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ROSENYA MICHELY CINTRA FILGUEIRAS

**POTENCIAL DE *Neoseiulus barkeri* Hughes (ACARI: PHYTOSEIIDAE) COMO
BIOCONTROLADOR DE *Raoiella indica* Hirst (ACARI: TENUIPALPIDAE)**

FORTEZA

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Tese apresentada ao Programa de Pós-Graduação em Agronomia/Fitotecnia da Universidade Federal do Ceará, como requisito à obtenção do título de Doutora em Agronomia/Fitotecnia. Área de concentração: Entomologia/Acarologia.

Orientador: Prof. Dr. José Wagner da Silva Melo.

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BANCA EXAMINADORA

Prof. Dr. José Wagner da Silva Melo (Orientador)
Universidade Federal do Ceará (UFC)

Profa. Dra. Débora Barbosa de Lima
Universidade Federal de Pernambuco (UFPE)

Dra. Cristiane Ramos Coutinho
IN Soluções Biológicas

Prof. Dr. Professor Manoel Guedes Corrêa Gondim Jr.
Universidade Federal Rural de Pernambuco (UFRPE)

Dr. Fernando Rodrigues da Silva
TOPBIO - Insumos Biológicos Industria e Comércio Ltda

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RESUMO

Raoiella indica Hirst (Acari: Tenuipalpidae) é uma espécie exótica que, após a sua introdução nas Américas expandiu sua gama de hospedeiros, e tem ocasionando sérios prejuízos nas áreas onde tem se estabelecido. Com o intuito de reduzir as populações desta praga os produtores têm adaptado estratégias utilizadas no controle de outros ácaros-praga, pois ainda não existem métodos de controle registrados para *R. indica*. Uma dessas estratégias é a utilização de extratos botânicos, contudo, não há registros da eficiência dessa ferramenta. Outra possível estratégia é o uso de ácaros predadores. O controle biológico de *R. indica* é considerado uma estratégia promissora em diversos países. No entanto, *Amblyseius largoensis* (Muma) (Acari: Phytoseiidae) apontada como espécie mais promissora no combate à *R. indica* ainda não é produzida comercialmente, limitando a sua utilização em campo. Assim, surge a necessidade de busca por estratégias alternativas. Diante disso, objetivou-se avaliar o potencial do ácaro predador *Neoseiulus barkeri* Hughes (Acari: Phytoseiidae) como biocontrolador de *R. indica*, pois ele é um ácaro generalista, já comercializado e eficaz no manejo de algumas espécies-praga em diferentes culturas. Para determinar seu potencial avaliaram-se: o estágio de desenvolvimento de *R. indica* preferido por *N. barkeri*; o consumo e a oviposição de *N. barkeri* sobre diferentes estágios de desenvolvimento de *R. indica*; a performance de predação e oviposição de *N. barkeri* e *A. largoensis* quando alimentados com ovos de *R. indica*; a resposta funcional e numérica de *N. barkeri* sobre ovos de *R. indica*; o desenvolvimento, a sobrevivência de imaturos e a taxa instantânea de crescimento da população de *N. barkeri* alimentados com diferentes estágios de desenvolvimento de *R. indica*; a longevidade, reprodução e tabela de vida de fertilidade de fêmeas de *N. barkeri* sobre ovos de *R. indica*; e o desempenho em campo de *N. barkeri* no controle de *R. indica* quando liberado nas densidades de 900, 600 e 300 predadores por planta comparado à pulverização com extratos botânicos. *Neoseiulus barkeri* mostrou preferência por ovos em relação aos outros estágios de *R. indica*. O consumo de *N. barkeri* foi inversamente relacionado ao estágio de vida de *R. indica*, e todos os estágios de desenvolvimento de *R. indica* permitiram a reprodução de *N. barkeri*. A predação e oviposição de *N. barkeri* foi maior que a de *A. largoensis*. *Neoseiulus barkeri* exibiu uma resposta funcional do tipo II e todas as densidades testadas proporcionaram a oviposição das fêmeas, com uma média de 2 ovos/fêmea/dia. O predador também completou seu desenvolvimento e alcançou a fase adulta alimentando-se de todas as fases de *R. indica*. Todos os parâmetros biológicos obtidos, indicaram que além de *R. indica* ser um alimento adequado ao desenvolvimento de *N. barkeri*, também é adequado para suportar sua reprodução, possibilitando sua sobrevivência por

longos períodos e garantindo o crescimento da população em campo. Além disso, as liberações de *N. barkeri* nas densidades de 900 e 600 predadores/planta resultaram em reduções significativas nas populações de *R. indica*, o que sugere que o predador *N. barkeri* tem potencial para ser utilizado no controle desse ácaro-praga.

Palavras-chave: ácaro predador; controle biológico; fitoseídeo; espécie invasora.

ABSTRACT

Raoiella indica Hirst (Acari: Tenuipalpidae) is an exotic species that, after its introduction in the Americas, has expanded its host range, and has caused serious damage in the areas where it has established itself. In order to reduce the populations of this pest, producers have adapted strategies used to control other pest mites, as there are still no control methods registered for *R. indica*. One of these strategies is the use of botanical extracts, however, there are no records of the efficiency of this tool. Another possible strategy is the use of predatory mites. Biological control of *R. indica* is considered a promising strategy in several countries. However, *Amblyseius largoensis* (Muma) (Acari: Phytoseiidae) identified as the most promising species in the combat against *R. indica* is not yet commercially produced, limiting its use in the field. Thus, the need arises to search for alternative strategies. Therefore, the objective was to evaluate the potential of the predatory mite *Neoseiulus barkeri* Hughes (Acari: Phytoseiidae) as a biocontroller of *R. indica*, since it is a generalist mite, already commercialized and effective in the management of some pest species in different cultures. To determine its potential, the following were evaluated: the developmental stage of *R. indica* preferred by *N. barkeri*; consumption and oviposition of *N. barkeri* on different stages of development of *R. indica*; the performance of predation and oviposition of *N. barkeri* and *A. largoensis* when fed with eggs of *R. indica*; the functional and numerical response of *N. barkeri* on *R. indica* eggs; development, immature survival and instantaneous population growth rate of *N. barkeri* fed different stages of development of *R. indica*; longevity, reproduction and fertility life table of *N. barkeri* females on *R. indica* eggs; and field performance of *N. barkeri* in controlling *R. indica* when released at densities of 900, 600 and 300 predators per plant compared to spraying with botanical extracts. *Neoseiulus barkeri* showed a preference for eggs in relation to other stages of *R. indica*. The consumption of *N. barkeri* was inversely related to the life stage of *R. indica*, and all stages of development of *R. indica* allowed the reproduction of *N. barkeri*. Predation and oviposition of *N. barkeri* was higher than that of *A. largoensis*. *Neoseiulus barkeri* exhibited a type II functional response and all densities tested provided oviposition of females, with an average of 2 eggs/female/day. The predator also completed its development and reached adulthood feeding on all stages of *R. indica*. All the biological parameters obtained indicated that in addition to *R. indica* being a suitable food for the development of *N. barkeri*, it is also suitable to support its reproduction, allowing its survival for long periods and ensuring the growth of the population in the field. In addition, releases of *N. barkeri* at densities of 900

and 600 predators/plant resulted in significant reductions in populations of *R. indica*, which suggests that the predator *N. barkeri* has the potential to be used to control this pest mite.

Keywords: predatory mite; biological control; Phytoseiid; invasive species.

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1 INTRODUÇÃO

A família Phytoseiidae representa o principal grupo de ácaros predadores utilizados como agentes de controle biológico de ácaros-praga (GERSON *et al.*, 2003; MCMURTRY, 2010; MCMURTRY *et al.*, 2013, 2015). Essa família contém mais de 2.450 espécies (DEMITE *et al.*, 2019), as quais são comumente encontradas sobre plantas em associação com diferentes grupos de artrópodes como ácaros fitófagos, tripeiros e moscas-brancas (HELLE & SABELIS, 1985; GERSON *et al.*, 2003; ZHANG, 2003; MCMURTRY *et al.*, 2013). Essa associação aliada a outras características tais como baixo requerimento alimentar, rápido desenvolvimento, alta persistência em plantas com baixa infestação de presas e capacidade de sobrevivência em substratos alternativos, certamente contribuem para que estes organismos sejam amplamente utilizados em programas de controle biológico em todo mundo (MORAES; FLECHTMANN, 2008).

