequence of behavioral transitions was tested for consistency across treatments using Cochran-Mantel-Haenszel statistics (CMH; $P < 0.05$) (PROF FREQ, SAS v. 9) (SAS 2008), and eventual differences in the proportion of behavioral transitions between treatments were compared using the χ^2 test ($P < 0.05$). The eventual differences in the recorded time budgets were also subjected to individual analysis of variance and Tukey's HSD test ($P < 0.05$), when appropriate (PROC GLM; SAS v. 9) (SAS 2008).

Daily mite fecundity (no. eggs daily laid/female) was subjected to linear regression analysis against female lifetime using the curve-fitting procedure of TableCurve 2D (Systat, San Jose, CA, USA). The significant regression models ($P < 0.05$) were tested from the simplest (linear and quadratic) to more complex peak models and the model selection was based on parsimony, high F-values (and mean squares), and steep increases in R^2 with model complexity. Residual distribution was also checked for each analysis to validate parametric assumptions.

Results

Overall mite group activity. The profile of overall mite group activity through time, exhibited in Fig. 1A, is suggestive of higher activity levels among untreated predatory mites, which was confirmed with subsequent analysis of variance for the average overall activity during the assessment period ($F_{1,14} = 10.09$, $P = 0.007$) (Fig. 1B). The duration spent in each level of activity, either inactive, or under slow or fast activity, also varied significantly between azadirachtin-treated and untreated predatory mites ($F_{1,14} \geq 7.97$, $P \leq 0.01$). Fast activity prevailed in untreated mites, in contrast with azadirachtin-treated predatory mites, which remained inactive and under slow activity for longer lengths of time (Fig. 1C). Furthermore, there were significant differences in the change of overall activity patterns in groups of mites either azadirachtin-treated or

untreated, with the former experiencing significantly higher changes in activity ($F_{1,14} \geq 9.30$, $P \leq 0.009$) (Fig. 1D).

Male mate-searching behavior. The length of time it took virgin male predatory mites to first find virgin females was subjected to analysis of variance (after data transformation); however, no significant difference was found between treatments (i.e., untreated mites of both sexes, azadirachtin-treated mites of either sex, and azadirachtin-treated mites of both sexes) (overall mean: 2.41 ± 0.30 min to first find the females) ($F_{3,76} = 1.54$, $P = 0.21$).

Mating behavior and associated fecundity. The sequential analysis of the first order of behavioral transitions for each treatment involving azadirachtin-treated and untreated mites was significant and consistent across treatments (CMH non-zero correlation = 30.53, df = 1, $P< 0.001$). Regarding the individual behavioral transitions, a significant difference was detected for the transition between the male meeting the female and either mounting or returning to walk, with significantly larger failure to mount when azadirachtin-treated males were attempting to mate ($\chi^2 = 4.1$, df = 1; $P = 0.04$) (Fig. 2).

The time budgets were also recorded for each mating treatment and are exhibited in Fig. 3. The length of time spent walking and in mounting attempts when male mites were exposed to azadirachtin is notable (i.e., when only males were exposed and when both male and females were exposed), with mites incurring up to three attempts of mounting the female before copulating (Fig. 3CD). Among the three recorded durations of the behaviors leading to mating, walking and copulating were significantly different among treatments ($F_{3,76} \geq 2.65$, $P \leq 0.05$), in contrast with mounting, which was similar among treatments (overall mean = 0.41 ± 0.04 min; $F_{3,76} = 0.68$, $P = 0.56$). Azadirachtin-treated males spent a significantly longer amount of time walking than did untreated males before mounting untreated females. The time spent by treated males walking

before mating with azadirachtin-treated females, however, led to intermediate results (Fig. 4A). A distinct trend was apparent for the time spent in copulation. Untreated couples and azadirachtin-treated couples copulated for longer periods of time, while copulation was quickest between azadirachtin-treated males and untreated females (Fig. 4B).

Total female fecundity (no. eggs laid/female) did not differ among treatments ($F_{3,76} = 0.12$, $P = 0.95$) probably due to the high variability among females within each treatment. However, and more importantly, the observed differences in mating among azadirachtin-treated couples, azadirachtin-treated individuals of either sex (i.e, the male or the female of each pair), and untreated couples led to significant differences in daily fecundity (Table 1, Fig. 5). Females from untreated couples exhibited a higher and earlier peak of egg-laying, which was observed approximately 2 days after mating. Azadirachtin-treated females that mated with untreated males exhibited a 0.5 day delay in peak fecundity, with levels that were 25% lower than females from the untreated couples. Females mated with azadirachtin-treated males exhibited even longer delays in peak fecundity, which occurred 5.0 days after mating, and reached levels as low as half that of untreated couples (Fig. 5). Such differences in daily fecundity are more important than total fecundity due to their greater impact in the rate of population growth, as evidenced in life-table and population studies (Stark *et al.* 1997, Stark & Banken 1999, Stark & Banks 2003).

Discussion

Azadirachtin is a chemical compound representative of the botanical biopesticides, whose safety to non-target arthropods has been a matter of debate, largely due to its reported deleterious effects on natural enemies of arthropod pest species (Qi *et al.* 2001, Medina *et al.* 2004, Cordeiro *et al.* 2010). The few studies that have been published investigated a rather small number of

species and did not explore the potential impact of detected impairments on the reproductive output of the natural enemies studied (Cote *et al.* 2002, Castagnoli *et al.* 2005, Duso *et al.* 2008, Bernardi *et al.* 2013). Even for the coconut mite predator, to which azadirachtin exhibited low acute lethal effect, behavioral avoidance to this limonoid triterpene was detected, but its impact on the natural enemy longevity and reproduction is not known.

Here, we assessed the impact of azadirachtin in the overall group activity and mating behavior of the predatory mite *N. baraki* and assessed its impact on the predator's fecundity. Azadirachtin is one of the pesticides used against the coconut mite in Brazil, and it is the sole pesticide allowed in organic coconut production systems, where the beneficial control provided by the predatory mite *N. baraki* is particularly important (Melo *et al.* 2011, MAPA 2014). Our study has both environmental and agricultural relevance. Azadirachtin is reported to exhibit arthropod sterilant activity, in addition to anti-feedant and growth regulator activity. However, behavioral impairment may also compromise exposed arthropods when in low doses.

Azadirachtin reduced the overall activity in groups of the predatory mite. The group determination is consistent with the aggregate pattern of distribution associated with phytoseiid mites and *N. baraki* in particular (Zhang & Sanderson 1997, Fernando *et al.* 2003, Reis *et al.* 2008). The low activity level detected with sublethal levels of azadirachtin exposure was due to a reduced rate of activity, with mites remaining inactive or under low levels of activity for longer, and frequently changing the pattern of activity from higher to lower levels. Reduced activity may have diverse consequences for the predatory mites, ranging from reduced foraging, to lower dispersal, and possibly compromised mating. To address the later potential consequence, the reproductive behavior of the predatory mite species *N. baraki* was investigated after azadirachtin exposure.

Azadirachtin did not compromise female searching for the first meeting between males and females. However, azadirachtin exhibited significant effects on exposed males, extending their latent period before copulation, often requiring multiple mounting attempts before eventual copulation. Untreated males coupled for longer periods of time with females (both azadirachtin-treated and untreated) than did azadirachtin-treated males except when mated with treated females. Therefore, azadirachtin impairs copulation and the end result is reduced fecundity of treated couples, particularly when the males are exposed to this biopesticide.

Azadirachtin does not seem to affect sex pheromone communication between males and females of *N. baraki*, as no difference was observed in the time necessary for the males to first locate the females. The observed reproductive impairment likely has endocrine origin, which is consistent with the growth regulator and sterilant activity reported for azadirachtin (Mordue (Luntz) *et al.* 2010). The synthesis, transport, and release of morphogenic peptide hormones in the arthropod brain are major components of the azadirachtin mode of action (Mordue (Luntz) & Nisbet 2000, Mordue (Luntz) *et al.* 2010). The detected reproductive effect of azadirachtin is stronger in male mites, impairing mating and compromising fecundity, in contrast with the more frequent reports on female fecundity reduction (Mordue (Luntz) *et al.* 2010, Bernardi *et al.* 2013). Here the impact of azadirachtin-treated females was smaller, unlike reports on spider mites (Duso *et al.* 2008, Bernardi *et al.* 2013). The reduction in male fertility caused by azadirachtin has been reported in few instances and only for a few arthropod pest species to the best of our knowledge. These effects have been reported as either a consequence of reduced potency, spermatocyte degeneration, or blocked cell division in developing spermatocytes. The findings have differed depending on the model insect pest species studied (Dorn 1986, Shimizu 1988, Linton *et al.* 1997), but there have not yet been any studies on male mites.

The low acute mortality of azadirachtin towards the predatory mite *N. baraki* previously reported (Lima *et al.* 2013a, Lima *et al.* 2013b) contrasts with its significant (sublethal) reproductive effects reported in the present study. This later finding has potential practical consequences since such reproductive effects may compromise the predator field performance against the coconut mite. The low daily fecundity can lead to changes in the numerical response of the predator, which is the change in predator density as a function of change in prey density (Solomon 1949), and consequently may result in a bigger time lag between prey and predator populations. Although azadirachtin exhibits a safer lethal profile to the predator *N. baraki* than alternative compounds used against the coconut mite, the range of choices available for organic coconut production is restricted to this botanical pesticide. Azadirachtin sparks behavioral avoidance in the coconut mite predator *N. baraki*, as also reported in lacewings and in contrast with earwigs (Cordeiro *et al.* 2010, Campos *et al.* 2011). This avoidance may potentially favor predator survival while reducing exposure, but may lead the predators to leave the area, compromising the biological control of the coconut mite (Lima *et al.* 2013a). More importantly, azadirachtin reduces the predatory mite fecundity, compromising the population growth potential of exposed individuals. Therefore, this phenomenon should be a matter of concern when designing management programs for the coconut mite and gives credence to the recent concerns with the significant deleterious effects of the biopesticide azadirachtin on non-target arthropod species.

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Table 1. Summary of the non-linear regression analyses of the daily fecundity curves (Fig. 5) of females of the coconut mite predator *Neoseiulus baraki* with and without exposure to azadirachtin ($n = 20$). All parameter estimates were significant at $P < 0.01$ by Student's *t*-test.

Model	Treatments	Parameter estimates (± SE)			df_{error}	F	*P*	R^2
		a	*b*	*c*				
Pulse (3-parameter)	Untreated couple	1.94 ± 0.09	0.90 ± 0.03	1.75 ± 0.09	19	258.12	< 0.001	0.96
$y = 4anwhere\ n = \exp(-(x - b)/c)$	Azadirachtin-treated male	1.53 ± 0.11	0.95 ± 0.08	2.64 ± 0.20	19	96.62	< 0.001	0.91
Gaussian (3-parameter)	Azadirachtin-treated female	1.42 ± 0.09	4.81 ± 0.17	2.19 ± 0.18	19	114.37	< 0.001	0.92
$y = a \exp(-0.5((x - b)/c)^2)$	Azadirachtin-treated couple	1.15 ± 0.08	5.18 ± 0.20	2.40 ± 0.21	93	201.32	< 0.001	0.91

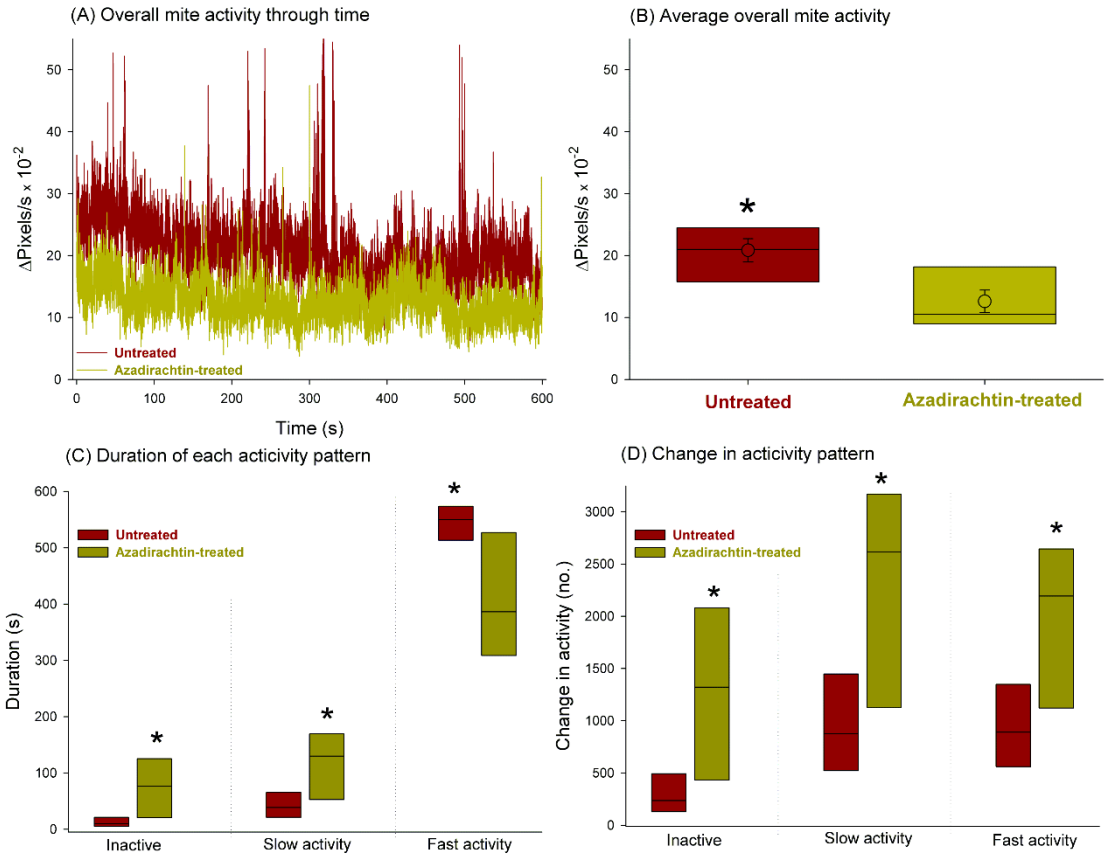

Figure. 1. Overall activity of groups of the coconut mite predator *Neoseiulus baraki* exposed to azadirachtin represented as: (A) activity profile through time: (B) average overall activity; (C) duration of each activity pattern; (D) changes in activity pattern. Individual group profiles are represented (A), while box plots with median (and mean ± SE for (B)) and lower and upper quartiles are exhibited in the remaining plots (B, C, D). Box plots with an asterisk indicate significant differences between azadirachtin-treated and untreated mites (Fisher's F test at $P< 0.05$).

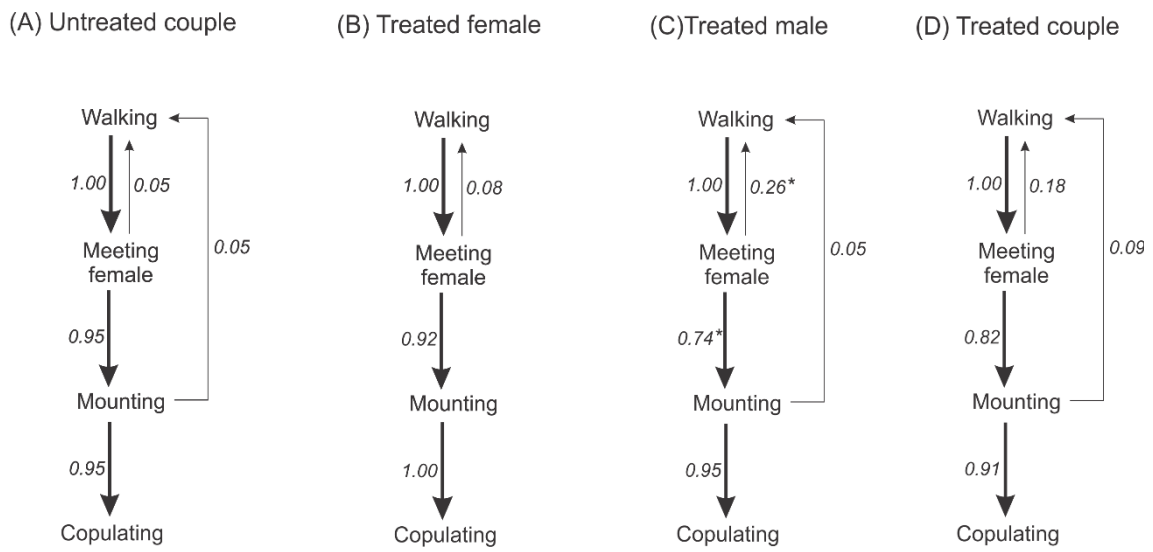

Figure. 2. Ethogram of the mating behavior of the coconut mite predator *Neoseiulus baraki* with and without exposure to azadirachtin represented as first order transition diagrams. The solid arrows indicate each behavioral transition. The relative thickness of each arrow represents the frequency of each behavioral transition (n = 20). Asterisk indicates significant difference in behavioral transition by the χ^2 test ($P < 0.05$).