Um dos gêneros mais diversos da família Phytoseiidae é *Neoseiulus* Hughes, com aproximadamente 400 espécies descritas (DEMITE *et al.*, 2019). De acordo com o comportamento alimentar e com o nível de especialização em relação às presas que atacam, os *Neoseiulus* podem ser classificados em 2 dos 4 grupos de fitoseídeos, grupos II e III (MCMURTRY *et al.*, 2013). Segundo McMurtry *et al.* (2013), os fitoseídeos do grupo II são caracterizados por alimentarem-se preferencialmente de ácaros da família Tetranychidae, enquanto que os fitoseídeos do grupo III são caracterizados por apresentar hábito alimentar generalista, alimentando-se de ácaros de diferentes grupos, certos insetos e outros tipos de alimento como pólen. O gênero *Neoseiulus* concentra algumas das principais espécies de ácaros predadores utilizados como agentes de controle biológico, como exemplo tem-se *Neoseiulus barkeri* Hughes (Acari: Phytoseiidae) espécie utilizada para o controle de ácaros tarsonemídeos e tripeiros presentes em várias culturas (HANSEN, 1988; BAKKER; SABELIS, 1989; FAN & PETITT, 1994a, JAROSIK & PLIVA, 1995; JAFARI *et al.*, 2013; WU *et al.*, 2014; WU *et al.*, 2015; RODRÍGUEZ-CRUZ *et al.*, 2017).

Neoseiulus barkeri é um fitoseídeo do grupo III, apresentando, portanto, hábito alimentar generalista (MCMURTRY *et al.*, 2013). Dentre as suas presas tem-se: ácaros tetraniquídeos (FAN; PETITT, 1994b; JAFARI *et al.*, 2010; HEKMAT *et al.*, 2017), ácaros tarsonemídeos (FAN; PETITT, 1994a), ácaros de produtos armazenados (grãos e farinhas) (XIA *et al.*, 2012; LI *et al.*, 2015), pequenos artrópodes como tripeiros (BAKKER; SABELIS, 1989; WU *et al.*, 2014; WU *et al.*, 2015) e estágios imaturos de mosca-branca (NOMIKOU *et al.*, 2001). Além disso, *N. barkeri* pode se desenvolver e reproduzir alimentando-se

exclusivamente de grãos de pólen de várias espécies de plantas (VAN RIJN, 1991; REZAIE; ASKARIEH, 2016), o que permite sua persistência em ambientes com escassez de presas (FAN; PETITT, 1994b).

O ácaro predador *N. barkeri* já foi relatado em diversas culturas e países, podendo ser encontrado em todas as regiões zoogeográficas (MORAES *et al.*, 2004; XIN, 1988; ZHANG, 2003; WU *et al.*, 2014; DEMITE *et al.*, 2019). Este predador é capaz de sobreviver em diferentes condições climáticas. Jafari *et al.* (2010) demonstraram que imaturos de *N. barkeri* quando alimentados com ninfas de *Tetranychus urticae* Koch (Acari: Tetranychidae) conseguiram alcançar a fase adulta em temperaturas entre 15 e 37°C. No entanto, os extremos de temperatura testadas provocaram efeito adverso sobre a biologia de *N. barkeri*. No limite inferior de temperatura (15°C) algumas fêmeas não conseguiram realizar oviposição enquanto que no limite superior de temperatura (37°C) foi observada elevada mortalidade em imaturos de *N. barkeri*. Informações semelhantes foram obtidas por Xia *et al.* (2012) ao avaliar o efeito da temperatura sobre *N. barkeri* alimentados com *Aleuroglyphus ovatus* (Troupéau) (Acari: Acaridae). Em temperaturas de 16°C não foi observado oviposição e em temperatura de 32°C foram observadas reduções na longevidade e fecundidade das fêmeas, consequentemente comprometendo a taxa líquida de reprodução (R_0) das mesmas.

As pequenas divergências entre os estudos citados podem ser explicadas, em parte, devido a distinção entre as presas ofertadas como alimento aos predadores. No que diz respeito a umidade, *N. barkeri* ocorre em ambientes áridos ou úmidos. O estudo conduzido por Negm *et al.* (2014) ilustra bem o desempenho de *N. barkeri* em condições de baixa umidade. Quando alimentados com os diferentes estágios de desenvolvimento de *Oligonychus australiacus* (McGregor) (Acari: Tetranychidae) e sob condições de 35°C e 35% de umidade relativa, formas imaturas de *N. barkeri* conseguiram alcançar a fase adulta e fêmeas apresentaram valores de R_0 superiores a 13 fêmeas. Tais características contribuem para a grande atenção que este predador tem recebido.

Neoseiulus barkeri é considerado um eficiente agente de controle biológico. Após a sua liberação em pomares de citros foram verificadas reduções significativas das populações do ácaro-vermelho, *Panonychus citri* (McGregor) (Acari: Tetranychidae) (WU *et al.*, 1997; XU; WANG, 2007). Também foi verificado sucesso deste predador contra outras espécies de ácaros tais como contra o ácaro-branco, *Polyphagotarsonemus latus* (Banks) (Acari: Tarsonemidae) (FAN; PETITT, 1994a), e contra o ácaro-rajado *T. urticae* (KARG *et al.*, 1987) ambos na cultura do pepino (*Cucumis sativus L.*). *Neoseiulus barkeri* também foi utilizado com sucesso

no controle biológico de tripes, *Thrips tabaci* Lind. (Thysanoptera: Thripidae) em cebola (*Allium cepa* L.) (BEGLYAROV; SUCHALKIN, 1983; BONDE, 1989).

Devido ao seu grande potencial de uso como agente de controle biológico, diversos estudos têm sido conduzidos buscando encontrar presas adequadas para a criação massal de *N. barkeri* (BARBOSA; MORAES, 2015). Adicionalmente, uma vez que o preço das espécies comercialmente disponíveis é altamente influenciado pelo custo de produção, estudos tem concentrado esforços na busca por presas viáveis. Um estudo recente que exemplifica bem essa busca por presas viáveis foi conduzido por Barbosa & Moraes (2015). No estudo as seguintes presas foram testadas: *A. ovatus*, *Austroglycyphagus lukoschusi* (Fain) (Acari: Aeroglyphidae), *Blomia tropicalis* Bronswijk, de Cock and Oshima (Acari: Echimyopodidae), *Chortoglyphus arcuatus* (Tropeau) (Acari: Chortoglyphidae), *Cosmoglyphus oudemansi* (Zachvatkin) (Acari: Acaridae), *Dermatophagoides pteronyssinus* (Trouessart) (Acari: Pyroglyphidae), *Glycyphagus domesticus* (De Geer) (Acari: Glycyphagidae), *Sancassania berlesei* (Michael) (Acari: Acaridae), *Suidasia nesbitti* Hughes (Acari: Suidasiidae), *Suidasia pontifeca* Oudemans (Acari: Suidasiidae), *Thyreophagus n. sp.* (Acari: Acaridae) e *Tyrophagus putrescentiae* (Acari: Acaridae). Dentre as espécies testadas aquela considerada mais adequada considerando desenvolvimento e viabilidade de formas jovens bem como longevidade e fecundidade de adultos foi a espécie *Thyreophagus n. sp.*.

Percebe-se que ao se procurar presas viáveis para a criação de ácaros predadores, sobretudo fitoseídeos, o foco tem sido espécies de astigmas. De um modo geral, astigmas podem ser facilmente produzidos em larga escala em recipientes relativamente pequenos contendo farinhas, farelos, ou substratos similares (GRIFFITH, 1964; SINHA, 1964; HUGHES, 1976; RAMAKERS; VAN LIEBURG, 1982). Resulta em processo de criação mais baratos em comparação aqueles que utilizam ácaros fitófagos como alimento (GERSON *et al.*, 2003). Atualmente, *N. barkeri* é criado de forma massal sendo alimentado com *T. putrescentiae*, uma presa alternativa viável que frequentemente é multiplicada em farelo de trigo.

Neoseiulus barkeri vem sendo comercializado em alguns países (GERSON *et al.*, 2003; LI *et al.*, 2015). O produto comercial de *N. barkeri* é vendido, por exemplo, pela Entocare Biologische Gewasbeschermering, sendo encontrado pelo nome de *Amblyseius barkeri* (ENTOCARE, 2020). Esse produto é disponibilizado em tubo de papelão, onde são fornecidos 50.000 ácaros predadores em vermiculita. A comercialização por essa empresa recomenda que *N. barkeri* seja utilizado para o controle de ácaros tarsonemídeos e tripes presentes em várias culturas (ENTOCARE, 2020).

Apesar de seu potencial como agente biológico, especialmente contra os ácaros, *N. barkeri* não foi submetido a testes de histórico de vida com ácaros tenuipalpídeos como presa. Os Tenuipalpidae têm sido negligenciados como uma família de importância econômica (JEPSSON *et al.*, 1975). No entanto, algumas espécies de tenuipalpídeos, como *Raoiella indica* Hirst (Acari: Tenuipalpidae), surgiram como uma ameaça à agricultura em vários países do hemisfério ocidental. *Raoiella indica* é uma espécie invasora que tem causado sérios prejuízos nas áreas onde tem se estabelecido, especialmente na América do Sul (NAVIA *et al.*, 2015; MELO *et al.*, 2018). Desde sua introdução nas Américas no ano de 2004 (FLECHTMANN; ETIENNE, 2004), vem sendo observada uma expansão no número de espécies de hospedeiros de *R. indica*, sendo reportadas atualmente cerca de 90 espécies, as quais estão distribuídas em 58 gêneros de plantas, incluindo espécies cultivadas e não cultivadas (CARRILLO *et al.*, 2012a; GONDIM JR. *et al.*, 2012; GÓMEZ-MOYA *et al.*, 2017).

As perdas ocasionadas por *R. indica* ainda não foram estimadas, contudo, os relatos indicam que, na cultura do coqueiro (*Cocos nucifera* L.), o ataque dessa praga pode causar reduções de cerca de 70% na produção (Informações não publicadas de Philippe Agostine, presidente da Associação de Produtores de Trinidad e Tobago, relatadas por RODA *et al.*, 2012). Nas áreas recentemente invadidas por *R. indica* estratégias de controle ainda estão sendo desenvolvidas visando minimizar as perdas ocasionadas por esse ácaro. O emprego de produtos químicos ainda não representa uma alternativa para o combate a essa praga, especialmente devido ao porte das plantas atacadas (tais como coqueiro e outras palmeiras), ao custo envolvido com essa prática (a maioria das culturas atacadas são produzidas por pequenos agricultores) e sobretudo devido à escassez de produtos registrados nas culturas afetadas (NAVIA *et al.*, 2015; AGROFIT, 2019).

Para alguns produtores de coco onde o emprego do controle químico não é proibitivo, tem-se utilizado extratos vegetais como o extrato de nim ou óleo de algodão (PEÑA *et al.*, 2006; TEODORO *et al.*, 2016) com o intuito de reduzir as populações de *R. indica*, contudo, não existem estudos que confirmem a eficácia dessa estratégia de controle. Neste cenário, a estratégia mais promissora, e consequentemente mais estudada, para reduzir as perdas ocasionadas por *R. indica* é o controle biológico (PEÑA *et al.*, 2009; CARRILLO *et al.*, 2012b; TAYLOR *et al.*, 2012; HOY, 2012; DOMINGOS *et al.*, 2013; MELO *et al.*, 2018).

Dentre as espécies de inimigos naturais encontradas em associação com *R. indica*, destaca-se o ácaro predador *Amblyseius largoensis* Muma (Acari: Phytoseiidae). Este predador é frequentemente encontrado em associação com populações de *R. indica* em campo

(GALLEGO *et al.*, 2003; PEÑA *et al.*, 2009; GONDIM JR *et al.*, 2012); é capaz de se desenvolver e reproduzir alimentando-se exclusivamente de *R. indica* (GALLEGO *et al.*, 2003; RAMOS *et al.*, 2010; DOMINGOS *et al.*, 2013); e apresenta resposta funcional tipo II, sugerindo eficiência em baixas densidades populacionais da praga (CARRILLO; PEÑA, 2012; MENDES *et al.*, 2018). Adicionalmente, o potencial deste predador já foi testado em casa de vegetação onde foram verificadas reduções significativas das populações de *R. indica* após a sua liberação (CARRILLO *et al.*, 2014). Ademais, tem sido relatado que em áreas recentemente colonizadas por *R. indica* as populações de *A. largoensis* tem aumentando naturalmente (PEÑA *et al.*, 2009). Apesar do potencial deste predador, o mesmo ainda não possui uma metodologia de criação massal estabelecida, não se conhecendo presas viáveis para sua multiplicação em larga escala, e consequentemente não se encontra disponível comercialmente, o que inviabiliza a sua utilização aplicada no manejo de *R. indica*.

Diante de todo o potencial que *N. barkeri* apresenta para o controle biológico de pragas, espera-se que este predador também seja capaz de predar e se desenvolver utilizando *R. indica* como alimento, e que possa vir a ser utilizado como agente de controle biológico dessa praga.

2 ARTIGO DA TESE 1

***Neoseiulus barkeri* Hughes (Acari: Phytoseiidae) as a potential control agent for *Raoiella indica* Hirst (Acari: Tenuipalpidae)¹**

Rosenya Michely Cintra Filgueiras², Jairo de Almeida Mendes², Eduardo Pereira de Sousa Neto², Neville Vieira Monteiro², José Wagner da Silva Melo²

²Departamento de Fitotecnia, Universidade Federal do Ceará, Pici, 60455-760, Fortaleza, CE, Brasil.

Abstract

Raoiella indica Hirst (Acari: Tenuipalpidae) is a pest species that has expanded not only in geographical distribution but also in the number of host plants. Control measures are still being evaluated in recently invaded countries. In some countries and for some crops, spraying with botanical extracts has been used to reduce *R. indica* populations. Laboratory and field studies point to the predatory mite *Amblyseius largoensis* (Muma) (Acari: Phytoseiidae) as promising for combating *R. indica*. However, this predator has not yet been commercially available. Thus, the present study aimed to evaluate the potential of another predatory mite *Neoseiulus barkeri* Hughes, a commercially available generalist predator as a biological control agent of *R. indica*. The consumption and oviposition rate of *N. barkeri* were determined across the developmental stages of *R. indica*. The predation and oviposition capacity of *N. barkeri* and *A. largoensis* when fed *R. indica* eggs in the laboratory were compared. In the field, releases of the predator *N. barkeri* at densities of 900, 600 and 300 predators per plant were compared to spraying with botanical extracts such as cottonseed oil and azadirachtin to control *R. indica*. Our results suggested that *N. barkeri* is an effective predator to control *R. indica*. The consumption of *N. barkeri* was inversely related to the life stage of *R. indica*, and all developmental stages of *R. indica* enabled the reproduction of *N. barkeri*. The predation and oviposition of *N. barkeri* were higher than those of *A. largoensis*, and the releases of *N. barkeri* at densities of 900 and 600 predators/plant resulted in significant reductions in *R. indica* populations

Keywords: Predatory mite. Biological control. Invasive species.

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Introduction

The red palm mite *Raoiella indica* Hirst (Acari: Tenuipalpidae) is considered an invasive species that has caused serious damage in areas where it has been established, especially in South America (NAVIA *et al.*, 2015; MELO *et al.*, 2018). Since its introduction

in the Americas in 2004 (FLECHTMANN; ETIENNE, 2004), there has been an expansion in the number of *R. indica* hosts, with approximately 90 species belonging to 58 genera including cultivated and non-cultivated plant species currently being reported (CARRILLO *et al.*, 2012; GONDIM JR. *et al.*, 2012; GÓMEZ-MOYA *et al.*, 2017).

Plants attacked by *R. indica* present yellowing, dark spots and leaf desiccation (OCHOA *et al.*, 2011; BEARD *et al.*, 2012). The losses caused by *R. indica* have not yet been estimated. However, reports showed that production can be reduced by up to 70%, in coconut (*Cocos nucifera* L.) (unpublished information of Philippe Agostine, President of Trinidad and Tobago Growers Association, reported by RODA *et al.*, 2012).

In areas recently invaded by *R. indica*, control strategies are still being developed to minimize losses caused by this mite. The use of chemicals is not yet an alternative to combat this pest, especially due to the size of the host plants such as coconut and other palms, the cost involved with this practice (most crops are produced by small farmers) and the scarcity of products for the infested crops (NAVIA *et al.*, 2015; AGROFIT, 2019). For some coconut farmers where the use of chemical control is not prohibitive, combating this pest has included the use of plant extracts such as neem extract or cottonseed oil (PEÑA *et al.*, 2006; TEODORO *et al.*, 2016).