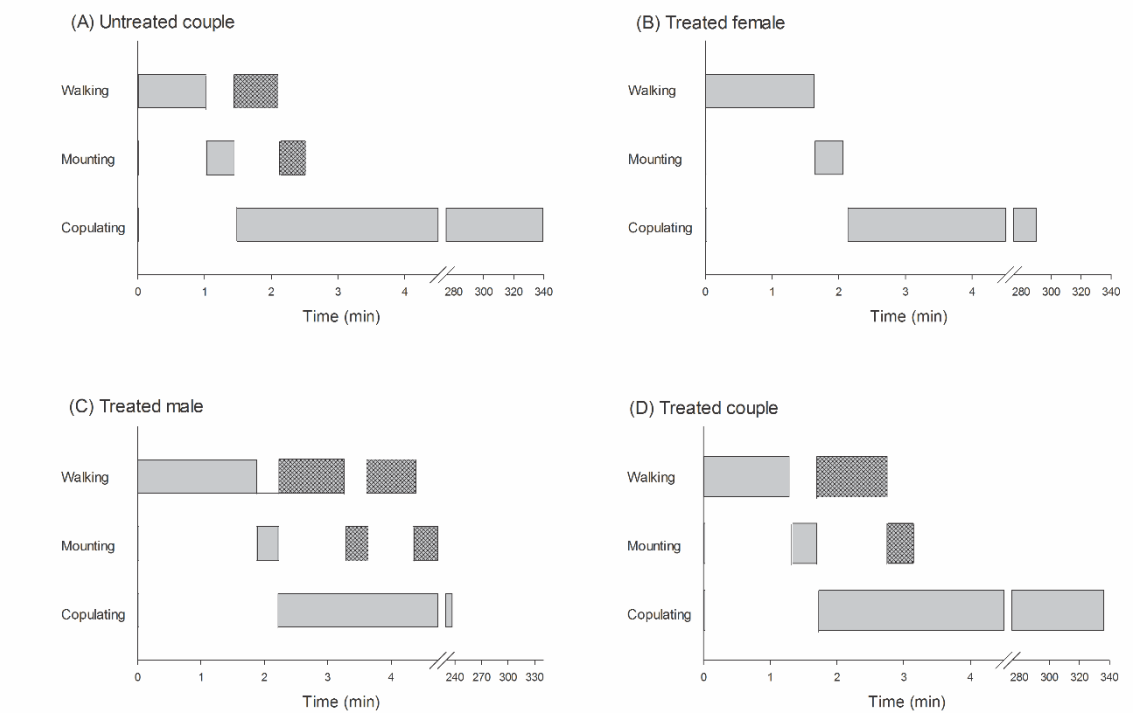

Figure. 3. Schematic representation of time budgets of the mating behavior of the coconut mite predator *Neoseiulus baraki* with and without exposure to azadirachtin (n = 20). The horizontal histogram bars indicate the average duration of each behavior. The dashed bars indicate events that were repeated before copulation eventually occurred, as indicated in the transition diagrams of Fig. 2.

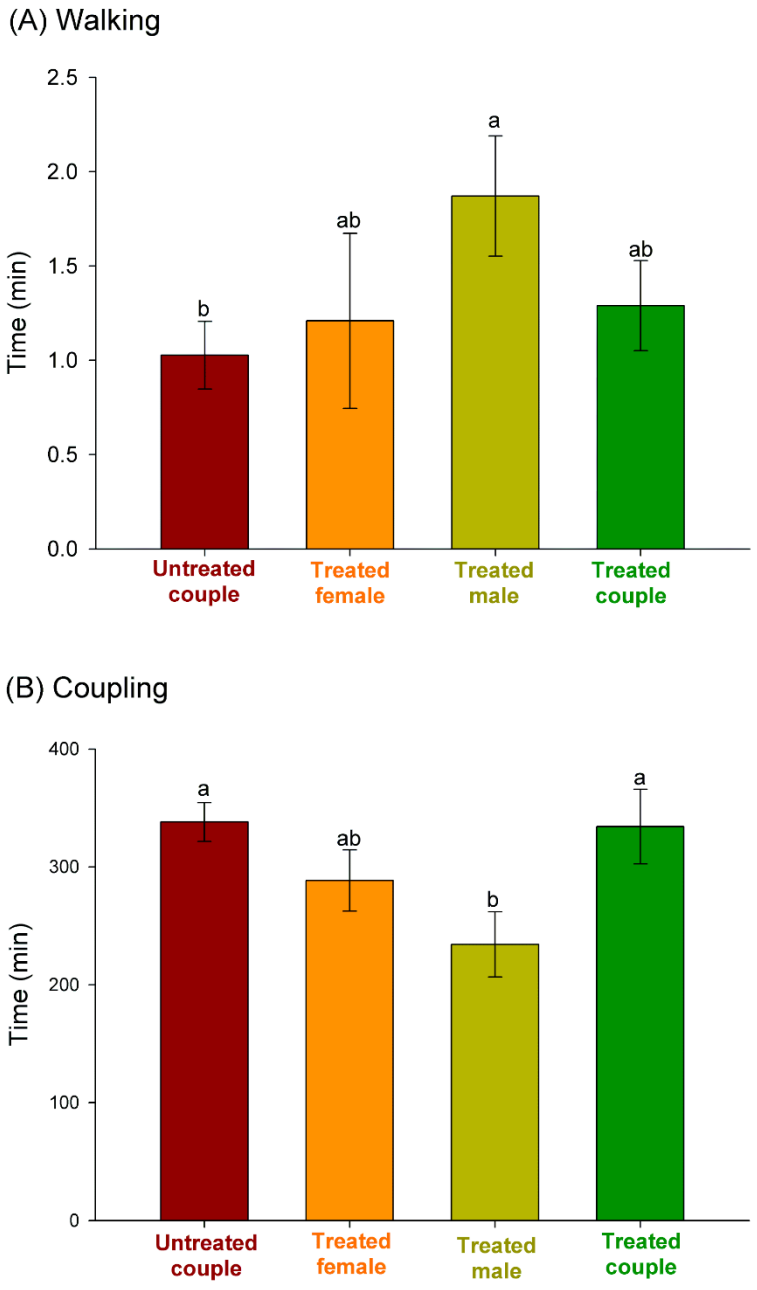

Figure. 4. Duration of walking (± SE) of male mites (A) and duration of copulation (± SE) in pairs of the coconut mite predator *Neoseiulus baraki* with and without exposure to azadirachtin (n = 20). Different letters at the top of the histogram bars indicate significant differences by Tukey's HSD test ($P < 0.05$).

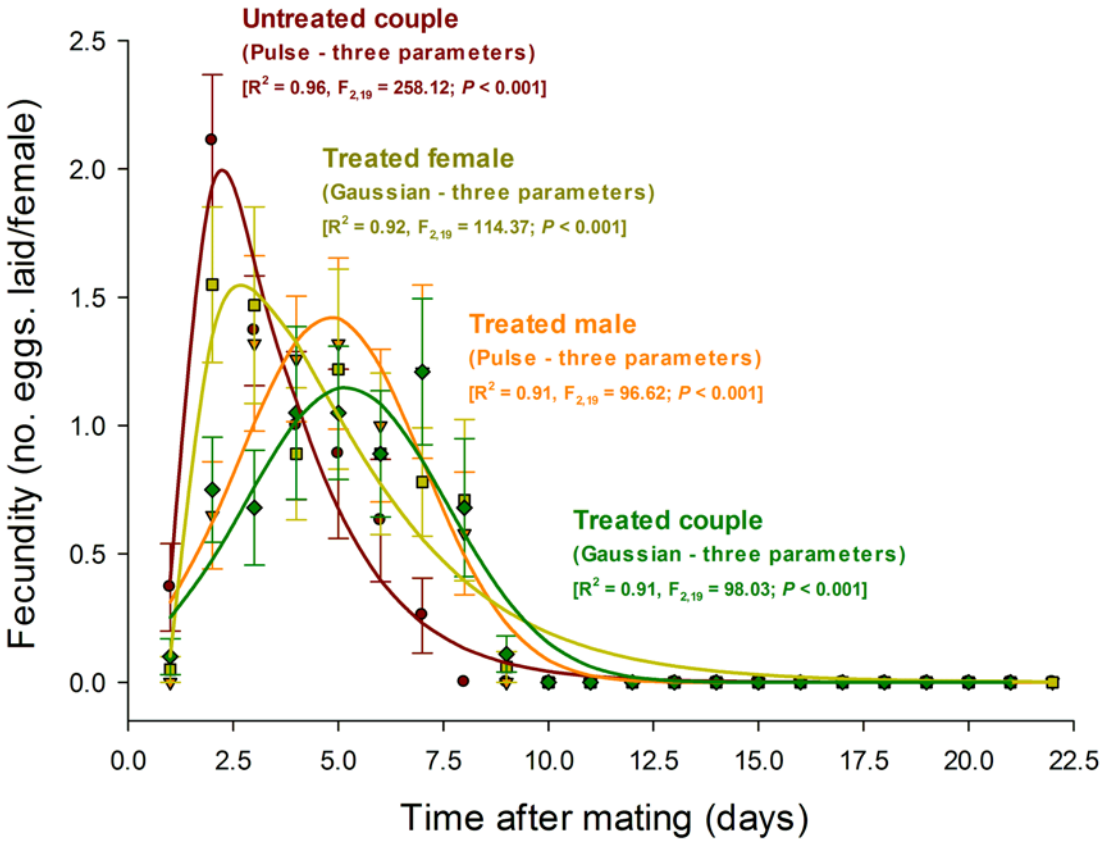

Figure. 5. Daily fecundity of females of the coconut mite predator *Neoseiulus baraki* with and without exposure to azadirachtin (n = 20). Each symbol indicates the mean (± SE) observed values.

CAPÍTULO 3

ACARICIDE-IMPAIRED FUNCTIONAL PREDATION RESPONSE OF THE PHYTOSEIID MITE *Neoseiulus baraki* TO THE COCONUT MITE A*ceria guerreronis*

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RESUMO - Acaricidas podem interferir em uma miríade de interações entre artrópodes, particularmente interações predador-presa. O ácaro do coqueiro, Aceria guerreronis Keifer (Acari: Eriophyidae), e seu fitoseídeo predador, Neoseiulus baraki (Athias-Henriot) (Acari: Phytoseiidae), fornecem uma oportunidade de explorar esse tipo de interação, pois o primeiro é uma praga-chave de coco que requer tanto predação quanto aplicação acaricida para a seu manejo. O objetivo do presente estudo foi avaliar o efeito dos acaricidas abamectina, azadiractina e fenpiroximato sobre a resposta funcional de N. baraki a densidades de A. guerreronis. As seguintes densidades de presa foram testadas: 5, 10, 20, 40 e 80 presas. O tipo de resposta e tempo de manipulação da presa (Th) não foram alteradas pelos acaricidas. No entanto, a taxa de ataque (a') foi modificada por abamectina e fenpiroximato, e o pico de consumo foi reduzido pelo abamectina. Todos os acaricidas permitiram a manutenção do predador em campo, mas a exposição a abamectina e fenepiroximato comprometeram o consumo presa.

PALAVRAS-CHAVE: Acaricidas, consume de presas, interação presa-predador, taxa de ataque

ACARICIDE-IMPAIRED FUNCTIONAL PREDATION RESPONSE OF THE PHYTOSEIID MITE *Neoseiulus baraki* TO THE COCONUT MITE A*ceria guerreronis*

ABSTRACT -Acaricides may interfere with a myriad of interactions among arthropods, particularly predator-prey interactions. The coconut mite, *Aceria guerreronis* Keifer (Acari: Eriophyidae), and its phytoseiid predator, *Neoseiulus baraki* (Athias-Henriot) (Acari: Phytoseiidae), provide an opportunity to explore such interference because the former is a key coconut pest species that requires both predation and acaricide application for its management. The objective of the present study was to assess the effect of the acaricides abamectin, azadirachtin and fenpyroximate on the functional response of *N. baraki* to *A. guerreronis* densities. The following prey densities were tested: 5, 10, 20, 40 and 80 preys. The type of functional response and prey handling time (*Th*) were not altered by the acaricides. However, the attack rate (*a'*) was modified by abamectin and fenpyroximate, and the consumption peak was reduced by abamectin. All of the acaricides allowed for the maintenance of the predator in the field, but exposure to abamectin and fenpyroximate compromised prey consumption.

KEYWORDS: Acaricides, prey consumption, predator-prey interaction, attack rate

Introduction

Pesticides may interfere with a myriad of arthropod interactions, but the focus on mortality in the assessment of pesticide impacts on biological systems may preclude the recognition of important sublethal effects of these compounds. This trend is shifting, but broader assessments have remained limited to relatively few species and realistic scenarios (Desneux *et al.* 2007, Cutler 2013, Guedes & Cutler 2014). The coconut production system provides an interesting model for studying acaricide-mediated predator-prey interactions and their potential consequences for integrated pest management.

Aceria guerreronis Keifer (Acari: Eriophyidae) is one of the main pests of coconut palm, *Cocos nucifera* L., worldwide (Moore & Howard 1996, Haq *et al.* 2002, Negloh *et al.* 2011). This mite inhabits the perianth of the coconut and feeds on the meristematic tissue, causing necrosis of the epidermis and the coconuts to fall. Biological control of *A. guerreronis* by predators has been extensively studied (Aratchige *et al.* 2007, Lawson-Balagbo *et al.* 2008, Negloh *et al.*2011, Lima *et al.* 2012), and *Neoseiulus baraki* (Athias-Henriot) is one of the most common predators associated with *A. guerreronis* within the perianth (Aratchige *et al.* 2007, Negloh *et al.* 2011, Lima *et al.* 2012).

Neoseiulus baraki can complete its development with *A. guerreronis* as its sole food source (Lawson-Balagbo *et al.*2008, Domingos *et al.* 2010); the mite is its preferred prey, and it is able to detect cues from this pest (Melo *et al.* 2011). *Neoseiulus baraki* has high predatory capacity and morphological traits that facilitate its entry into the perianth region, which optimises its foraging and predation of the coconut mite (Lima *et al.* 2012). However, although the use of predators as biological control agents represents a promising alternative to pesticides, acaricide

spraying remains the control method most used against *A. guerreronis* (Monteiro *et al.* 2012, Lima *et al.* 2015).

Acaricides are only effective if applied frequently, beginning when the coconuts are still developing (Moore & Howard 1996, Ramaraju *et al*. 2002). However, frequent use of acaricides can lead to selection for insecticide-resistant populations, pest resurgence, secondary pest outbreaks, or even compromised performance of natural enemies (Cranham & Helle 1985, Omoto *et al.* 2000, van Leeuwen *et al.* 2010, Cordeiro *et al.* 2013). Recent studies have suggested the possible compatibility of natural enemies and acaricides in controlling *A. guerreronis*, which could lead to higher mortality rates of this pest (Lima *et al.* 2013a, 2013b).

Integrated pest management aims to reduce pest populations to levels that do not cause economic losses through a combination of control methods, especially biological control and chemical control (Croft 1990), so knowledge of the effects of pesticides on biological control agents is important (Desneux *et al.* 2007). Predators can be exposed to pesticides through direct spraying, contact with contaminated surfaces during foraging, or feeding on contaminated prey (Jepson 1989, Ahmad *et al.* 2003, Hua *et al.* 2004, Torres & Ruberson 2004).

The toxicity, selectivity, and sublethal effects of pesticides have been studied in diverse mite pest and predator systems (Poletti *et al.* 2007, Teodoro *et al.* 2009, Hamedi *et al.* 2011, Lima *et al.* 2013a, 2013b). Pesticides may have lethal or sublethal effects on predators, the latter of which has been receiving increasing attention (e.g., Desneux *et al.* 2007, Guedes & Cutler 2014). Sublethal effects allow individuals to survive exposure to pesticides (Desneux *et al*. 2007), but these effects can lead to physiological and/or behavioural processes that may compromise the efficiency of a natural enemy by, for example, altering functional response, life table parameters, and foraging (Wang & Shen 2002, Poletti *et al.* 2007, Nadimi *et al.* 2009, Teodoro *et al.* 2009,

Rezác *et al.* 2010, Hamedi *et al.* 2011). Previous studies have been conducted with *N. baraki* exposed to acaricides used for controlling *A. guerreronis* and have revealed altered instantaneous rate of increase, walking patterns, and survival in the predator (Lima *et al.* 2013a, 2013b). Additionally, some acaricides have been shown to repel and/or irritate the predator (Lima *et al.* 2013a). Thus, this study aimed to evaluate the compatibility of the acaricides abamectin, fenpyroximate, and azadirachtin with the use of *N. baraki* by observing the action of these products on the predator's functional response.

Materials and Methods

Rearing *N. baraki.* *Cocos nucifera* coconuts were collected from Itamaracá Island, the state of Pernambuco, Brazil (07°46'S, 34°52'W) and transported to the Laboratory of Acarology of the Federal Rural University of Pernambuco (Universidade Federal Rural de Pernambuco - UFRPE). The plants from which the coconuts were taken have not been sprayed with pesticides for more than 10 years. The coconuts were kept in the laboratory (27 ± 1.0 ^{o}C, 75 ± 10% R.H., and a 12-hour photoperiod) until used. Approximately 100 *N. baraki* females were collected from the perianth of the coconuts and transferred to 16 cm diameter rearing units consisted of plastic trays containing 1cm thick polyethylene foam, on to which was placed a filter paper and 1 mm thick black PVC. In each unit, the PVC disk was surrounded by hydrophilic cotton moistened with distilled water to prevent mites from escaping. *Aceria guerreronis* was provided as food on perianth fragments (~0.5 cm^3) containing approximately 300 individuals in different stages of development. The food was replenished every 2 days as 5 perianth fragments per rearing unit. The *A. guerreronis* were removed from coconuts and stored up to 7 days. The rearing units were kept in an incubator at 27 ± 1.0 ^{o}C, 75 ± 10% RH, and a 12-hour photoperiod.

Acaricides. The following acaricides were administered in their respective registered concentrations to control *A. guerreronis* in coconut palms (Agrofit 2014): abamectin (Vertimec 18 CE, 18 g a.i. (active ingredient) l^{-1}, emulsifiable concentrate, Syngenta, São Paulo, São Paulo, Brazil) at 13.5 mg a.i. l^{-1}, azadirachtin (AzaMax, 12 g a.i. l^{-1}, emulsifiable concentrate, DVA Brazil, Campinas, São Paulo, Brazil) at 30 mg a.i. l^{-1}, and fenpyroximate (Ortus 50 SC, 50 g a.i. l^{-1}, suspension concentrate, Arysta LifeScience, Salto de Pirapora, São Paulo, Brazil) at 100 mg a.i. l^{-1}.

Testing the consumption of dead prey. Before performing the functional response experiment, dead prey without acaricide residue were offered to *N. baraki* to assess whether they would be consumed because some acaricides can kill *A. guerreronis* before they are predated. The method was adapted from the one used by Lima et al. (2012), and the experimental unit was similar to the rearing unit previously described, except for the black PVC which was replaced by fragments of *Canavalia ensiformis* L. leaves (4 x 4 cm). The preys were killed by touching them with a single-bristle brush and considered dead when no movement was observed in the legs. Ten dead prey individuals were offered on perianth fragments of 1 cm^3, and one visually healthy, fertilised *N. baraki* female was transferred from the rearing unit to each experimental unit. Each experimental unit comprised one replicate, and a total of ten replicates were performed. After 24 hours, the number of dead prey consumed was calculated, and it was observed that *N. baraki* consumes dead prey. Thus, the *A. guerreronis* individuals found during the evaluation of the functional response were not consumed due to the effect of the acaricides. The entire experiment was conducted under the same conditions of temperature, humidity, and photoperiod used for rearing.

Effect of acaricides on the functional response. The method used to evaluate the effect of insecticides on the functional response was the same as used in the dead prey consumption test.

However, the fragments of *C. ensiformis* leaves and perianth fragments were dipped (for 5 seconds) into distilled water or solutions containing the recommended concentrations of the acaricides for controlling *A. guerreronis*. After immersion, the leaf and perianth fragments were left to dry for 30 minutes. Next, *A. guerreronis* were transferred to perianth fragments at densities of 5, 10, 20, 40, and 80 individuals per fragment. Each experimental unit that contained one fertilised predator female comprised one replicate, and there were a total of 20 replicates for each density. After 24 hours, the number of prey (alive or dead) present in the arena was calculated, and the number of prey consumed was obtained by subtraction.

The evaluation method described above can lead to an overestimation of prey consumption because acaricides can repel and/or irritate the prey, causing them to try to escape the experimental arena. To correct for the overestimated prey consumption, a blank test was performed for each density of each treatment, following the same method described above, but without introducing the predator. Thus, the percentage of prey lost during the experimental period was quantified as a possible correction of the predator's consumption, which was only used when the total percentage of mites lost was higher than 10%. This correction was only performed for the density of 80 prey individuals/arena treated with azadirachtin, for which Abbot's (1925) formula was used.

Data analyses. For each treatment (control and each acaricide), logistic regression curves were fitted between the proportion of prey consumed and the density of prey, following the protocol by Juliano (1993) (Proc CATMOD, SAS Institute 2002), to determine the significance of the regression coefficients and the sign of the linear coefficient, which determined the type of functional response. Holling (1959, 1961) characterised 3 functional responses: Type I – a linear increase in the number of prey ingested by the predator up to a maximum as prey density

increases; Type II – the number of prey attacked by the predator quickly increases because of high prey availability followed by a gradual decrease until stabilisation (plateau); Type III – the response is sigmoid and approaches a higher asymptote. Using a modified version of the same protocol, the estimated proportion of dead prey was obtained (modification: Proc PRINT instead of Proc PLOT), and the proportion of prey consumed was plotted as a function of prey density using SigmaPlot® (Systat Software, San Jose, CA). The attack rate *(a')* and handling time (*Th*) parameters were calculated with nonlinear least squares regression (Proc NLIN of the SAS software, SAS Institute 2002) using the "full model" of the protocol by Juliano (1993) for a Type II functional response. The *a'* and *Th* values were compared among the treatments using 95% confidence intervals.

Logistic regression curves were fitted for each treatment (control and each acaricide) between the number of consumed prey and prey density using PROC REG in SAS software (SAS Institute 2002). After fitting the linear model ($P < 0.05$), the slopes of the regressions were compared using PROC MIXED in SAS software (SAS Institute 2002).

Peak consumption was calculated for each treatment based on the reciprocal of *Th* ($\frac{1}{Th}$) and compared based on the confidence interval. The mean variation in prey consumption for each predator at each density (ΔNa) was calculated following the equation of Poletti *et al.* (2007): $\Delta Na = \frac{(Na_{Nmax} - Na_{Nmin})}{(N_{max} - N_{min})}$ where $Na_{N\text{min}}$ and $Na_{N\text{max}}$ are the minimum and maximum number of prey consumed by the predator, respectively, and N*min* and N*max* correspond to the minimum and maximum densities. The consumption variation data were analysed using ANOVA, and the means were compared using Tukey's HSD test ($P = 0.05$) in SAS (SAS Institute 2002). The mean variation in consumption was plotted as a function of handling time; variations closest to the control indicated little or no effect on prey consumption by *N. baraki*.

Results

Regardless of acaricide exposure, the model of the variation in the number of prey killed by predators remained unaltered, and prey consumption stabilised at higher densities (Fig. 1a). This model corresponded to a Type II functional response (Table 1, Fig. 1a). Prey handling time by *N. baraki* (*Th*) remained unaltered when the predator was exposed to acaricides and ranged from 0.26 and 0.58 hours. However, the acaricides fenpyroximate and abamectin altered the *N. baraki* attack rate (*a'*), which was lower than that observed in the control treatment (Table 2).

Differences in the slopes of the regressions between prey consumption and the tested densities were observed among the treatments ($-21.37 \geq t \leq 7.61$; $P \leq 0.01$). A higher slope was observed for azadirachtin followed by the control, fenpyroximate, and abamectin (Fig. 1b).

Significant differences at the 5% level occurred in peak consumption when the 95% confidence intervals of the estimates did not overlap [control: 3.57 (3.35 - 3.68); azadirachtin: 3.23 (3.16 - 3.26); fenpyroximate: 3.85 (3.19 - 4.16) and abamectin: 1.72 (1.45 - 1.86)]. The peak consumption of prey estimated for the predator in the control treatment was 3.57 prey/hour which decreased by 52% when the predator was exposed to abamectin, distinct from the other acaricides. The peak consumption values were similar to the control when the predator was exposed to fenpyroximate and azadirachtin.

There was a difference in the mean variations in prey consumption by *N. baraki* (DF = 3; $F_{3,\ 73} = 59.24$; $P < 0.0001$). A higher degree of variation was observed in the control and azadirachtin treatments, and there was no difference between the treatments. This indicates little or no effect of azadirachtin on *N. baraki* predatory activity. Fenpyroximate and abamectin caused variations in prey consumption lower than the control, which indicates impairment of predatory

activity by these acaricides. The acaricide abamectin exhibited the lowest variation in prey consumption (Fig. 2).

Discussion

Functional response describes changes in a predator's feeding rate as a function of food density (Holling 1959). In this study, the acaricides azadirachtin, fenpyroximate, and abamectin did not alter the functional response type of *N. baraki*. However, fenpyroximate and abamectin compromised *N. baraki* predatory activity by altering the attack rate and consequently reducing the mean consumption of *A. guerreronis* by *N. baraki*.

Attack rate determines the capture ability of a predator within a certain area (Holling 1959), and this parameter was lower when *N. baraki* was exposed to fenpyroximate and abamectin, indicating that exposure to such products decreases the predator's potential for capturing prey. This decrease may be due to irritation and/or altered locomotion parameters. According to Lima *et al.* (2013a), fenpyroximate irritates *N. baraki*, causing a behavioural change that leads the predator to avoid the pesticide after contact with its residue (Cordeiro *et al.* 2010, Lima *et al.* 2013a). Thus, contact with fenpyroximate residue may have prevented encounters with *A. guerreronis* and, consequently, reduced prey consumption. Abamectin does not appear to cause irritability, but this product may compromise behavioural parameters in *N. baraki*, especially walking speed (Lima *et al.* 2013a). Due to the possible changes in these parameters, the search for prey by *N. baraki* was hindered.

Handling time did not significantly increase following the exposure of *N. baraki* to acaricides. Although statistically similar, the handling time by *N. baraki* when exposed to abamectin was 2.7 times higher than that observed in the absence of acaricide. This may explain

the 52 % decrease in the *N. baraki* consumption peak. Handling time includes the time needed by the predator to identify, capture, attack, and consume prey (Holling 1959). Therefore, high *Th* values suggest that the predator spends more time with a particular prey item and thus takes longer to go in search of another. This can be observed through the variation in prey consumption where the abamectin treatment, which results in the highest handling time, exhibited lower variation in the number of prey consumed. Altered *Th* values in mites and insects have been observed after exposure to neurotoxic pesticides (Wang & Shen 2002, Polleti *et al.* 2007, Rezac *et al.* 2010).

Fenpyroximate and abamectin decreased *A. guerreronis* consumption by *N. baraki* and also exhibited lower mean variations in consumption. This decrease was expected as these acaricides can alter the behavioural parameters of this predator (Lima *et al.* 2013a). Additionally, both of these acaricides altered the *N. baraki* attack rate. Among all of the acaricides, azadirachtin was the only one that did not decrease consumption, so it is possible that azadirachtin does not have a significant effect on *N. baraki* predation.

Contact of *N. baraki* with acaricide may take place since the onset of colonization (when the plants are approximately 2 months old), while walking on the coconut surface and penetrating it via the bract. This contact exposure likely lasts until mites disperse away from coconuts frequently treated with acaricides [about 15 days apart, but longer intervals have been suggested (Melo *et al.* 2012)]. According to Lima *et al.* (2013b), the acaricides studied here lead to low (acute) toxicity to *N. baraki*, but fenpyroximate and abamectin are lethal to *A. guerreronis* at levels lower than those used in this study (Monteiro *et al.* 2012, Lima *et al.* 2013b). Thus, *N. baraki* may reduce the population of *A. guerreronis* under field conditions (i.e., under acaricide use).

All of the acaricides from our study allowed for the maintenance of the predator in the field, but exposure to abamectin and fenpyroximate compromised prey consumption. This means that combining these acaricides with biological control is possible for *A. guerreronis* management, but predator efficiency is reduced with exposure to abamectin and fenpyroximate. However, evaluating the effects of acaricides on predator foraging and biology through further experiments is necessary to evaluate the risks from these compounds to *N. baraki* and the effectiveness of *N. baraki* as a biological control agent with the simultaneous use of these acaricides. In addition, the compatibility of the acaricides with the use of *N. baraki* should be further investigated under field conditions.

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Table 1. Holling disc equation and type of functional response of *Neoseiulus baraki* eating *Aceria guerreronis* in each treatment.

Treatments	Holling Disc Equation	χ^2	DF	P	Coefficient of LogisticRegression				Type
					I[1] (P)	L[2] (P)	Q[3] (P)	C[4] (P)	FR[5]
Control	$y = \frac{(0.0005x^2)-(0.09x)+4.32}{1+[(0.0005x^2)-(0.09x)+4.32]}$	219.59	97	<.0001	4.32 (<.0001)	- 0.09 (<.0001)	0.0005 (0.0007)	-	II
Azadirachtin	$y = \frac{-(0.00005x^3)+(0.0067x^2)-(0.25*x)+4.42]}{1-[(0.00005x^3)+(0.0067x^2)-(0.25*x)+4.42]}$	194.12	96	<.0001	4.42 (<.0001)	-0.25 (0.0003)	0.0067 (0.0003)	-0.00005 (0.0002)	II
Fenpyroximate	$y = \frac{-(0.00004x^3)+(0.0054x^2)-(0.21x)+2.78}{1-[(0.00004x^3)+(0.0054x^2)-(0.21x)+2.78]}$	458.89	96	<.0001	2.79 (<.0001)	-0.21 (<.0001)	0.0054 (<.0001)	-0.00004 (<.0001)	II
Abamectin	$y = \frac{-(0.00002x^3)+(0.0029x^2)-(0.11x)+0.56}{1-[(0.00002x^3)+(0.0029x^2)-(0.11x)+0.56]}$	260.79	96	<.0001	0.56 (0.0649)	-0.11 (0.0017)	0.0029 (0.0065)	-0.00002 (0.0085)	II

[1]Intercept

[2] Linear

[3]Quadratic

[4]Cubic

[5]Type of Functional response

Table 2. Parameters (±SE) of functional responses of *Neoseiulus baraki* fed with *Aceria guerreronis* and respective confidence intervals in each treatment.

Treatment	*a'* ± SE (95% CI)	*Th* ± SE (95% CI)
Control	0.13 ± 0.02 (0.09-0.17)	0.28 ± 0.03 (0.22-0.33)
Azadirachtin	0.13 ± 0.02 (0.10-0.17)	0.31 ± 0.02 (0.27-0.36)
Fenpyroximate	0.05 ± 0.01 (0.03-0.07)*	0.26 ± 0.07 (0.12-0.40)
Abamectin	0.02 ± 0.01 (0.01-0.03)*	0.58 ± 0.15 (0.29-0.87)

Attack rate coefficient *a'* (in units of the proportion of prey captured by predator per unit of searching time) and handling time *Th* (in units of the proportion of 24 h exposure period)

*Significantly different from control at 5% level when 95% confidence intervals of estimates did not overlap.