The most promising strategy, and consequently more studied, is biological control (PEÑA *et al.*, 2009; ZANNOU *et al.*, 2010; CARRILLO *et al.*, 2011; TAYLOR *et al.*, 2012; HOY, 2012; VÁSQUEZ; DE MORAES, 2012; DOMINGOS *et al.*, 2013; MELO *et al.*, 2018). Among the species of natural enemies found in association with *R. indica*, the predatory mite *Amblyseius largoensis* (Muma) (Acari: Phytoseiidae) stands out. This predator is frequently found in association with *R. indica* populations in the field (PEÑA *et al.*, 2009; GONDIM JR *et al.*, 2012). It is able to develop and reproduce by feeding exclusively on *R. indica* (RAMOS *et al.*, 2010; DOMINGOS *et al.*, 2013), and has a type-II functional response, suggesting efficacy in areas with low pest densities (CARRILLO; PEÑA, 2012; MENDES *et al.*, 2018). Additionally, the ability of this predator has already been tested in greenhouses, where significant reductions in *R. indica* populations were observed after their release (CARRILLO *et al.*, 2014). Moreover, it has been reported that in areas recently colonized by *R. indica*, *A. largoensis* populations have increased naturally (PEÑA *et al.*, 2009). Despite the potential of *A. largoensis*, there was no established mass rearing method, and thus, it is not commercially available, making its application for pest management in a large scale not feasible.

The species *Neoseiulus barkeri* Hughes (Acari: Phytoseiidae) stands out among the species of commercially available phytoseiids. This predator has a generalist feeding habit and feeds on mites of various families (PEÑA; OSBORNE, 1996; LI *et al.*, 2017; RODRÍGUEZ-CRUZ *et al.*, 2017; LI *et al.*, 2018), thrips (JAROSIK; PLIVA, 1995; GERSON *et al.*, 2003; MOUDEN *et al.*, 2017), whitefly eggs (NOMIKOU *et al.*, 2001) and pollen (HOY, 2016; NOMIKOU *et al.*, 2001). *Neoseiulus barkeri* was already used for the augmentative biological control of thrips (*Thrips tabaci* Lind.) as well as onion cultivation (HANSEN, 1988; BONDE, 1989), and was considered an efficient strategy for the control of the broad mite *Polyphagotarsonemus latus* (Banks) (Acari: Tarsonemidae) (FAN; PETITT, 1994a) and two-spotted spider mite *Tetranychus urticae* Koch (Acari: Tetranychidae) in cucumber (KARG, 1987). Furthermore, *N. barkeri* can develop and reproduce using pollen as an alternative food source and it can survive in the field even under prey scarcity (FAN; PETITT, 1994b). Thus, the objective of this study was to evaluate the potential of the *N. barkeri* as a biological control agent of *R. indica*. Data on the predation and oviposition capacities of *N. barkeri* were obtained from laboratory tests and were compared with those observed for *A. largoensis*. In the field, the potential of *N. barkeri* was investigated and compared to the strategies which is currently adopted to control *R. indica* in Brazil (spraying with cottonseed oil or azadirachtin every 15 days).

Material and methods

Collection and mass rearing of predators

Neoseiulus barkeri individuals were collected in bell peppers (*Capsicum annuum* L.) in the municipality of Icapuí, Ceará, Brazil (4°51'S, 37°21'W). *Amblyseius largoensis* individuals were collected from coconut palms (*C. nucifera* L.) in the city of Fortaleza, Ceará, Brazil (3°44'S, 38°34'W). Both species were raised starting with approximately 200 adults of each species.

Neoseiulus barkeri rearing units consisted of plastic pots (approximately 60 cm in height and 25 cm in diameter, with a capacity of 7 L) with two lateral orifices (7 cm diameter) sealed with voile fabric to allow the entry of air. The pots were filled with 3 L of rice husks and vermiculite at a 1:1 ratio. Predators were fed with populations of *Tyrophagus putrescentiae* (Schrank) (Acari: Acaridae) obtained from stock rearing. The *T. putrescentiae* population was kept in wheat flour moistened with distilled water. One litre of wheat bran infested with *T. putrescentiae* was provided to the predators weekly as a food source. *Amblyseius largoensis*

was reared and feed according to the methodology described by de Alfaia *et al.* (2018). The rearing units of *A. largoensis* consisted of plastic trays (16 cm diameter and 2.5 cm height) containing polyethylene sponges (15 cm diameter and 1 cm thickness), on which a sheet of black PVC (10 x 10 cm) was placed. The edge of the PVC sheet was covered with hydrophilic cotton, and both sponge and cotton were moistened daily with distilled water. The predators were fed (~ every 2 days) with a solution of honey (10%), castor bean pollen (*Ricinus communis* L.) and *T. urticae* in their different life stages (eggs, nymphs and adults). The *T. urticae* individuals were obtained from stock rearing. The rearing of *T. urticae* was performed in *Canavalia ensiformis* L. leaves arranged on filter paper and polyethylene foam (1 cm thick) and kept in a rectangular plastic tray (18 x 10 x 3.5 cm). The edge of the *C. ensiformis* leaf was covered with cotton moistened with distilled water to prevent mites from escaping. The rearing units were kept at 25 ± 2 °C, with $60 \pm 10\%$ relative humidity (RH) and a 12 h photoperiod.

Experimental units and climatic conditions

The experimental units consisted of individual cells from bioassay trays (128 cells, Bio-Serv, Frenchtown, NJ, USA) with transparent adhesive lids (see LIMA *et al.* 2013 for details). All laboratory experiments were conducted at 25 ± 2 °C, with $60 \pm 10\%$ relative humidity (RH) and a 12 h photoperiod.

Predation and oviposition of Neoseiulus barkeri across the developmental stages of R. indica
Fertilized *N. barkeri* females (approximately 10 days of age) were deprived of food for 4 h in experimental units. After starvation, the females were transferred to new experimental units containing 40 *R. indica* specimens (eggs, larvae, nymphs (protonymphs and deutonymphs) or adult females). All developmental stage of *R. indica* used in the experiments were collected on naturally infested coconut leaflets and were transferred to experimental units. Prey stages were replenished day after day during 5 consecutive days and the number of prey consumed as well as the number of eggs laid by each predator were recorded daily. Each *N. barkeri* female represented one replicate and twenty replicates (i.e., females) were used for each prey stage. Data obtained from the first day was not considered in the analysis to avoid the effect of previous feeding (SABELIS, 1990). As predation and oviposition data did not satisfy the normality assumption, the non-parametric Kruskal-Wallis test was used (SAS INSTITUTE, 2002). In addition, previous bioassays with all developmental stages of *R. indica* and without predator revealed that mortality due to handling was very low (<5%), and thus, no correction was applied.

Predation and oviposition of Neoseiulus barkeri and Amblyseius largoensis

The predation and oviposition potential of both predators was evaluated under exclusive feeding of *R. indica* eggs under the same environmental conditions used for predator rearing. Eighty *R. indica* eggs were transferred to PVC plates (3 cm diameter), which were introduced into the experimental units. The eggs used in the experiments were collected on naturally infested coconut leaflets and were transferred to experimental units. Then, each experimental unit received one gravid females of *N. barkeri* or *A. largoensis* (approximately 10 days of age each predators), with 20 replicates for each species of predator. The assessments were performed every 24 h for 5 days, and during the assessments, the PVC plates were replaced by new plates containing *R. indica* eggs. Additionally, a blank test without the addition of predators was performed to evaluate the mortality of eggs as a function of their handling. Because egg mortality from handling was lower than 5%, no correction was applied.

Data obtained from the first day of the assessment were disregarded to avoid the effect of previous feeding (SABELIS, 1990). Predation and oviposition of *N. barkeri* and *A. largoensis* were compared by a Student's t-test at the 5% probability level (SAS INSTITUTE, 2002).

Releases of Neoseiulus barkeri in the field

The experiment was conducted in a commercial coconut palm farm (dwarf green variety) in Trairi, Ceará, Brazil ($3^{\circ}14'S$, $39^{\circ}18'W$). The crop plants were approximately four years old and had no history of spraying. Initially, the plants were visually inspected to confirm *R. indica* infestation. Next, the initial infestation level of the plants were determined, for which 6 groups and 10 plants for each group were arranged in parallel rows. The plants were randomly selected and marked. Between each group of plants, a row of plants was left to serve as a physical barrier; thus mite contamination between the plants of different groups was avoided. Each of the 60 plants was sampled, collecting three leaflets from leaf 10 to count the number of mobile stages (larvae, nymphs and adults) of *R. indica*. The data obtained were subjected to normality and homogeneity tests and then to an analysis of variance.

After checking the initial infestation level, the following treatments were used: Control treatment - no treatment was applied, allowing the free development of *R. indica* colonies; Chemical treatment 1 - plants sprayed with cottonseed oil (Agro-Oil, 880 g ia l⁻¹ EC, Vittia Fertilizantes and Biológicos Ltda, São Joaquim da Barra, São Paulo, Brazil), at 1,760 g ia 100

l^{-1} ; Chemical treatment 2 - plants sprayed with azadirachtin (AzaMax, 12 g ia l^{-1} water, EC, UPL do Brasil Indústria and Comércio de Insumos Agropecuários SA, Ituverava, São Paulo, Brazil), at 3.0 g ia 100l^{-1} water; Predator Treatment 1 - Plants inoculated with mixed stages of *N. barkeri* at a density of 300 predators per plant; Predatory treatment 2 - plants inoculated with mixed stages of *N. barkeri* at a density of 600 predators per plant; Predatory treatment 3 - plants inoculated with mixed stages of *N. barkeri* at a density of 900 predators per plant.

Cottonseed oil and azadirachtin were sprayed using a backpack sprayer, directing the spray to the abaxial surface of the leaflets. Since the experimental area had no history of spraying and under this conditions *R. indica* can reach high populations, such as those observed by Gondim Jr *et al.* (2012) upper than 300 mites/leaflet (about 900,000 mites/plant - taking into account an average number of 200 leaflets/leaf and 15 leaves/plant), the density of predators used were: 300, 600 and 900 predators/plant (1:3,000, 1:1,500 and 1:1,000 respectively). The predator's densities were obtained through preliminary counts in the stock rearing. The number of predators was estimated by dispersing 5 samples of the same volume of substrate into 50 ml of water each, and then taking 3 subsamples of 1 ml for counting the number of predators using a Peter's chamber. The material was shaken before taking each subsample, to homogenize the distribution of the mites in the liquid. It was observed that each 1 ml of substrate contained an average of 15 predators (immature + adults), thus 20, 40 and 60 ml of substrate were released during each application, corresponding to 300, 600 and 900 predators.

The predators were stored in pots containing vermiculite and rice husks and, with the aid of a beaker, were transferred to small cardboard boxes previously fixed to the plants. At 15-day intervals for 2 months (4 releases) the treatments were reapplied, and the plants were sampled following the same methodology.

The infestation levels were transformed into percentages considering the initial infestation of each group as 100%. Then, the data were subjected to repeated-measures ANOVA (analysis of variance) using the Wilks' lambda indicator for significance of the isolated effects (treatment effect and time effect) and interaction (SAS INSTITUTE, 2002). For the effect of time in each group, the data were subjected to regression analysis, in which the infestation level of *R. indica* (mites/leaflet) was the dependent variable (y) and the time was the independent variable (x). Regressions were obtained using the TableCurve 2D "curve-fitting" procedure (Systat, San Jose, CA, USA). Among the significant models ($P < 0.05$), a single model representing all applied treatments was chosen based on simplicity, parsimony, and high values

of F and R². The distribution of the residuals was also verified for each analysis to validate the parametric assumptions.

Results

Predation and oviposition of Neoseiulus barkeri across the developmental stage of R. indica

The average consumption of *N. barkeri* differed across the developmental stage of *R. indica* ($\chi^2 = 73.80$; df = 3; P < 0.0001). The highest average of prey consumed was observed for eggs, followed by nymphs and adults of *R. indica* (Figure 1A). The exclusive consumption on a prey stage affected the predator's oviposition ($\chi^2 = 13.36$; df = 3; P = 0.0039). The highest oviposition rate was observed on nymphs, followed by eggs, larvae and adults. However, there was no significant difference between larvae and adults neither among eggs and the other prey stages (Figure 1B).

Predation and oviposition of Neoseiulus barkeri and Amblyseius largoensis

Predators differed both in the consumption of *R. indica* eggs and in the number of eggs deposited (Figure 2). *Neoseiulus barkeri* consumed more *R. indica* eggs (56.2 ± 2.0) and deposited more eggs per day (1.91 ± 0.09) than *A. largoensis* (51.1 ± 1.3 eggs consumed and 1.55 ± 0.08 eggs deposited per day) (T = -2.12; P = 0.0404 - egg consumption; T = -2.95; P = 0.0054 - oviposition).

Releases of N. barkeri in the field

In the initial assessment of the infestation level (assessment before the use of treatments), no difference was observed among treatments, showing a uniformity in the initial infestation level ($F_{54.5} = 1.78$; P = 0.1328), the highest average number was 178 ± 16.65 mites/leaflet while the lowest was 105.9 ± 14.11 mites/leaflet.

Both treatment ($F_{5.54} = 4.56$; P = 0.0015) and time (Wilks' lambda = 0.84; $df_{den}/df_{num} = 15/143.95$; F = 3.27; P = 0.0284) influenced the infestation levels of *R. indica*, but there were no interactions between these factors (Wilks' lambda = 0.72; $df_{den}/df_{num} = 15/143.95$; F = 1.18; P = 0.29).

At the end of the assessment period (2 months after the start of the experiment), differences were observed between the infestation levels in the different treatments ($F_{5.54} = 3.51$; P = 0.0081) (Figure 3). In plants in which *N. barkeri* was released at densities of 900 and 600 predators/plants and where chemical treatments (cottonseed oil and azadirachtin) were used, the percentage of *R. indica* per leaflet was significantly reduced, with approximately 46, 37, 36

and 44% fewer *R. indica* than in the control, respectively. The release of 300 predators per plant showed intermediate control efficiency, which did not differ from the control or other treatments.

Except for the control treatment, fluctuations in infestation levels of *R. indica* (number of mobile stages/leaflets) over time fit the same polynomial growth model ($y = a + bx + cx^{1.5} + dx^{0.5}$) ($P < 0.043$), explaining at least 97% of the observed variation ($R^2 > 0.97$) (Table 1). The *R. indica* infestation levels remained constant in plants where no control measures were used (control treatment) (Figure 3). In the treatment with 900 predators, a reduction in the pest infestation was observed soon after predator release and levels remained constant throughout the experimental period (Figure 3). In the other treatments, the same initial reduction was observed; however, increases in infestation levels were observed at 30 and 45 days, with a subsequent reduction at 60 days after treatment application (Figure 4).

Discussion

The results of this study suggested that the predator *N. barkeri* could be effective for the biological control of *R. indica*. In the laboratory, this predator was able to preyed on all stages of *R. indica*, and all developmental stages of *R. indica* enabled the reproduction of *N. barkeri*. In addition, it showed superior performance (consumption and oviposition) to that observed for *A. largoensis*, a predator considered promising for *R. indica* control (CARRILLO *et al.*, 2010; 2011; 2014; CARRILLO; PEÑA, 2012; GONDIM JR *et al.*, 2012; DOMINGOS *et al.*, 2013; LIMA *et al.*, 2018). In the field, the performance of *N. barkeri* was similar to the methods such as azadirachtin and cotton oil currently adopted by farmers to control *R. indica*.

The predation on all developmental stages of *R. indica* by *N. barkeri* and consequent oviposition is not surprising because *N. barkeri* is a type III generalist predator. According to McMurtry *et al.* (2013), predator species of type III feed on a wide range of prey species, including mites of different families as well as small insects such as thrips, whiteflies, cochineals and nematodes. The consumption of *N. barkeri* was inversely related to the life stage of *R. indica*. This result could be explained by some reasons such as: (i) an innate trend to feed on smaller prey; (ii) the lower biomass of eggs when compared to other stages and thus the predators need to feed on a higher number of eggs to get the same amount of nutrients; and (iii) the ease to handle and subdue eggs. A higher consumption of eggs may be a main factor from a pest management point of view as this predator will kill eggs before they hatch and begin to

feed. Moreover, all developmental stages of *R. indica* enabled the reproduction of *N. barkeri*, which suggests that all prey stages of *R. indica* may be considered adequate to *N. barkeri*. The reproduction of mites is directly correlated with the quantity and quality of the food they consume. Sabelis (1985a, b) report that the reproduction of predatory mites requires a lot from their feeding, not only due to the number of eggs produced but also because of the amount of food invested per egg.