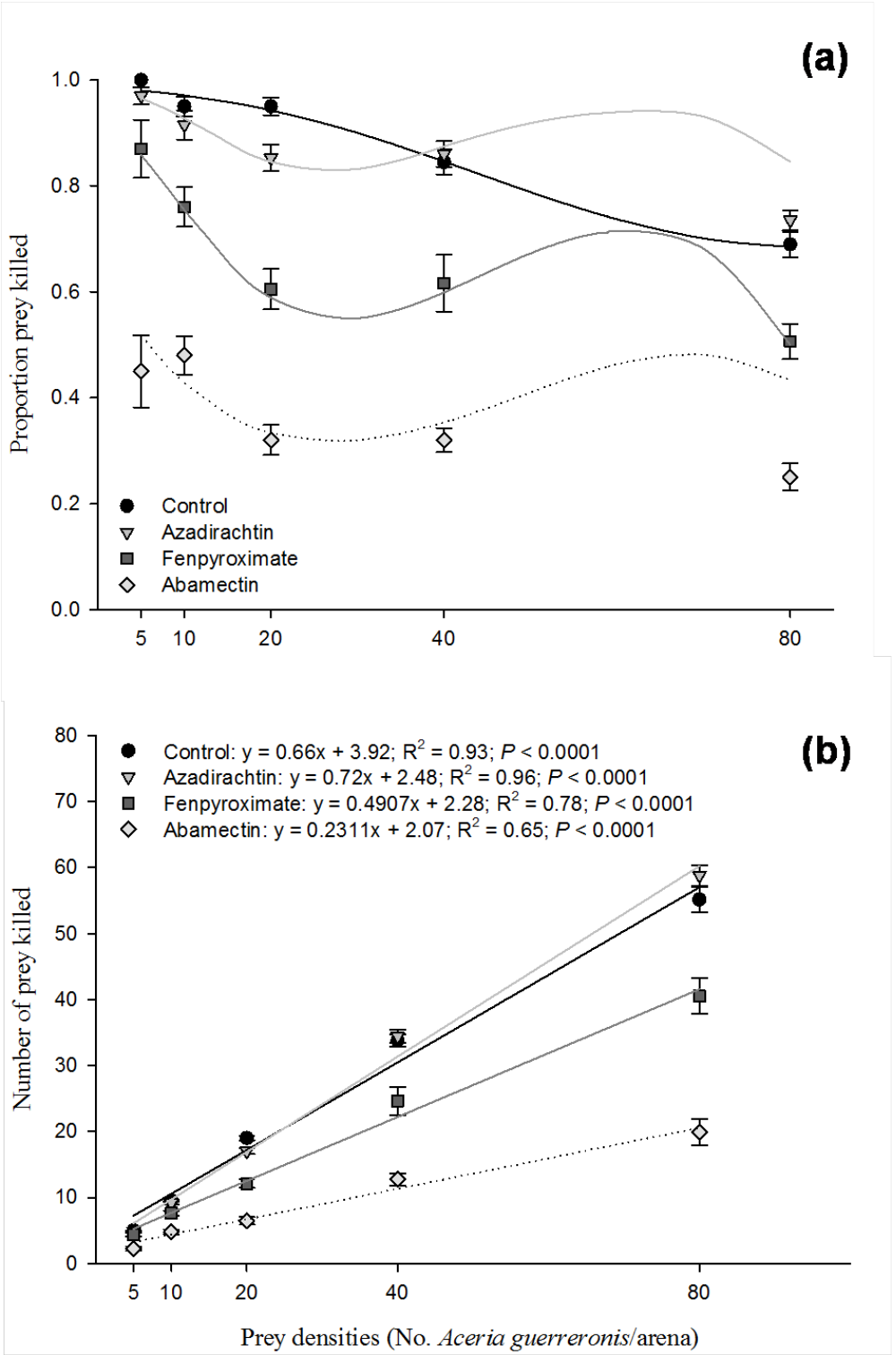

Figure 1. Regression of proportion (Mean ± SE) (a) and number (Mean ± SE) (b) of *Aceria guerreronis* killed per *Neoseiulus baraki*.

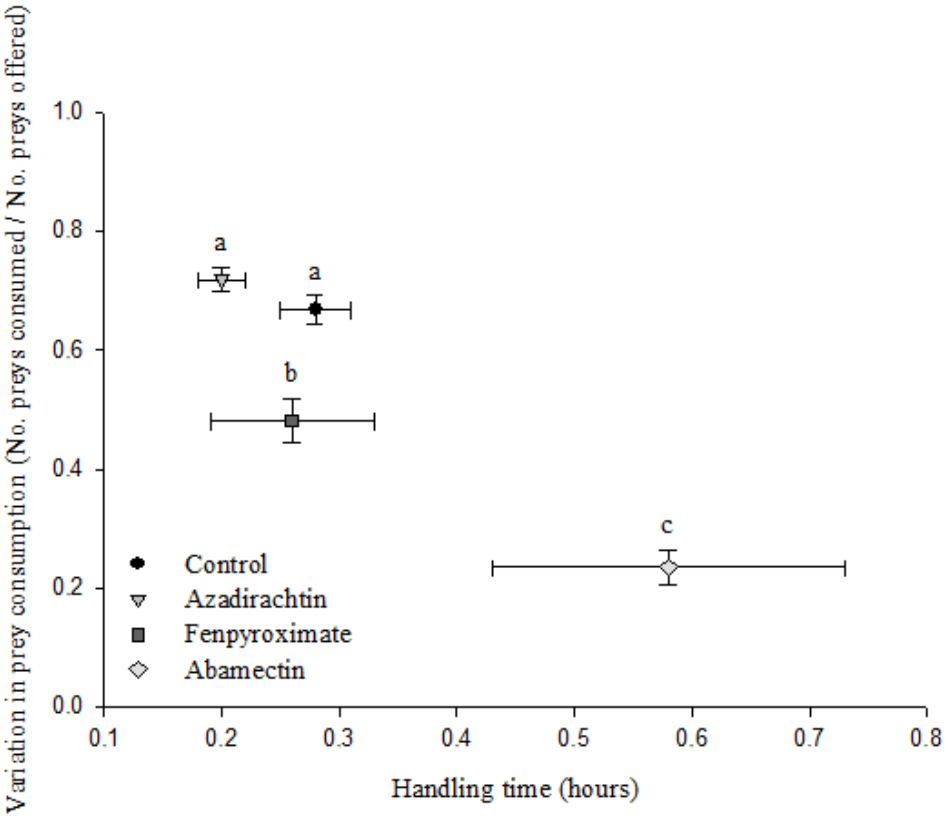

Figure 2. Mean variation in consumption of *Aceria guerreronis* (Mean ± SE) as a function of variation in handling time (Mean ± SE) by *Neoseiulus baraki*. Different letters indicate significant differences between treatments by the Tukey's HSD test ($P < 0.05$).

CAPÍTULO 4

ACARICIDE POPULATION-LEVEL EFFECTS ON THE PREDATORY MITE *Neoseiulus baraki*

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Lima, D. B., Melo J.W.S., M.G.C. Gondim JR., R.N.C. Guedes & J.E.M. Oliveira. Acaricide population-level effects on the predatory mite *Neoseiulus baraki*. Em revisão na Exp. Appl. Acarol.

RESUMO – O sistema de produção de coco, em que o ácaro do coqueiro *Aceria guerreronis* é considerado uma praga-chave, fornece um modelo interessante de integração entre o controle biológico e químico. No Brasil, o agente de controle biológico mais promissor contra o ácaro do coqueiro é o fitoseídeo predador *Neoseiulus baraki*. No entanto, aplicação de acaricidas é o controle mais utilizado contra o ácaro do coqueiro, embora eles produzam frequentemente resultados insatisfatórios. Neste estudo, avaliou-se o efeito direto do contato com resíduo seco e ingestão de presa contaminada com os principais acaricidas utilizados em coqueiros (abamectina, azadiractina e fenpiroximate) sobre a biologia de *N. baraki* e seus descendentes. Estes acaricidas são registrados, recomendados e amplamente utilizada contra esta espécie de praga no Brasil; as doses de campo destes foram aqui testadas. A segunda geração dos predadores expostos aos acaricidas também foi avaliada pela estimativa da taxa instantânea de crescimento populacional (*ri*). Abamectina comprometeu o desempenho das fêmeas, enquanto fenpiroximato não afetou as fêmeas expostas aos acaricidas (F0). No entanto, fenpiroximato comprometeu fortemente os descendentes (F1), taxa líquida de reprodução (R0), taxa intrínseca de crescimento populacional (*ri*), e tempo de duplicação da população (DT). Em contraste, fenpiroximate não teve efeitos sobre a segunda geração de predadores expostos a acaricidas. A azadiractina não afectou os predadores, sugerindo que este acaricida pode ser utilizado em associação com o controle biológico com esta espécie predadora. Em contraste, o uso de abamectina e fenpiroximate é susceptível a consequências adversas para o controle biológico de *A. guerreronis* usando *N. baraki*.

PALAVRAS-CHAVES: Toxicologia demográfica, tabela de vida, *Aceria guerreronis*, Phytoseiidae, Manejo Integrado de Pragas

ACARICIDE POPULATION-LEVEL EFFECTS ON THE PREDATORY MITE *Neoseiulus baraki*

ABSTRACT –The coconut production system, in which the coconut mite *Aceria guerreronis* is considered a key pest, provides an interesting model of integration between biological and chemical control. In Brazil, the most promising biological control agent for the coconut mite is the phytoseiid predator *Neoseiulus baraki*. However, acaricides are the control agent most widely used against the coconut mite, although they frequently produce unsatisfactory results. In this study, we evaluated the simultaneous direct effect of dry residue contact and contaminated prey ingestion of the main acaricides used on coconut palms (i.e., acaricides abamectin, azadirachtin and fenpyroximate) on the life history traits of *N. baraki* and their offspring. These acaricides are registered, recommended and widely used against this pest species in Brazil, and they were tested at their label rates. The 2nd generation of the exposed predators was also evaluated by estimating the instantaneous rate of population increase (*ri*). Abamectin compromised female performance, while fenpyroximate did not affect the exposed females (F0). Nonetheless, fenpyroximate strongly compromised the offspring (F1) net reproductive rate (R_0), intrinsic rate of population growth (*ri*), and doubling time (DT). In contrast, fenpyroximate did not have such effects on the 2nd generation of predators with acaricide-exposed parents. Azadirachtin did not affect the predators, suggesting that this acaricide can be used in association with biological control for this predatory species. In contrast, the uses of abamectin and fenpyroximate are likely to lead to adverse consequences in the biological control of *A. guerreronis* using *N. baraki*.

KEYWORDS: Demographic Toxicology, Life Table, *Aceria guerreronis*, Phytoseiidae, Integrated Pest Management

Introduction

Predatory mites are considered effective natural enemies of phytophagous mites (Helle & Sabelis 1985, McMurtry & Croft 1997, McMurtry *et al.* 2013). Many predatory mite species are specialized feeders and are able to respond to the population dynamics of particular species (Solomon *et al.* 2010), such as predatory type I species (specialized mite predators) (McMurtry *et al.* 2013). However, they are frequently unable to fully and reliably prevent pest damage when used as a sole management tactic (Solomon *et al.* 2010). In such cases, alternative strategies are needed to control mite pests. The control of phytophagous mites is frequently performed almost exclusively using acaricides (Watanabe *et al.* 1994). Nevertheless, this is an expensive management tactic that requires periodic acaricide applications and the purchase of suitable equipment and supplies. Thus, given its high cost, the use of acaricides can be economically prohibitive for small-scale farmers. In such low-input production systems (systems that receive no or low cash inflows), the integration between predatory mites and acaricides becomes an economically viable option. For this integration to be possible, acaricides with low (or no) negative impacts on predatory mites are required. As the coconut production system permits the potential integration of these management tactics, it is a suitable model for studying such integration.

The coconut *Cocos nucifera* L. suffers from attack by several pests. Among these, the coconut mite *Aceria guerreronis* Keifer (Acari: Eriophyidae) is considered a key pest of coconut palms (Moore & Howard 1996, Haq *et al.* 2002). Several countries have reported losses of up to 60% due to this pest (Negloh *et al.* 2011, Navia *et al.* 2013, Rezende 2014). The coconut mite causes damage by feeding on the fruit perianth, an enclosed region under the bracts where the mite develops. The fruit surface becomes necrotic and can eventually result in premature fruit fall,

reducing the number of fruits in the bunch (Moore & Howard 1996). The most common method of control for this pest is based on intensive acaricide use, but because this pest lives well protected under the floral bracts, the efficacy achieved using acaricides is not always satisfactory (Moore & Howard 1996, Ramaraju *et al.* 2002, Monteiro *et al.* 2012). Monteiro *et al.* (2012) suggest that control of the coconut mite using pesticides should only be implemented while the mites are dispersing by walking over sprayed fruit.

Biological control is gaining attention as a management alternative for coconut mites (Domingos *et al.* 2010, Lima *et al.* 2012). The most promising biological control agent for the coconut mite is *Neoseiulus baraki* (Athias-Henriot) (Acari: Phytoseiidae) (Aratchige *et al.* 2007, Negloh *et al.* 2008, Domingos *et al.* 2010, Melo *et al.* 2011, Lima *et al.* 2012). This predator has easier access to the microhabitat inhabited by the pest compared with others predators, and it exhibits a progressively higher predation rate of the coconut mite (Lima *et al.* 2012). *Neoseiulus baraki* has been tested in field inundative releases by Fernando *et al.* (2010), who reported that a single release of *N. baraki* in coconut palm sprovides significant reduction of the coconut mite population.

Within this context, understanding the potential effects of the acaricides used against the coconut mite on its predator *N. baraki* is fundamental for the integration of chemical and biological control. Fenpyroximate and chlorfenapyr appear to be selective to *N. baraki* over *A. guerreronis* (Lima *et al.* 2013a). However, there is no information on the potential impacts of acaricides on the life history traits and demography of *N. baraki*. Information on demographic toxicology involves the ecological and toxicological parameters that predict the overall effect of toxins at the population level (Ahmadi 1983, Stark & Wennergren 1995). Fecundity and population growth have been used as indicators of mite population performance. The latter is a

more robust toxicological endpoint than typical mortality assessments because it includes assessments of fertility and the mortality and birth rates in a given population (Saito 1979, Sabelis 1985, Stark & Wennergren 1995, Stark *et al.* 1997, Ansaloni *et al.* 2007, Walthall & Stark 2007). The evaluation of the life table parameters of predators after exposure to acaricides is helpful for the selection of suitable acaricides with minimal non-target toxicity. In this study, we evaluated the effects of widely used acaricides (abamectin, azadirachtin and fenpyroximate) that are recommended by the Brazilian Ministry of Agriculture against *A. guerreronis* (Agrofit 2015) on the life history traits of *N. baraki* and their 1st and 2nd generation offspring.

Materials and methods

Establishment and maintenance of predator colonies. Coconuts were collected from the coastal island of Itamaracá ("Ilha de Itamaracá"), State of Pernambuco, Brazil (07°46'S, 34°52'W), and transported to the laboratory. The area is not commercially used; thus, it has not been subjected to pesticide applications or coconut mite control for over 10 years. Coconuts and mites were collected from 10 plants in the area. The fruits were maintained under controlled laboratory conditions (27 ± 1 °C, 70 ± 10 % relative humidity [RH] and a 12-h photoperiod). *Neoseiulus baraki* colonies were established using approximately 100 females collected from coconut fruits and transferred to rearing units. Each rearing unit consisted of a black PVC disk (13 cm in diameter, 1 mm thick) laid on to a foam mat disk that lined the bottom of a plastic tray. The margin of the PVC disk was covered using a band of hydrophilic cotton, and both the foam mat and the cotton band were kept wet by daily additions of distilled water to the tray. Coconut mites (*A. guerreronis*) were provided as food on a piece of perianth (approximately 1 cm^2) obtained from the collected coconut fruits, as previously described. Three hundred coconut mites were

placed on each piece of perianth, which was replaced every two days to prevent the perianth from drying out, which would cause the coconut mites to starve to death. Five pieces of perianth were placed in each rearing unit. The units were maintained in a rearing chamber under the environmental conditions described above.