Neoseiulus barkeri consumed an average of 56 *R. indica* eggs, which was higher than that observed for other mites, and demonstrates the effectiveness of this predator on *R. indica*. When fed with the eggs of *Eotetranychus kankitus* (Acari: Tetranychidae), for example, this predator consumed only approximately 20 eggs per day (LI *et al.*, 2017), and when *T. urticae* eggs were offered, the consumption was 40.7 eggs (FAN; PETITT, 1994b) with the same egg density used in the present study. The consumption of *A. largoensis* obtained in the present study was similar to that observed for the same prey species. Here, an average intake of 51 eggs per day was observed, similar to Mendes *et al.* (2018), who observed an average of 52 *R. indica* eggs.

The oviposition rates observed for both predators were similar to those reported by other authors. In the present study, *N. barkeri* deposited 1.91 eggs when fed *R. indica* eggs; similar values (1.97 eggs) were observed for *N. barkeri* when fed *T. urticae* eggs (JAFARI *et al.*, 2010). *Amblyseius largoensis* deposited 1.55 eggs (present study) and 1.7 eggs (CARRILLO; PEÑA, 2012) when feeding on *R. indica* eggs. Importantly, the experience of a predator feeding on a particular prey may cause an increase in consumption and oviposition rates compared to those for inexperienced predators (MENDES *et al.*, 2018). Thus, the consumption and oviposition of *N. barkeri* may be even greater considering the inexperience of the predators tested.

There are no reports demonstrating the performance of *N. barkeri* as a biological control agent of *R. indica*. In the present study, *N. barkeri* and *A. largoensis* were only compared on egg stage of *R. indica* in the lab, where *N. barkeri* showed higher consumption and oviposition rates than *A. largoensis*. Carrillo and Peña (2012) evaluated the daily consumption of *A. largoensis* when offered *R. indica* stages in a no-choice condition under similar environmental conditions to those used in the present study. They found that the consumption of *A. largoensis* was inversely related to the life stage of *R. indica*, as observed in the present study to *N. barkeri*. By comparing our results with those obtained by Carrillo and Peña (2012), *N. barkeri* rate of consumption was at least twice as many as those observed for *A. largoensis* for each developmental stage of *R. indica*.

When released into the field at densities of 900 and 600 predators per plant, *N. barkeri* was able to significantly reduce the average number of mobile forms of *R. indica*. The reduction observed was similar to that obtained by spraying with plant extracts (cottonseed oil and azadirachtin). These results may reflect both the predation by *N. barkeri* as well as the avoidance of pests due to the presence of predators. Some studies have shown that the presence of a natural enemy can cause the dispersal/escape of the pest (LESNA *et al.*, 2004; LAWSON-BALAGBO *et al.*, 2007; GALVÃO *et al.*, 2012). Additionally, our study did not control the *R. indica* population on the plants, and because aerial dispersion appears to be the main form of dispersion of *R. indica*, it is possible that the reduction in the percentages of infestation observed are underestimated because the migration of mites was not considered. Carrillo *et al.* (2014) demonstrated the potential of *A. largoensis* to control *R. indica* populations in short-time experiments (3 months). Different densities of *A. largoensis* were released in coconut plants to evaluate their ability to control *R. indica* in the greenhouse, and the results indicated that *A. largoensis* could reduce *R. indica* densities and the damage inflicted on coconut under greenhouse conditions. Long-term experiments under field conditions are needed to be sure about the effectiveness of a biocontrol agent. That kind of experiment has not been carried out for any natural enemy of *R. indica* in coconut plants. There are several possible explanations for this, including the cost associated with experiments and also the absence of established mass rearing method for some agents.

Despite being commonly found in the soil, *N. barkeri* can move to the upper part of plants (MCMURTRY, 2010; MCMURTRY *et al.*, 2013) and perform arthropod pest control. This predatory mite has been found in all zoogeographic regions (DE MORAES *et al.*, 2004; XIN, 1988; ZHANG, 2003; WU *et al.*, 2014; DEMITE *et al.*, 2019). Laboratory and greenhouse experiments have shown this predator to be potentially useful for the control of several pest species, such as *Tetranychus urticae* Koch (Acari: Tetranychidae) (FAN; PETITT, 1994a; JAFARI *et al.*, 2012; HEKMAT *et al.*, 2017), *Eotetranychus kankitus* Ehara (Acari: Tetranychidae) (LI *et al.*, 2017), *Polyphagotarsonemus latus* (Banks) (Acari: Tarsonemidae) (FAN; PETITT, 1994b), *Stenotarsonemus laticeps* (Halbert) (Acari: Tarsonemidae) (MESSELINK; VAN HOLSTEIN-SAJ, 2006), *Aleuroglyphus ovatus* Toupeau (Acari: Acaridae) (XIA *et al.*, 2012), *Oligonychus afrasiaticus* (McGregor) (NEGM *et al.*, 2014), *Bemisia tabaci* Gennadius (Hemiptera: Aleyrodidae) (NOMIKOU *et al.*, 2001), *Thrips tabaci* Lind (Thysanoptera: Thripidae) (HANSEN, 1988; BONDE, 1989; JAFARI *et al.*, 2013; WU *et al.*, 2014; WU *et al.*, 2015) and *Frankliniella occidentalis* (Thysanoptera: Thripidae)

(RAMAKERS; VAN LIEBURG, 1982; WU *et al.*, 2014). Since *R. indica* has been reported in almost 100 host plants, including cultivated and non-cultivated species (CARRILLO *et al.*, 2012; GONDIM JR. *et al.*, 2012, GÓMEZ-MOYA *et al.*, 2017), it is possible that *N. barkeri* be effective against *R. indica* in some of the host plants and/or in some situations, such as in seedling nurseries, greenhouses or even in field.

This is the first study to suggest the potential of *N. barkeri* as a biological control agent of *R. indica*. Importantly, the conditions tested included high *R. indica* populations (outbreaks). It is possible that in newly infested areas (or those with lower densities than those evaluated, likely to be found in seedling nurseries), *N. barkeri* could be even more effective at controlling *R. indica*. However, before *N. barkeri* is considered a biological control agent other aspects should be evaluated such as: (i) the feeding preference of *N. barkeri*, because in field conditions multiple prey species may be present and whether *R. indica* is not its preferred prey, its release may not be effective; (ii) whether *R. indica* can support the development and reproduction of *N. barkeri*; (iii) whether *N. barkeri* can become established in the aerial parts of the plants; and (iv) whether the release of this predator could displace other species present in coconut leaflets, such as *Euseius alatus* De Leon (Acari: Phytoseiidae) and *A. largoensis*, which are important for the control of *Aceria guerreronis* Keifer (Acari: Eriophyidae), another key pest of coconut cultivation (MELO *et al.*, 2015).

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Disclosure

The authors declare no conflicts of interest.

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Figures & Tables

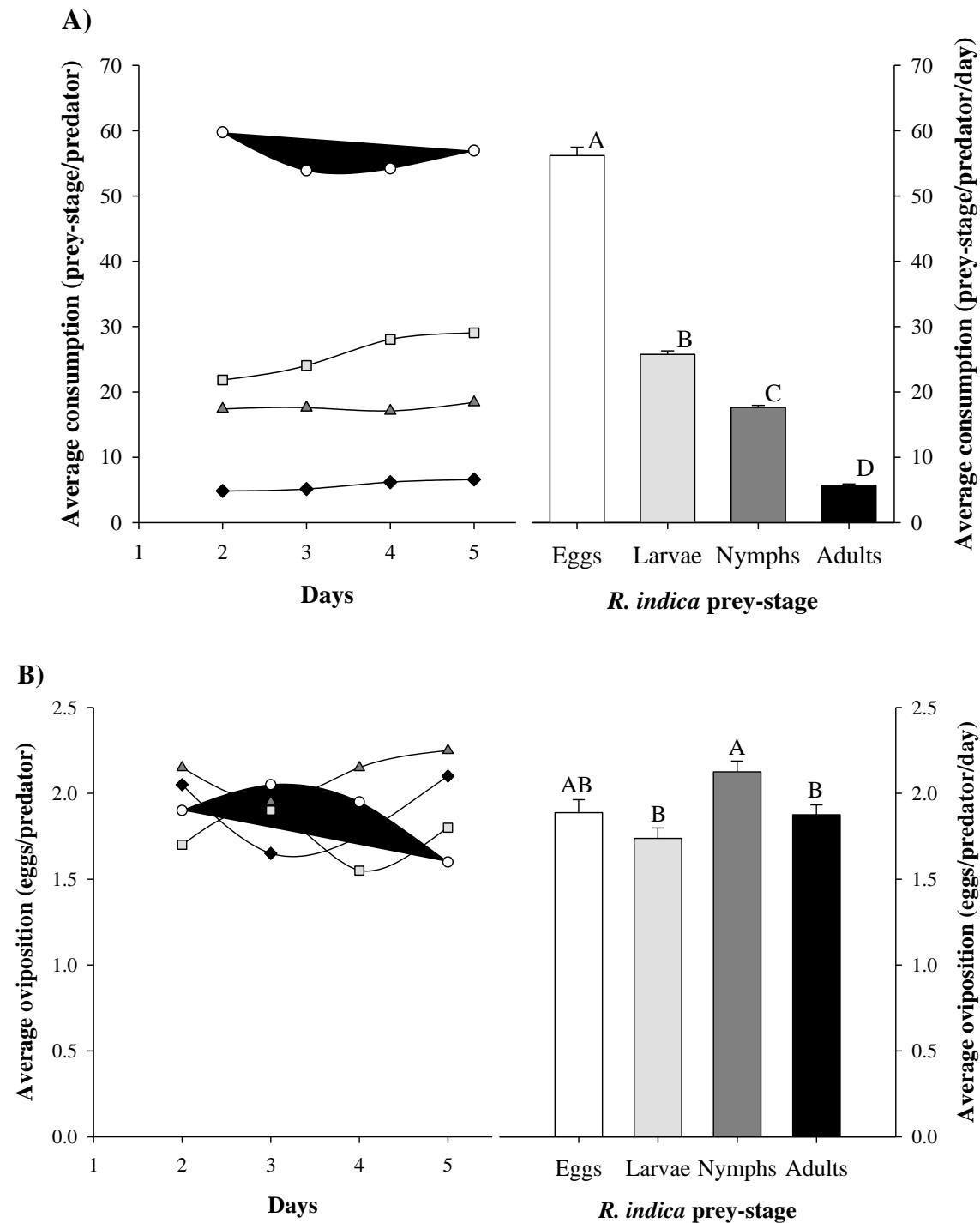


Figure 1. Consumption (A) and oviposition (B) by *Neoseiulus barkeri* when offered *Raoiella indica* stages in a no-choice condition. Error bars represent standard error of the mean. Different letters indicate significant differences between the treatments ($P < 0.05$) by Kruskal-Wallis test.

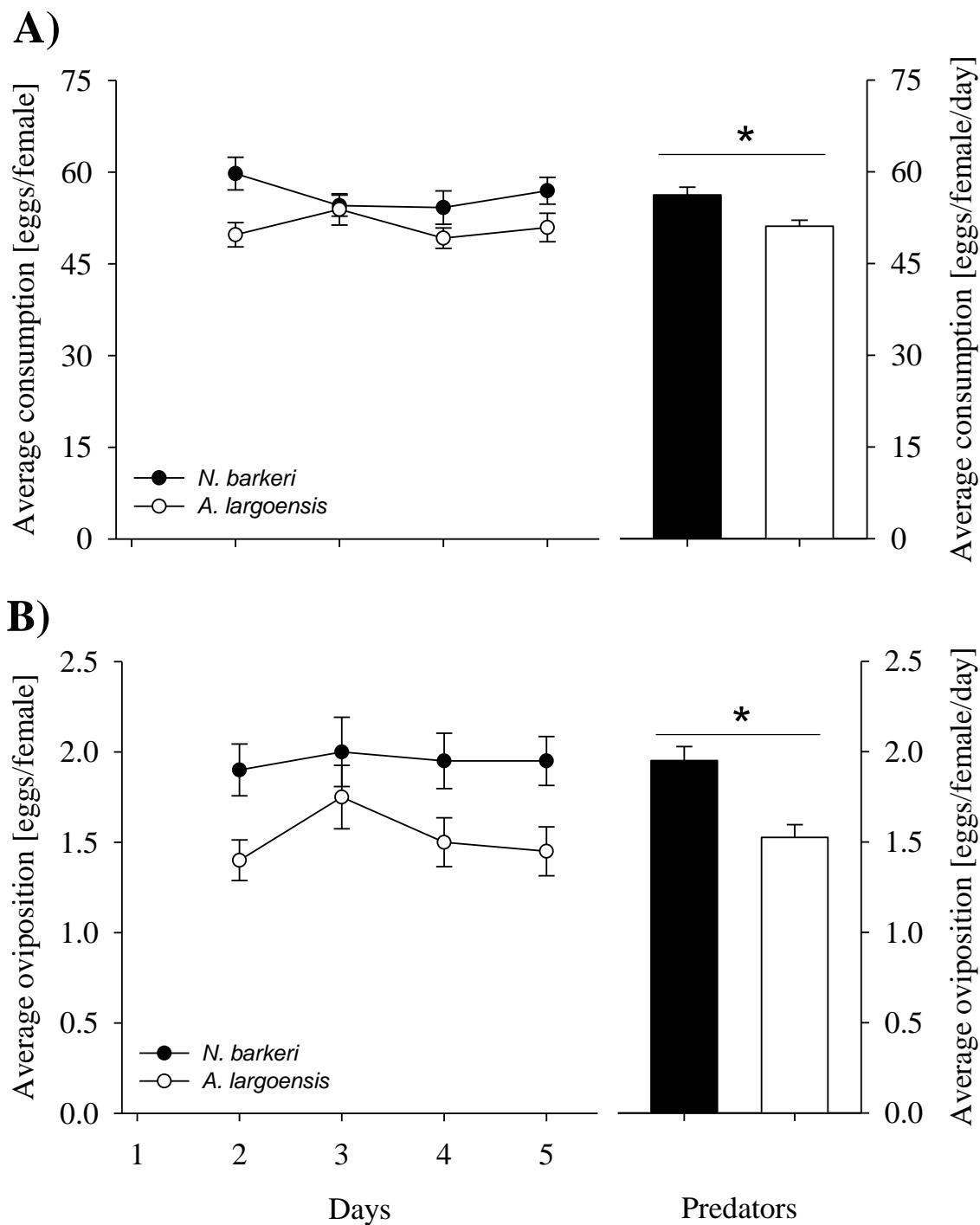


Figure 2. Consumption (A) and oviposition (B) of *Neoseiulus barkeri* and *Amblyseius largoensis* on *Raoiella indica* eggs. Error bars represent standard error of the mean. Asterisks indicate significant differences between treatments according to Student's t test ($P < 0.05$).