Acaricides. Azadirachtin, fenpyroximate and abamectin were the acaricides used in the experiments. These acaricides are registered and recommended by Brazilian Ministry of Agriculturefor use against *A. guerreronis* and are widely used by coconut farmers (Agrofit 2015). These compounds were used in their respective commercial formulations as follows: azadirachtin (Azamax, 1.2 g a.i. [active ingredient]/L, emulsifiable concentrate, DAV Agro, Ituverava, SP, Brazil), fenpyroximate (Ortus, 50 g a.i./L, suspension concentrate, Arysta Lifescience, Salto de Pirapora, SP, Brazil), and abamectin (Vertimec, 18 g a.i./L, emulsifiable concentrate, Syngenta, São Paulo, SP, Brazil). The acaricides were tested at a single rate, the maximum label rate for the coconut mite in Brazil (Agrofit 2015), and their corresponding concentrations used in our experiments were 30 mg a.i./L for azadirachtin; 100 mg a.i./L for fenpyroximate, and 13.5 mg a.i./L for abamectin (Agrofit 2015).

Effects of acaricides on the life history traits of the treated unmated females (F_0). Pieces of coconut perianth (0.5 cm^3), individual wells (i.e., cells) of bioassay trays and adhesive covers (128 cells; Bio-Serv, Frenchtown, NJ, USA) were immersed in water (control) or one of each of the acaricide solutions recommended for the coconut mite for 5 s and allowed to air-dry for 2 h (Lima *et al.* 2013a). Then, a treated piece of perianth was placed in each cell and 300 *A. guerreronis* were transferred onto each treated piece of perianth food source for the predators. One *N. baraki* female (12 days old) was transferred to each well and confined for 24 h. Treated adhesive covers were used to seal each well to prevent the mites from escaping. The wells were placed in a rearing

chamber and maintained at 27 ± 1 °C and 70 ± 10 % RH and a 12-h daily photoperiod. After 24 h, the surviving females were transferred to untreated wells. An untreated male was also transferred to each (untreated) well containing a female to allow mating. The food was replenished every day with a new piece of perianth with coconut mites. Forty replicates were performed for each acaricide following a completely randomized design where each female represented one replicate.

Fertility and the pre-oviposition, oviposition, and post-oviposition periods were monitored, and data were recorded daily until female death. The males that died were replaced with new ones. For each treatment, the number of eggs per female (mx) on each oviposition date (x) was calculated considering the total number of females, the cumulative survival rate of females (lx) during the oviposition period, and the number of adult offspring of x age in the next generation (lx.mx). Using this information (mx, lx and lx.mx), the following parameters were estimated: net reproductive rate ($R_0 = \sum lxmx$), mean generation time ($T = \frac{\sum xlxmx}{\sum lxmx}$), intrinsic rate of increase ($r_m = \frac{Ln(R_0)}{T}$) and doubling time ($DT = \frac{Ln(R_0)}{r_m}$); the latter refers to the time needed to double the initial population.

The fertility and the pre-oviposition, oviposition, and post-oviposition periods were analysed using the Kruskal-Wallis test with the NPAR1WAY procedure (SAS Institute 2008). The survival data were used to construct time-mortality curves using Kaplan–Meier estimators with the LIFETEST procedure (SAS Institute 2008), and log-rank tests were used for pairwise planned comparisons. The median survival times were analysed using the Kruskal-Wallis test employing the NPAR1WAY procedure (SAS Institute 2008). The "jackknife" technique was used to estimate the confidence intervals to compare life table parameters (Maia *et al.* 2000). A chi-square analysis was performed to determine whether there was any deviation from the expected sex ratio of 1:1 using the FREC procedure (SAS Institute 2008).

Effects of acaricides on the development and reproductive performance of the offspring (F_1) of treated females. Eggs from untreated (control) and treated females were transferred individually into untreated wells. Each subsequent stage was checked daily, and the developmental time and survival were recorded. When the adults emerged, they were sexed, and the females were individualized. Males were subsequently added and paired with the females. Coconut mites were provided as food on a piece of perianth (approximately 1 cm^2) containing nearly 300 individuals. The food was changed every day.

The developmental time (from egg to adulthood) and juvenile survival (proportion of eggs reaching adulthood) were subjected to Kaplan-Meier survival analysis using the LIFETEST procedure (SAS Institute 2008) to identify the overall effect, and log-rank tests were used for planned pairwise comparisons (Hosmer & Lemeshow 1999). The fertility, pre-oviposition, oviposition, and post-oviposition periods were analysed using the Kruskal-Wallis test employing the NPAR1WAY procedure (SAS Institute 2008). The survival data of the adults were used to construct time-mortality curves using Kaplan–Meier estimators, employing the LIFETEST procedure (SAS Institute 2008), and log-rank tests were used for planned pairwise comparisons. Using the developmental time, viability and female oviposition data, lifetables were constructed and analysed for each treatment as specified in the experiments described in subsection "Effects of acaricides on biological parameters of the treated unmated females (F_0)".

Effects of acaricides on the offspring (F_2) of F_1 females of *N. baraki*. The eggs laid by the F_1females (Bioassay: "Effect of acaricides on the developmental and reproductive performance of the offspring of treated females") were collected daily to determine and compare the instantaneous rate of increase (r_i) of the following generation (F_2). Every egg was transferred to a new untreated well, placed in a rearing chamber and maintained at 27 ± 1 °C and 70 ± 10 % RH

and a 12-h daily photoperiod until they reached adulthood. Coconut mites were provided as food on a piece of perianth (approximately 1 cm^2) containing nearly 300 individuals. When the females emerged, one male was added to each well. Fifteen replicates were performed for each acaricide, and every female represented one replicate. The numbers of eggs, larvae, nymphs and adults were recorded for 10 days. The instantaneous rate of increase (r_i) was estimated using the following equation: $r_i = \ln {(N_f/N_0)}/{\Delta t}$, where N_f is the final number of live mites, N_0 is the initial number of live mites and Δt is the time interval between the start and end of the bioassay (Stark et al. 1997; Walthall and Stark 1997), which was carried out for 10 days. Positive r_i values indicate that the population is growing, $r_i = 0$ indicates that the population is stable, and negative r_i values indicate that the population is in decline. The instantaneous rate of increase was analysed using the Kruskal-Wallis test employing the NPAR1WAY procedure (SAS Institute 2008).

Results

Effects of acaricides on the biological parameters of the treated unmated females (F_0). The exposure of *N. baraki* females to the acaricides did not affect the pre-oviposition period ($\chi^2 \geq 0.17$; d.f. = 1; $P \geq 0.06$). However, significant differences were observed for the oviposition ($\chi^2 = 28.10$; d.f. = 3; $P < 0.001$) and post-oviposition periods ($\chi^2 = 18.11$; d.f. = 3; $P < 0.001$). Both periods (oviposition and post-oviposition) were shorter when the females were treated with abamectin. In addition, this product was the only one that affected the number of eggs per female ($\chi^2 = 28.15$; d.f. = 3; $P < 0.001$), which decreased by 96 % (Table 1).

Abamectin was the only acaricide that caused changes in the survival curves ($\chi^2 = 134.32$; d.f. = 3; $P < 0.001$) and consequently decreased the median survival time ($\chi^2 = 27.15$; d.f. = 3; $P < 0.001$) of *N. baraki* (Fig. 1 A and B). This pesticide reduced survival by 76.7 % and the median

survival time by 84 %. Adult *N. baraki* did not survive longer than 7 days after exposure to abamectin, and the median survival time was 3.5 ± 0.4 days.

The R_0 was negatively affected by abamectin and was reduced by 96.52%. Although the other parameters were not affected when *N. baraki* was exposed to abamectin, these parameters were consistently (numerically) lower, and r_m and DT were negative. The values of r_m, T and DT did not differ among the treatments (Table 2). The number of female offspring of *N. baraki* was consistently higher than the number of male offspring, except when females were exposed to abamectin, in which case the only two viable eggs produced males (Table 3).

Effects of acaricides on the development and reproductive performance of the offspring (F_1) of treated females. It was not possible to analyse the developmental time, juvenile survival or life-table parameters of offspring of abamectin-treated females of *N. baraki* because only two F_0 eggs were viable, and these eggs produced males. For the other acaricides, there was no effect of the exposed *N. baraki* females on the developmental rate of their offspring (Fig. 2; $\chi^2 = 0.66$, d.f. = 2, $P = 0.72$), but there was a significant effect on juvenile survival (Fig. 2; $\chi^2 = 6.84$, d.f.= 2, $P = 0.03$). This was caused by the lower survival of juveniles from fenpyroximate-exposed females compared with unexposed females (control) or azadirachtin-exposed females (Fig. 2; $\chi^2 > 4.38$, d.f. = 1, $P < 0.04$).

The survival curves of the offspring of acaricide-exposed females were not significant different from those of the offspring of unexposed females ($\chi^2 = 1.40$; d.f. = 2; $P = 0.49$). There were significant acaricide effects on the R_0, r_m and DT of the offspring of the exposed *N. baraki* females from those of the offspring of unexposed females (Table 4). Fenpyroximate was the only acaricide that significantly reduced the R_0 and r_m of the offspring compared with the offspring of azadirachtin-treated and untreated females. As a consequence, the offspring of females exposed to

fenpyroximate had a higher DT. There was no effect of acaricide exposure on the generation time (T) of *N. baraki* females from those of the offspring of unexposed females.

Effects of acaricides on the second generation of exposed *N. baraki.* The r_i value of the second generation of the acaricide-exposed females was not significantly different with the control (χ^2 = 4.70; d.f. = 2; P = 0.09). The r_i values were as follows: control = 0.22 ± 0.01, azadirachtin = 0.20 ± 0.01 and fenpyroximate = 0.21 ± 0.01.

Discussion

Three acaricides registered at the Ministry of Agriculture and widely used against the coconut mite in Brazil (Agrofit 2015) were evaluated with regard to their effects on the predator *N. baraki* upon realistic exposure via contact with contaminated surfaces and ingestion of contaminated prey to identify which compounds are likely to be compatible with biological control of the coconut mite, exhibiting little or no negative impact on the biocontrol agent. The results of our research showed negative effects of abamectin on the oviposition, post-oviposition, survival curves and R_0 of female *N. baraki* after exposure. Reductions in R_0 and r_m as well as an increase in DT were observed in the offspring of fenpyroximate-exposed *N. baraki* females. No biological parameter of *N. baraki* or their offspring was affected by azadirachtin.

Abamectin decreased the oviposition of *N. baraki* as well as its fertility. The adverse effect of abamectin on the fecundity of phytoseiid predators has been demonstrated in several studies (Zhang & Sanderson 1990, Ibrahim & Yee 2000, Bostanian & Akalach 2006, Nadimi *et al.* 2009, Lima *et al.* 2013b). Lima *et al.* (2015a) observed that abamectin exposure can compromise the consumption of *A. guerreronis* by *N. baraki*. A reduction in predator feeding may compromise its fitness. Our results also showed that the population growth rate of the offspring of abamectin-

exposed females was significantly reduced. This product has been considered non-selective based on a comparison of its toxicity on *N. baraki* and coconut mite (Lima *et al.* 2013b).

Although fenpyroximate did not show negative effects on treated females (F_0), this product showed adverse effect on offspring of the treated females (R_0, r_m and DT). Our findings agree with those of Hamedi *et al.* (2010), who reported a decreased oviposition period of fenpyroximate-treated females of the predatory mite species *Phytoseius plumifer* (Canestrini & Fanzago) (Acari: Phytoseiidae), compromising its fertility and that of the subsequent generation. How the acaricides interfere with the offspring of predatory mites is unclear. However, fenpyroximate did not affect the 2nd generation. These results suggest that the impact of fenpyroximate is minimized over the generations. In our study, the predator was exposed only once to the acaricides, which we assumed to be a realistic condition because *N. baraki* lives under the bracts and is not in contact with acaricides that do not reach this area. Nevertheless, field acaricide application is performed at short intervals (at least two times per month) (Melo *et al.* 2012). Thus, the effects observed here will be more persistent in the field.

Azadirachtin also does not appear to affect the biological parameters of *N. baraki* through subsequent generations (e.g., life table parameters for the first generation and r_i for the second generation). Althought behaviour effect has been reported on *N. baraki* (walking behaviour, overall predator activity and mating behaviour) when exposed to azadirachtin (Lima *et al.* 2013a, 2015b). These authors suggested that doubts remain regarding the alleged environmental safety and selectivity of this bioinsecticide towards biological control agents.

The results obtained in this study indicate significant negative effects of abamectin and fenpyroximate on *N. baraki*. The use of abamectin and fenpyroximate resulted in adverse consequences for the biological control of *A. guerreronis* using *N. baraki* because both acaricides

decreased predator population growth, extending to the 1st but not the 2nd generation of exposed mites. Curiously, azadirachtin did not exhibit significant negative effects on the predator population, which suggests its potential compatibility with biological control for the management of coconut mites. However, field experiments need to be performed using periodic acaricide applications to account for this pattern of pesticide use and to allow for possible predator behavioural changes with exposure, which has been reported elsewhere (Melo *et al.* 2011, Lima *et al.* 2013a, 2015b) and may result in significant negative impacts on *N. baraki*.

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Table 1.Effects of sublethal exposure of acaricides on the reproductive performance and female longevity of *Neoseiulus baraki*.

Parameters	Treatments			
	Control	Abamectin	Azadirachtin	Fenpyroximate
Period of pre-oviposition (days ± SE)	1.3 ± 0.16a	2.9 ± 0.38a	4.9 ± 1.84a	2.0 ± 0.29a
Period of oviposition (days ± SE)	15.5 ± 1.7a	0.6 ± 0.16b	11.6 ± 0.16a	13.5 ± 0.16a
Period of post-oviposition (days ± SE)	1.7 ± 0.90a	0.1 ± 0.16b	1.7 ± 0.16a	2.3 ± 0.16a
Total number of eggs/females (eggs ± SE)	24.1 ± 2.71a	1.0 ± 0.64b	20.4 ± 3.23a	22.9 ± 4.10a

Means with in a row followed by different letters are significantly different by the Kruskal-Wallis test ($P > 0.05$).

Table 2. Life table parameters (± 95 % CI) of *Neoseiulus baraki* treated or not with acaricides at 27 ± 1 °C and 70 ± 10 % RH under a 12-h daily photoperiod.

Treatments	R_0 (females/female)	r_m(females/female/days)	T (days)	DT (days)
Control	13.21 (9.33 – 16.48)	0.20 (0.18 – 0.22)	12.76 (11.02 – 14.50)	3.42 (3.10 – 3.73)
Abamectin	0.46 (-0.17– 1.10)*	-0.02 (-0.25 – 0.20)	11.74 (7.82 – 15.65)	-6.90 (-21.56 – 7.76)
Azadirachtin	9.19 (6.16 – 12.21)	0.18 (0.15 – 0.20)	12.63 (11.08 – 14.19)	3.91 (3.27 – 4.55)
Fenpyroximate	11.23 (7.19 – 15.27)	0.18 (0.16 – 0.21)	13.39 (11.79 – 15.00)	3.81 (3.26 – 4.35)

The asterisks indicate significant differences between the treatment and control based on a 95 % confidence interval after estimating errors using the jackknife method

Table 3.Effects of sublethal exposure of acaricides on the sex ratio of *Neoseiulus baraki* tested by the chi-square goodness-of-fit test to a 1:1 (female:male) ratio*.

Treatments	Observed Frequency		Expected Frequency		df	χ^2	P-value	Female/Male
	Female	Male	Female	Male				
Control	15	4	9.5	9.5	1	6.36	0.01	3.75
Abamectin	0	2	1.0	1.0	-	-	-	-
Azadirachtin	15	2	8.5	8.5	1	9.94	0.002	7.50
Fenpyroximate	15	6	10.5	10.5	1	3.86	0.049	2.50

*The real sex ratio for *Neoseiulus baraki* is 80 % (Domingos et al. 2010); 50 % was used for statistical purposes.

Table 4. Life table parameters (± 95 % CI) for the offspring of *Neoseiulus baraki* females treated or not with acaricides at 27 ± 1 °C and 70 ± 10 % RH undera 12-h daily photoperiod

Treatments	R_0 (females/female)	r_m(females/female/days)	T (days)	DT (days)
Control	10.05 ± 3.01 (7.03 - 13.06)	0.20 ± 2.11 (0.16 - 0.24)	11.38 ± 2.11 (9.27 - 13.50)	3.39 ± 0.72 (2.68 - 4.11)
Azadirachtin	15.30 ± 5.75 (9.55 - 21.05)	0.24 ± 0.03 (0.21 - 0.27)	11.58 ± 1.63 (9.95 - 13.21)	2.92 ± 0.37 (2.55 - 3.29)
Fenpyroximate	3.47 ± 1.71 (1.76 - 5.17)*	0.11 ± 0.04 (0.07 - 0.15)*	11.50 ± 1.90 (9.95 - 13.21)	6.00 ± 2.26 (3.84 - 8.35)*

The asterisks indicate significant differences between the treatment and control according to a 95% confidence interval after estimating errors using the jackknife method.

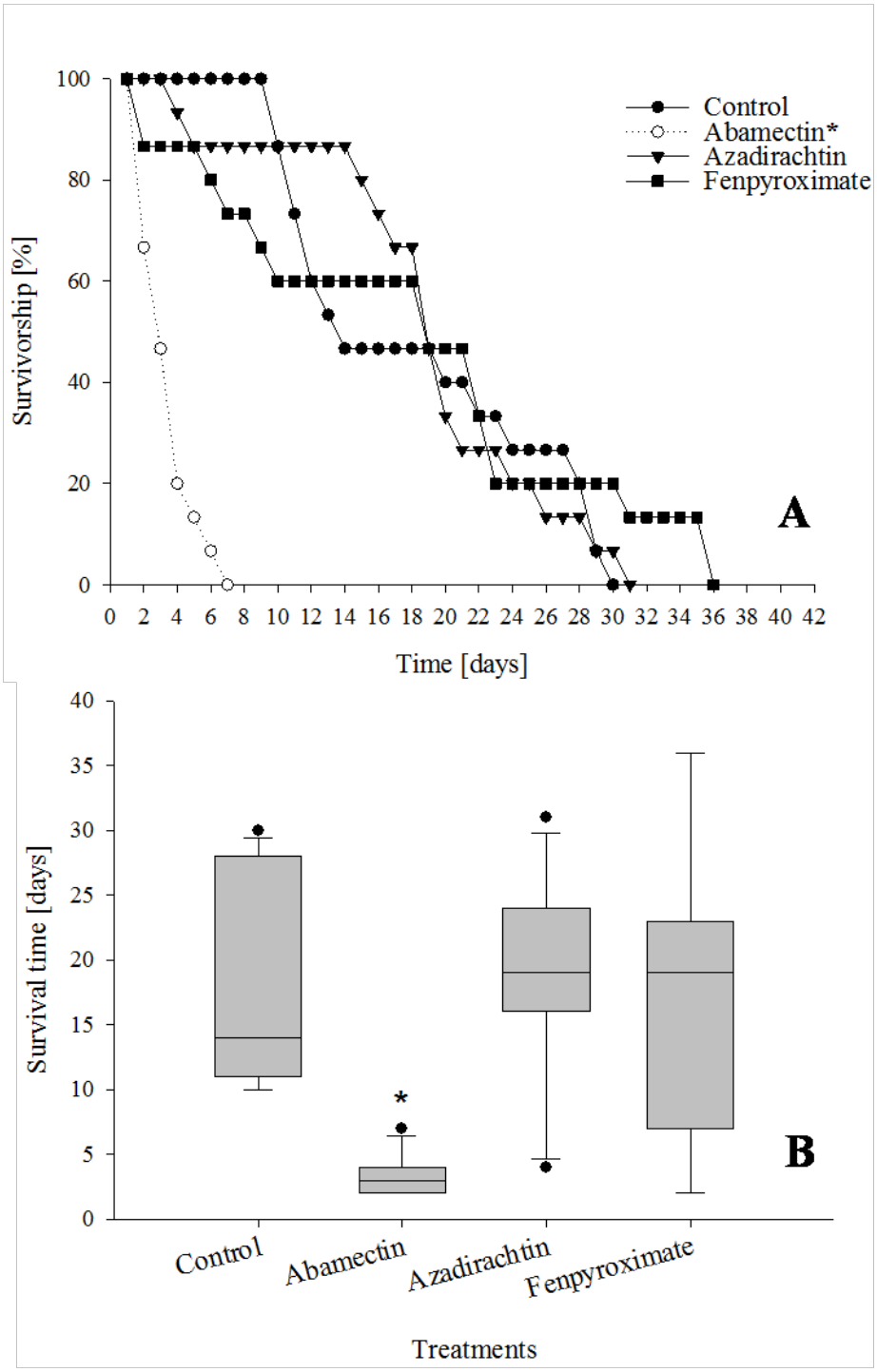

Figure 1. Observed survival curves of *Neoseiulus baraki* (A) and respective median survival times (B) when exposed to abamectin, azadirachtin and fenpyroximate. The asterisk indicates a significant difference between acaricide-exposed and unexposed predators ($P < 0.05$).

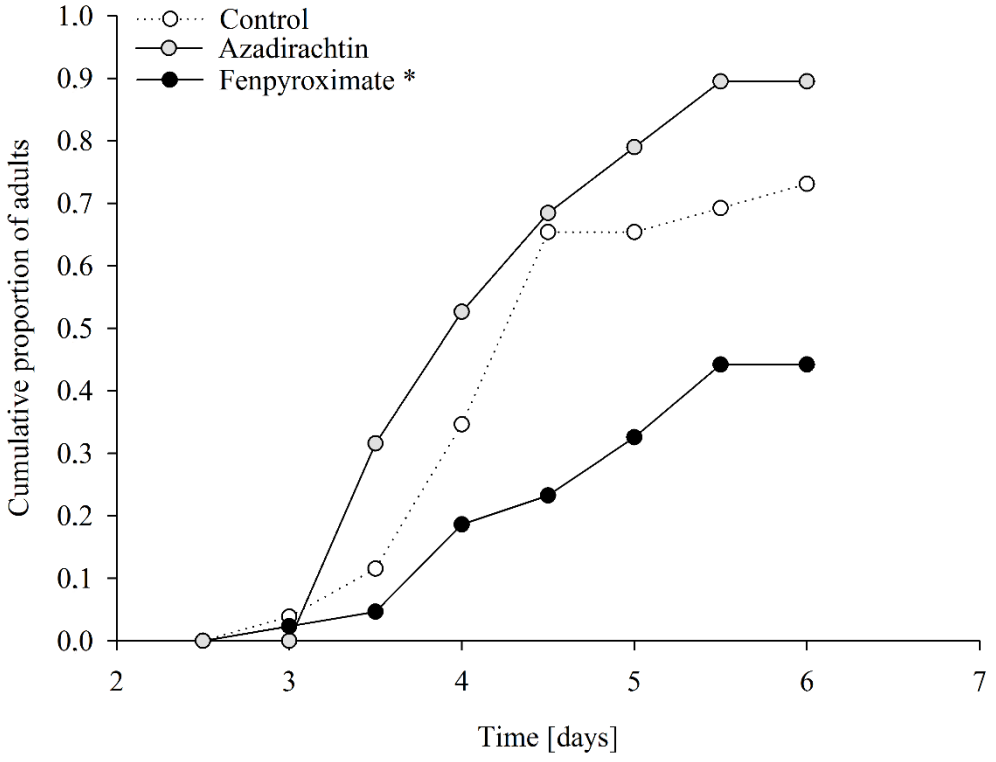

Figure 2. Proportion of offspring of *Neoseiulus baraki* developing to adults after female exposure to acaricides (azadirachtin and fenpyroximate) or water (control). There were no significant differences in offspring developmental time (days). The asterisk indicates a significant difference in the cumulative proportion of adults ($P < 0.05$).

CAPÍTULO 5

ACARICIDES IMPAIR PREY LOCATION IN A PREDATORY PHYTOSEIID MITE

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Lima, D.B., Oliveira, H.K.V., J.W.S. Melo, M.G.C. Gondim Jr, R.N.C. Guedes, A. Pallini & J.E.M. Oliveira. Acaricides impair prey location in a predatory phytoseiid mite. Submetido a Journal of Applied Entomology.

RESUMO – A utilização de ácaros predadores como a única tática de manejo em programas de controle biológico freqüentemente não evita danos de ácaros fitófagos nas plantas. Portanto, como uma alternativa, a integração de ácaros predadores com acaricidas pode proporcionar um controle mais eficiente de ácaros fitófagos do que apenas o uso de predadores. No entanto, para essa integração ser possível, acaricidas com mínimos impactos negativos sobre ácaros predadores são necessários. Neste estudo, foram avaliados os efeitos subletais de três acaricidas sobre o comportamento de forrageamento de *Neoseiulus baraki* (Athias-Henriot) (Acari: Phytoseiidae) em um sistema de produção de coco. Os acaricidas foram avaliados quanto a interferência da localização do habitat da presa através de um olfactômetro de tubo em Y e por interferência com a localização da colônia da presa dentro do habitat usando um sistema digitalizado de companhamento de movimentação. Adicionalmente a escolha da fonte de odor, o tempo requerido e a distância caminhada até a escolha foram avaliados. Os acaricidas testados foram abamectina, azadiractina e fenpiroximato. Ácaros predadores preferiram frutos infestados com o ácaro do coqueiro *Aceria guerreronis* Keifer (Acari: Eriophyidae) a frutos não infestados quando não expostos aos acaricidas. No entanto, quando exposto aos acaricidas, o predador não distinguiu entre frutos infestados e não infestados. Quando exposto a abamectina, *N. baraki* apresentou maior tempo de descanso e caminhou maiores distâncias antes de fazer a escolha de uma fonte de odor. Assim, os acaricidas prejudicaram a capacidade do ácaro predador *N. baraki* para localizar o habitat da presa e localizar a presa dentro do seu habitat. Os acaricidas diferentemente afetam o forrageamento da presa por interferir na percepção de odor.

PALAVRAS-CHAVE: Controle biológico, *Neoseiulus baraki*, comportamento, controle químico, manejo.

ACARICIDES IMPAIR PREY LOCATION IN A PREDATORY PHYTOSEIID MITE

ABSTRACT –The use of predatory mites as the sole management tactic in biological control programs frequently does not fully and reliably prevents damage of phytophagous mites on plants. Therefore, as an alternative, the integration of predatory mites with acaricides can provide more effective control of phytophagous mites than that of the predators only. However, for such integration, minimal negative impacts of acaricides on predatory mites are required. In this study, we evaluated the sublethal effects of three acaricides on the foraging behavior of *Neoseiulus baraki* (Athias-Henriot) (Acari: Phytoseiidae) in a coconut production system. The acaricides were assessed for interference with the location of prey habitat using a Y-tube olfactometer and for interference with the location of the prey colony within the habitat using a video-tracking system. In addition to the choice of odor source, the time required and the distance walked to make the choice were assessed. The acaricides tested were abamectin, azadirachtin and fenpyroximate. The predatory mite preferred coconuts infested with the coconut mite *Aceria guerreronis* Keifer (Acari: Eriophyidae) over uninfested coconuts when not exposed to acaricides. However, when exposed to acaricides, the predator did not distinguish between infested and uninfested fruits. When exposed to abamectin, *N. baraki* spent more time resting and walked greater distances before making the choice of an odor source. Thus, the acaricides impair the ability of the predatory mite *N. baraki* to locate a prey habitat and to locate a prey within that habitat. The acaricides differentially affect prey foraging by interfering with odor perception.

KEYWORDS: biological control, *Neoseiulus baraki*, behavior, chemical control, management

Introduction

Predatory mites are effective agents for the biological control of phytophagous mites (Helle & Sabelis 1985, McMurtry & Croft 1997, McMurtry *et al.* 2013). Among the mite predators, the Phytoseiidae family is recognized for rapid development, high foraging ability, persistence on plants with low prey infestations, high adaptability to different habitats and some predators have ability to survive on alternative substrates (McMurtry & Croft 1997, Moraes 2002). The success of the biological control can vary with crop species, plant spacing, the physical environment, and other factors, including the number and the distribution of predators released, which all influence the efficacy of biological control (Chant 1961, Hussey *et al.* 1965, Hussey & Bravenboer 1971, Osborne *et al.* 1985, van Lenteren & Woets 1988, Jarosik 1990, Gough 1991, Nihoul 1993, Opit *et al.* 2009). As a consequence, the use of biological control as sole management tactic frequently does not fully and reliably prevent damage and loss by pest species (Solomon *et al.* 2010). In such cases, other tactics are required to control mite pests.The integration of predatory mites with acaricides is an alternative approach for the control of phytophagous mites (Croft 1990). However, for the integration of the two tactics to be successful, acaricides with low (or no) negative effects on the predatory mites are required (Crof 1990).

Based on previous studies, *Neoseiulus baraki* (Athias-Henriot) is one of the most promising predators for controlling the coconut mite *Aceria guerreronis* Keifer (Aratchige *et al.* 2007, Domingos *et al.* 2010, Lima *et al.* 2012). The predator naturally occurs in the perianth of coconut fruits, which is the habitat of *A. guerreronis* (Lawson-Balagbo *et al.* 2008, Reis *et al.* 2008, Lima *et al.* 2012). The body morphology of *N. baraki* is slim and flat, which allows the mite to reach and colonize the habitat of *A. guerreronis*. However, the access of *N. baraki* usually takes place about a month after the coconut colonization by the coconut mite (Lima *et al.* 2012).

This delay is enough to *A. guerreronis* cause significant damage and start the dispersion to another coconut fruits spreading out the infestation (Lima *et al.* 2012). Thus, complementary control methods are necessary to control the coconut mite.

In a previous study, the lethal effect of acaricides on *N. baraki* was evaluated, and the authors demonstrated that some acaricides are promising to manage *A. guerreronis* in combination with *N. baraki* (Lima *et al.* 2013a). However, in addition to the lethal effects, acaricides may cause sublethal effects on *N. baraki* (Lima *et al.* 2013a,b, Lima *et al.* 2015a,b). These effects include changes in the life span, fertility, fecundity, sex ratio, population growth, and survival rate, in addition to changes in behavior such as repellence and irritability compromising mating (Cranham & Helle 1985, Omoto *et al.* 2000, Van Leeuwen *et al.* 2010, Guedes *et al.* 2016). Moreover, the functional response of the predatory species may be altered (Davidson 1953, Robertson & Preisler 1992, Poletti *et al.* 2007, Teodoro *et al.* 2009, Lima *et al.* 2013a, b, Lima *et al.* 2015a, b).

Before integrating any acaricides in a system, it is important to collect information on its compatibility with the predator. That is why we evaluated the sublethal effects of acaricides on the foraging behavior of *N. baraki*. The plants attacked by phytophagous mites produce volatiles that signal their presence to predatory mites (Dicke & Sabelis 1988, Dicke 1994, Dicke *et al.* 1998, Janssen *et al.* 1999, Sabelis *et al.* 2001, Arimura *et al.* 2005), and phytoseiid use chemoreceptors on their palps and foreleg tarsi to detect these chemical cues from the plants in the search for prey (Jagers op Akkerhuis *et al.* 1985). Thus, to successfully forage for their prey, these predators are dependent on chemical and/or tactile stimuli from this host plant. The interaction between predator and prey follows the subsequent hierarchical steps: (1) location of the habitat colonized by the prey, (2) location of the prey colony within the habitat, and (3) location of individuals

within the colony (Sabelis & Dicke 1985). The foraging behavior of *N. baraki* was described by Melo *et al.* (2011), who showed that *N. baraki* detected chemical cues emitted by coconut plants infested by *A. guerreronis*. That behaviour facilitated the predator-prey encounter and increased the predator efficiency by reducing the time required for prey location. Nevertheless, no information is available regarding the potential effects of acaricides on these first and two steps of the foraging behavior of *N. baraki*. Therefore, the objective of this study was to evaluate the effect of acaricides on the behavior of a mite predator, *N. baraki*, in the location of its prey.

Materials and methods

Rearing *N. baraki*. *Cocos nucifera* L. coconuts were collected from Itamaracá Island (State of Pernambuco, Brazil; 07°46'S, 34°52'W) and transported to the laboratory of the Federal Rural University of Pernambuco (Recife, State of Pernambuco, Brazil). The coconuts were collected from plants that were not sprayed with pesticides for more than 10 years. The coconuts were maintained under controlled laboratory conditions (27 ± 1.0 ^{o}C, 75 ± 10% R.H., and a 12-h photoperiod) until use. Approximately 100 *N. baraki* females were collected from the perianth of the coconuts. The females were transferred to rearing units with a diameter of 16 cm that consisted of plastic trays containing 1 cm thick polyethylene foam on which a filter paper and a 1 mm thick black piece of polyvinyl chloride (PVC) were placed. In each unit, the PVC disc was surrounded by hydrophilic cotton moistened with distilled water to prevent the mites from escaping. *Aceria guerreronis* was provided as food on meristematic tissue fragments from infested coconut fruits (~0.5 cm^3) that contained around 300 individuals at different developmental stages. The food was replenished every 2 days with 5 prey-infested perianth

fragments per rearing unit. The coconuts were stored for up to 7 days. The rearing units were placed in an incubator under the same conditions as described above.

Acaricides. The acaricides used in the experiments were azadirachtin, fenpyroximate and abamectin. These compounds were used as commercial formulations as follow: azadirachtin (Azamax, 12 g a.i. [active ingredient]/L, emulsifiable concentrate; DAV Agro, Ituverava, SP, Brazil), fenpyroximate (Ortus, 50 g a.i./L, suspension concentrate; Arysta Life science, Salto de Pirapora, SP, Brazil), and abamectin (Vertimec, 18 g a.i./L, emulsifiable concentrate; Syngenta, São Paulo, SP, Brazil). The concentrations used in our experiments were based on the registered and recommended label rates for coconut mite in Brazil, as follows: 30 mg a.i./L for azadirachtin; 100 mg a.i./L for fenpyroximate, and 13.5 mg a.i./L for abamectin (Agrofit 2015).

Olfactometer experiments. The response of *N. baraki* to odor sources was determined in a two-choice test using a Y-tube olfactometer (Sabelis & van de Baan 1983, Pallini *et al.* 1997, Janssen *et al.* 1999). The olfactometer consisted of a Y-shaped glass tube (27 cm long,3.5 cm inner diameter) with a Y-shaped metal wire fixed in the middle of the glass tube to channel the mites (Sabelis & van de Baan 1983). The base of the tube was connected to an air pump that produced airflow from the arms of the tube to the base. The airflow through both arms of the Y-tube was calibrated with a digital flow meter with needle valves between the air outlet of the containers with the odor sources and the arms of the olfactometer. When the airflow speeds in both arms were equal, the odors formed two cleanly separated fields at the base of the Y-tube with the metal wire as the interface. Glass boxes (50 cm x 36 cm x 43 cm) with the odor sources were connected by a transparent hose to the end of each of the two arms. The boxes were used for the following odor sources: (1) uninfested coconut fruits and (2) infested coconut fruits. The predators were starved for 2.5h before the experiments. The black PVC discs (3 cm in diameter and 1 mm thick)

were immersed for 5 seconds in 40 mL of an acaricide solution (treatments) or in distilled water (control). The discs were dried for 2 h (27 ± 1.0 °C and 75 ± 10% R. H.). The predators were transferred to the treated or untreated trays for 30 minutes without food. With the airflow from the boxes passing through the tube, a starved predatory mite (untreated or treated) was introduced at the base of the Y-tube to walk upwind along the wire inside the tube to choose one of the two odor sources at the Y-junction. The air speed inside the glass tube was 0.5 m/s in each arm, as measured with digital anemometers and calibrated with manual registers. Each treatment and the respective control were performed with identical fruits.

To obtain the odor sources from infested or uninfested coconut palms, the procedure described by Melo *et al.* (2011) was followed to minimize the chance of erroneously classifying lightly infested fruits as uninfested fruits. In this procedure, a bunch of each of three coconut palms was collected containing either infested or apparently uninfested fruits. The bunches were collected in coconut fields (cv. Dwarf Green) on Itamaracá Island (State of Pernambuco, Brazil). The fruits were examined under a stereomicroscope in the laboratory. The plants that showed no damaged fruits and no *A. guerreronis* were classified as uninfested. The plants that showed signs of damage or disease caused by other arthropods were discarded, as were those plants on which arthropods other than *A. guerreronis* were found during laboratory inspections.

The odor sources were obtained from 10 infested fruits (ca. 3 months old and with 16–32% damage, according to Galvão *et al.* 2008) and from 10 uninfested fruits (ca. 3 months old). The olfactometer experiment was performed at 24–27 °C and 60–80 % R. H. and was replicated three times. The females were observed for a maximum of 5 min. When the end of an arm was not reached within 5 min, no choice was recorded. The percentage of predators that did not choose in each replicate was very low (1%), and these predators were not included in the analysis. Each

replicated experiment was continued until 20 females responded to an odor source. After testing five mites, the position of the odor source was changed to avoid uncontrollable asymmetries in the experimental setup. In each replicate, we changed the odor sources to avoid pseudoreplication (Hurlbert 1984). The average time for the female to choose an odor source in the olfactomete rafter exposure to an acaricide or the distilled water (control) was also measured.

Viewpoint experiments. The methods used in this study were adapted from Lima *et al.* (2013a) as follow. Individual discs of black PVC (3 cm diameter) were glued to a piece of wood (1 cm thick) and placed in the center of a Petri dish (6 cm diameter) containing water (0.5 cm deep). With this setup, the PVC disc floated on the water surface. The experimental arena is shown in Fig. 1. To prevent the mites from escaping, the PVC disc was surrounded by a layer of glycerin. One disc of the meristematic tissue of infested coconut fruit containing about 360 active stages of *A. guerreronis* and another uninfested disc (both witha diameter of 7 mm) were placed at the edge of the PVC disc. The females of *N. baraki* were starved and simultaneously exposed to an acaricide (or not) as performed in the olfactometer experiments. One female was released on each PVC disc, and a digital tracking system with a video camera connected to a computer recorded the movements (ViewPoint Life Sciences, Montreal, Canada) at a temperature of 25-27 °C. The evaluation continued until the female chose one of the epidermal discs of a coconut fruit. The parameters recorded for each mite were as follow: total distance walked, walking time and walking velocity. Twenty replicates were performed for each acaricide, and each mite represented a replicate. The epidermal discs of the coconut fruit were switched to the opposite edge of the PVC disc after five mites were tested to correct for any unforeseen asymmetry in the experimental setup.

Data analyses. The differences in the number of *N. baraki* females choosing the odor sources in the olfactometer and viewpoint experiments were tested using the *G*-test with expected fractions of 0.5 for each odor source. The pooled results were tested with a replicated goodness-of-fit test (Sokal & Rohlf 1995). The average times for the females to choose an odor source at the olfactometer after exposure to an acaricide (or not, for the control) were compared using an unpaired Student's *t*-test (method of Satterthwaite for unequal variances; procedure TTEST; SAS Institute, 2008). The total distance walked, walking time and walking velocity of females in making the choice between the epidermal discs in the view-tracking experiments were subjected to multivariate analysis of variance (MANOVA) with the acaricides as the independent variable; if significant difference was detected, analysis of variance (univariate) was used (ANOVA; GLM procedure) with subsequent Tukey's HSD tests, when necessary.

Results

Olfactometer experiments. When not exposed to acaricides, *N. baraki* preferred coconut fruits infested by *A. guerreronis* over uninfested coconut fruits ($G \geq 5.48$; d.f. = 1; $P \leq 0.022$), with no significant differences among replicates ($G \geq 0.1$; d.f. = 2; $P \geq 0.74$). However, when the predatory mites were exposed to acaricides, no preference for infested or non-infested fruit was observed (abamectin: $G = 0.60$; d.f. = 1; $P = 0.44$; azadirachtin: $G = 2.41$; d.f. = 1; $P = 0.12$; and fenpyroximate: $G = 0.7$; d.f. = 1; $P = 0.80$). Among the replicates, no significant differences were detected ($G \geq 0.4$; d.f. = 2; $P \geq 0.43$; Fig. 2). Among the acaricides, only abamectin caused an increase in the time required to decide an odor source ($t_{110} = 4.87$; $P < 0.0001$; Fig. 3).

Viewpoint experiments. When not exposed to acaricides, *N. baraki* preferred infested discs of coconut epidermis over uninfested discs ($G = 6.79$; d.f. = 1; $P = 0.01$), without significant

differences among replicates ($G \geq 0.2$; d.f. = 2; $P \geq 0.24$). However, when the predatory mites were exposed to acaricides, no preference was observed (abamectin: $G = 0.60$; d.f. = 1; $P = 0.44$; azadirachtin: $G = 0$; d.f. = 1; $P = 1.00$; and fenpyroximate: $G = 0.60$; d.f. = 1; $P = 0.44$), and no significant differences among replicates were detected ($G \geq 0.2$; d.f. = 2; $P \geq 0.24$; Fig. 4).

Significant differences in the walking parameters were detected in the multivariate analysis of variance (MANOVA: $d.f._{num/den} = 9/570$; Wilks' Lambda = 0.92; F = 2.14; P =0.02). There was significant effect of acaricide treatment on the total distance walked ($F_{3,236} = 2.98$; P = 0.03) and the walking time ($F_{3,236} = 3.80$; $P = 0.01$). *Neoseiulus baraki* walked more when exposed to abamectin, where the opposite took place with azadirachtin exposure (Fig. 5). The walking velocity was not significantly affected by the exposure to acaricides ($F_{3,236} = 2.52$; P = 0.06).

Discussion

The foraging behavior of *N. baraki* was negatively affected by the acaricides. As observed in both the olfactometer and video-tracking experiments, the acaricides hampered the ability of the predator to locate the habitat colonized by the prey. Therefore, the first and second steps in the foraging hierarchy proposed by Sabelis & Dicke (1985) were compromised. Moreover, the acaricides interfered with the walking parameters of the predatory mites during foraging.

Based on the olfactometer experiments, the acaricides affected the olfactory response of *N. baraki* and impaired the ability of this predator to discriminate between prey-infested and uninfested coconut fruits. Similar results were found when Teodoro *et al.* (2009) assessed the effect of fenbutatin oxide on the foraging behavior of another predatory mite species, *Iphiseiodes zuluagai* Denmark & Muma. In our study, the exposure of *N. baraki* to the acaricides occurred

through contact with a surface contaminated with dried acaricide residue. Therefore, the changes in the response to the odors likely occurred because of either the impairment of chemoreception at the tarsi, and the neural processing of such signals, or the impairment of a behaviorally oriented response system (e.g., respiration or muscle activity, among others). As the consequence of such a change in odor perception, the foraging for prey by *N. baraki* was compromised. Field studies with acaricide applications on coconut plants indicate that spraying is directed to the coconut bunches (Oliveira *et al.* 2012), and mite contact with acaricide residues occurs with tarsal contact while walking on the contaminated fruit surface (Monteiro *et al.* 2012, Silva *et al.* 2013). However, contact with acaricides may also occur during predator dispersion through surface contact and, although less likely, through direct topic contact that could potentially enhance the effect of the acaricide.

The predatory mites exposed to acaricides did not discriminate between infested and uninfested epidermal discs of coconut fruit. Upon reaching a prey-infested coconut, the predator seeks more specific prey-associated chemical cues for the efficient location of the prey colony within the habitat. The kairomones produced by colonies of phytophagous mites (from webs, feces and exuviae) and the synomones released from parts of infested plants are stimuli for prey foraging (Hislop & Prokopy 1981, Sabelis *et al.* 1984a,b, Sabelis & Dicke 1985, Dicke 1988, Dicke *et al.* 1993a, b, Takabayashi *et al.* 1994). Based on our results, the acaricides altered predatory mite behavior and ability to search and reach the prey. Although the mechanisms for such a change in the olfactory response after acaricide exposure remain unclear, these mechanisms are likely associated with the nervous system (Gauthier 2010). Thus, the acaricides might impair foraging by the predator because of a neural impairment in the perception of an odor or in the subsequent processing of an odor signal.

In addition to compromising the detection of an odor source by *N. baraki*, some acaricides interfered with the walking behavior of the predator. Although all the acaricides impaired the recognition of the odor source, abamectin led to a particularly long time to select the odor source compared with azadirachtin and fenpyroximate-exposed predators. With abamectin exposure, the distance traveled to reach the prey colony was also greater. By contrast, fenpyroximate and azadirachtin did not change this behavioral parameter. According to Lima *et al.* (2013a), these products are selective because they did not affect the instantaneous rate of increase (*ri*) for *N. baraki*. Moreover, fenpyroximate is also selective because the LC_{50} to *A. guerreronis* is smaller than that of *N. baraki*.

In previous studies, the walking patterns of *N. baraki*, i.e., the total distance walked, walking velocity, resting time and number of stops, were compromised by acaricide exposure (Lima *et al.* 2013b). In this study, changes were also observed in the walking distance and the walking time after *N. baraki* was exposed to abamectin. The longer time required to select the prey-colonized arena was directly associated with the slower movements of *N. baraki*, which are a likely consequence of the neurotoxic activity of abamectin interfering with the GABA-gated receptors in inhibitory synapses (Yu 2008). According to Jansson & Dybes (1998), avermectins block γ-amino butyric acid-stimulated chloride channels and open non-neurotransmitter-gated chloride channels, which cause an ion imbalance in the nervous system and results in paralysis. The absence of orientation to the odor, in addition to the slower mobility caused by abamectin, might explain the longer distances walked and the longer times required by the predator to search for prey.

This study is the first to report on alterations in the foraging behaviors of *N. baraki*, and showed that exposure to acaricides differentially affected prey foraging by affecting odor

perception. The acaricide-induced changes in the predator foraging behavior impaired the predator-prey interactions because phytoseiid mites rely on odor perception to forage for prey. Thus, the use of acaricides might compromise the efficacy of this key biocontrol agent against the coconut mite.

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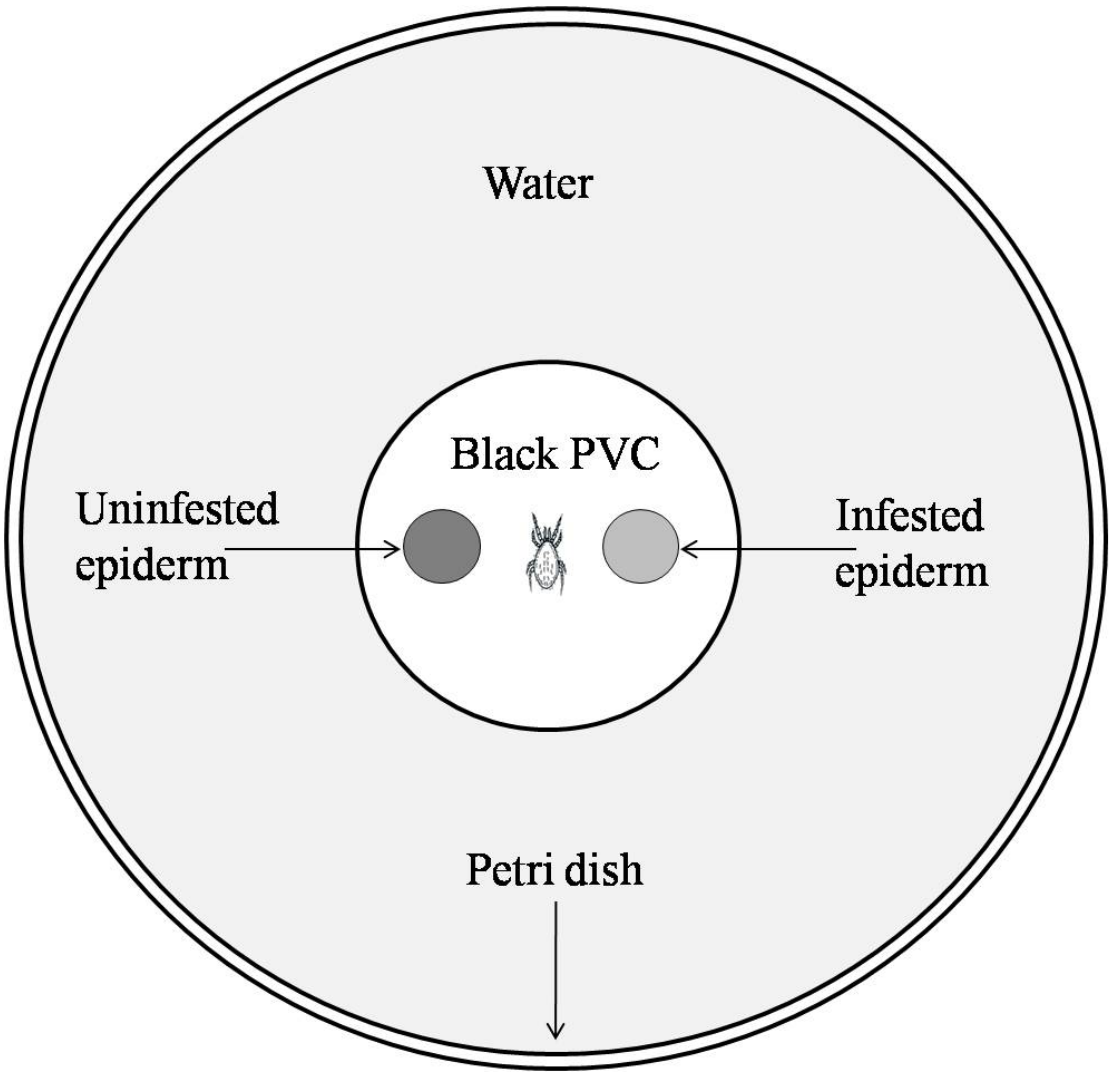

Figure 1.Experimental arena used in the digital video-tracking experiments.

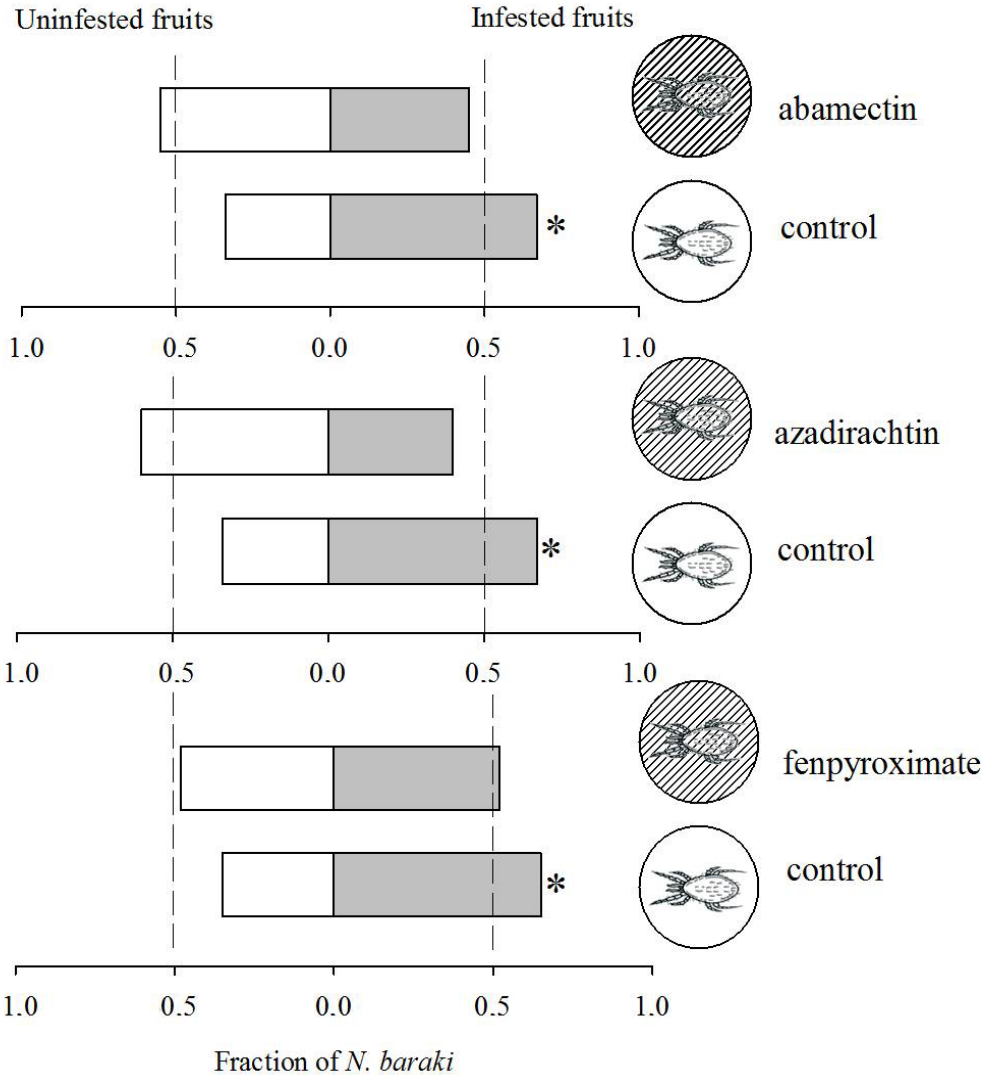

Figure 2. Response of the predators *Neoseiulus baraki* to odors from coconut fruits infested or not by *Aceria guerreronis* in a Y-tube olfactometer. Each bar represents the mean of three independent replicates (60 mites). Pooled results were tested using the *G*-test with expected fractions of 0.5 for each odor source. The vertical dashed lines represent the expected fraction for both arms. Bars with an asterisk are significantly different ($P < 0.05$).

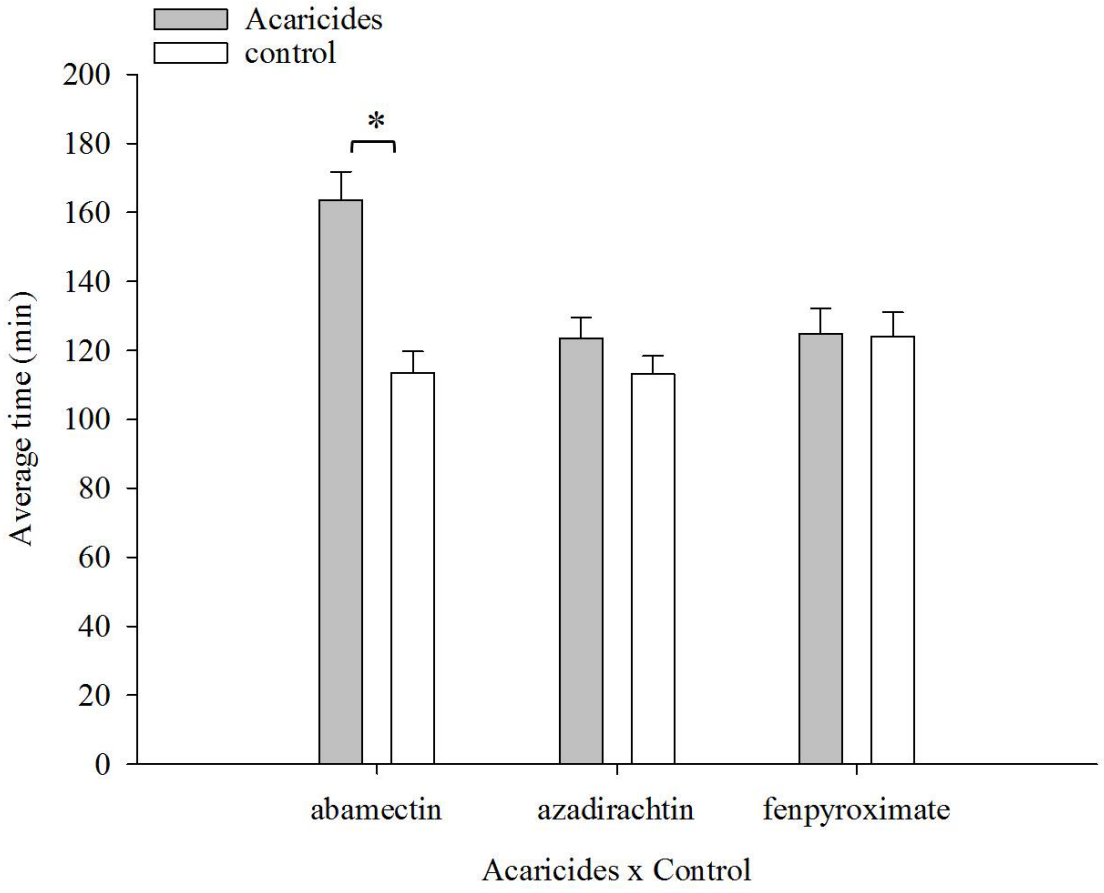

Figure 3.Average time (± standard error) of the *N. baraki* choice in olfatocmeter when exposed to acaricides. Bars with an asterisk are significantly different by Student *t*-test ($P < 0.05$).

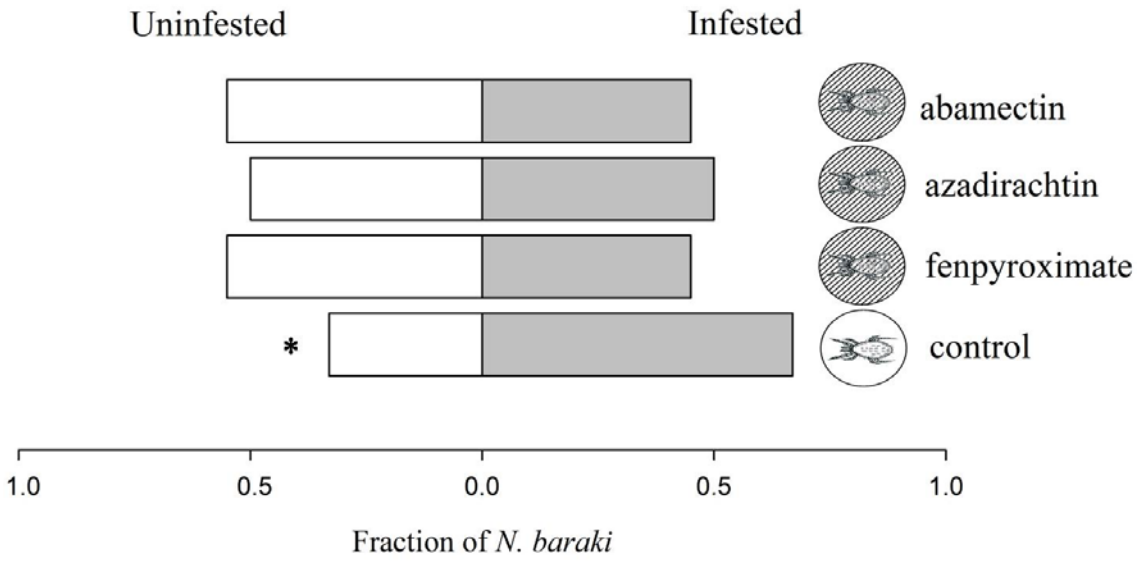

Figure 4. Walking response of the predators *Neoseiulus baraki* to coconut perianth discs infested by *Aceria guerreronis* using a digital videotracking system. Each bar shows the mean of three independent replicates (20 mites). Pooled results were tested with a replicated goodness-of-fit test. Bar with asterisk is significantly different ($P < 0.05$).

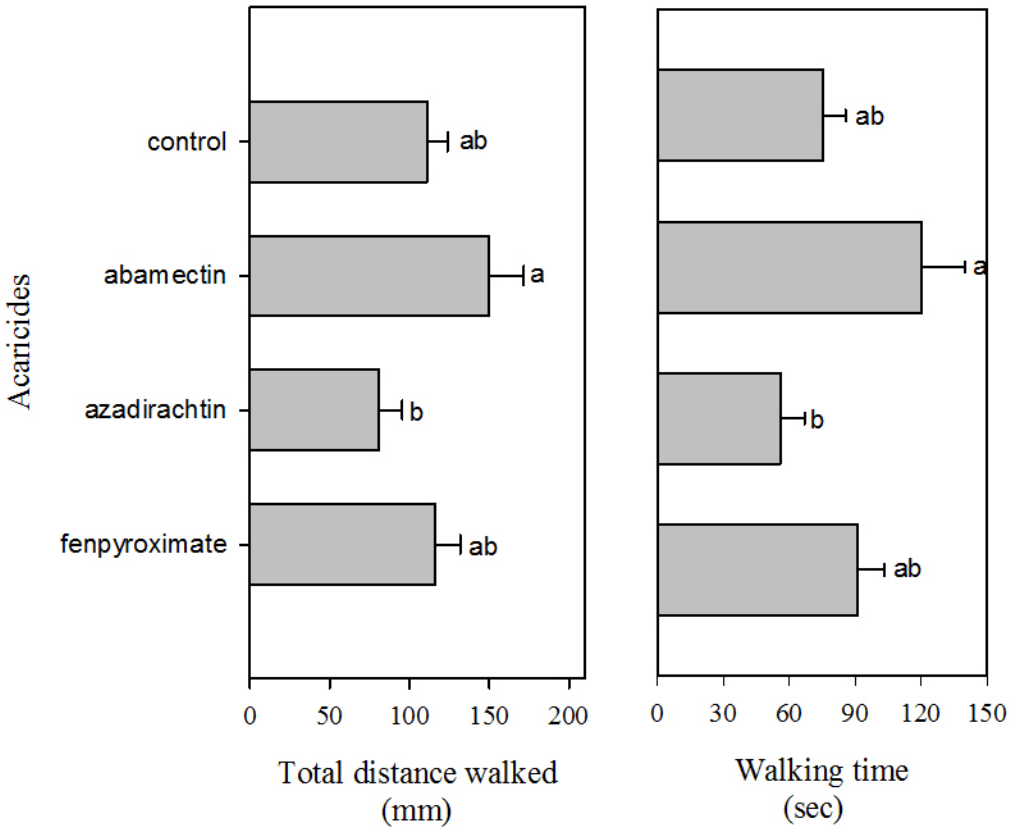

Figure 5.Total distance walked and walking time (+ standard error) by *N. baraki* after exposure to acaricides. Bars with the same letter do not differ significantly by Tukey´s HSD test ($P < 0.05$).

Considerações finais

Apesar de grande atenção ter sido despendida na busca de inimigos naturais eficientes para o controle de *Aceria guerreronis* Keifer (Acari: Eriophyidae), o uso de acaricidas tem sido a prática usual dos agricultores. É sabido, no entanto, que existe uma fauna benéfica associada a *A. guerreronis*, com potencial para regular ou pelo menos reduzir a população da praga. Como exemplo dessa fauna temos o ácaro predador *Neoseiulus baraki* (Athias-Henriot) (Acari: Phytoseiidae). Espécies deste gênero são consideradas as mais promissoras para o controle biológico de *A. guerreronis*. Portanto, a melhoria no manejo de *A. guerreronis* foi investigada, estudando-se os efeitos sub-letais dos acaricidas registrados e empregados contra *A. guerreronis* sobre *N. baraki*. Resultados destes estudos podem melhorar a integração entre o controle químico e o biológico. Verificou-se que todos os acaricidas afetaram de alguma forma o predador, seja alterando parâmetros de sua resposta funcional; tabela de vida de fertilidade de fêmeas expostas e/ou de sua prole, dificultando o encontro da presa (forrageamento); ou ainda modificando a atividade global da população. Enfim, apesar das alterações observadas, e tendo em mente o rol de produtos legalmente disponíveis, recomenda-se o controle de *A. guerreronis* com a rotação dos produtos azadiractina e fenpyroximato. Embora ainda não verificado em campo, espera-se que tais produtos proporcionem a integração entre os acaricidas e o predador de forma a obter melhores resultados no controle da praga.