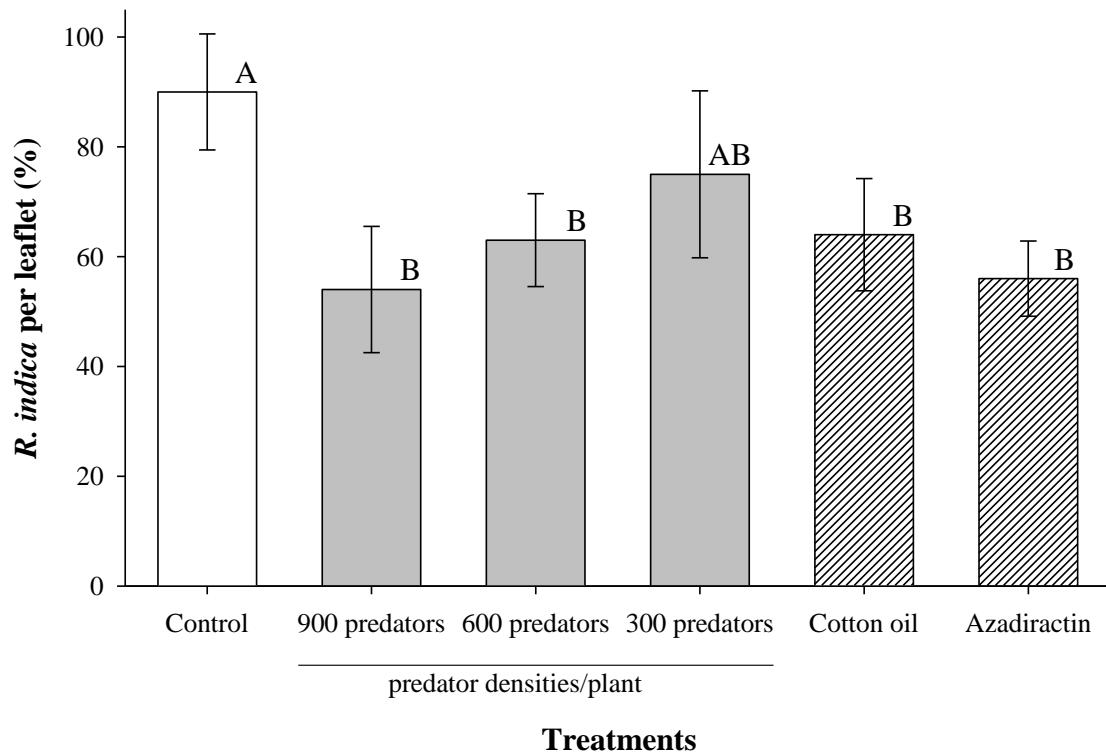


Figure 3. *Raoiella indica* populations (Mean \pm SE) on coconut leaflets after releasing *Neoseiulus barkeri*, and other control strategies after 2 months. Error bars represent standard error of the mean. Different letters indicate significant differences between the treatments ($P < 0.05$) by Tukey test.

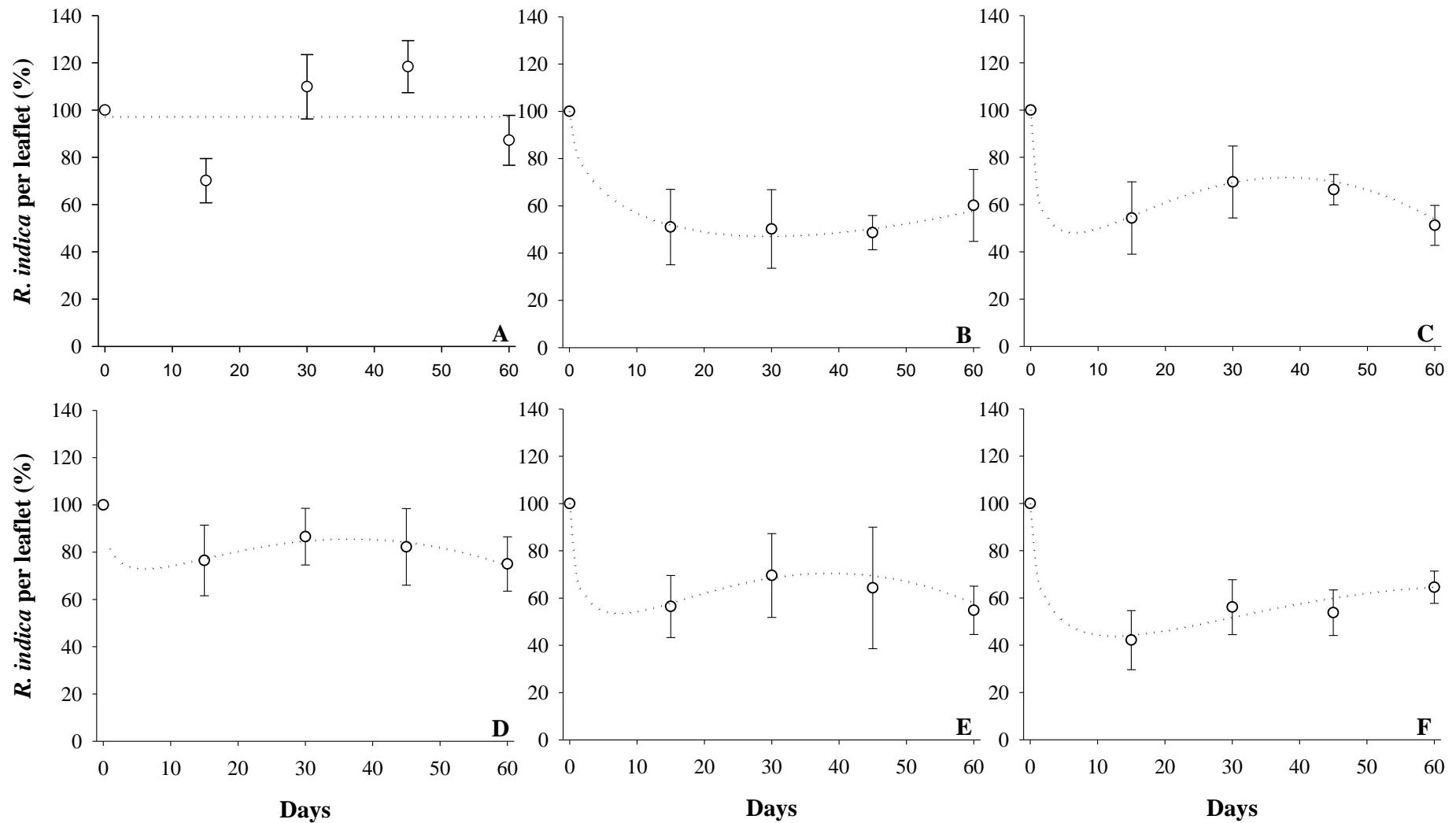


Figure 4. Population dynamics of *Raoiella indica* on coconut leaflets in *Neoseiulus barkeri* release and other treatments in Trairi-CE, Brazil. Control treatment (A), 900 predators release (B), 600 predators release (C), 300 predators release (D), Cotton oil application (E), azamax application (F)

Table 1. Parameters of regression analysis for population fluctuation of *Raoiella indica* on coconut leaflet over 60 days period.

Tratament	Model	Coefficient				R^2	F	P
		(a)	(b)	(c)	(d)			
Control	$y = \bar{x}$							
900 Predators		99.94	1.46	0.03	-18,54	0.99	41.73	0.011
600 Predators		99.96	13.07	-1.00	-47.19	0.99	117.87	0.004
300 Predators	$y = a + bx + cx^{1.5} + dx^{0.5}$	99.95	7.14	-0.55	-25.18	0.98	32.97	0.029
Cotton oil		99.94	10.59	-0.79	-40.04	0.98	32.36	0.015
Azadiractin		99.88	7.42	-0.42	-36.83	0.97	22.33	0.043

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3 ARTIGO DA TESE 2

Prey stage preference and functional and numerical responses of *Neoseiulus barkeri* Hughes (Acari: Phytoseiidae) to eggs of *Raoiella indica* Hirst (Acari: Tenuipalpidae)¹

Rosenya Michely Cintra Filgueiras², Jairo de Almeida Mendes², Francisco Wesller Batista da Silva², Eduardo Pereira de Sousa Neto², José Wagner da Silva Melo²

²Departamento de Fitotecnia, Universidade Federal do Ceará, Pici, 60455-760, Fortaleza, CE, Brasil.

Abstract:

Raoiella indica Hirst (Acari: Tenuipalpidae) is a polyphagous pest widely dispersed worldwide and a particular threat to crops from the Arecaceae family. Control measures are still being evaluated in recently invaded countries. A possible control strategy for this pest is the use of predatory mites. A recent study has suggested *Neoseiulus barkeri* Hughes (Acari: Phytoseiidae) as a potential biological control agent of *R. indica*. In the present study we determined the prey stage preference of *N. barkeri* when offered different stages of *R. indica* besides its functional response and numerical over its prey stage preferred. The predatory mite *N. barkeri* showed a marked preference for eggs over other stages of the prey. The regression analysis indicated that the predatory mite *N. barkeri* exhibited a Type II functional response. The prey density needed to start the oviposition was 10 *R. indica* eggs. The number of eggs laid by *N. barkeri* females increased with an increase in the prey density and tended to stabilize when prey availability was greater than 80 *R. indica* eggs, with average oviposition of 2 eggs/female. Our study suggests that *N. barkeri* shows potential to reduce populations of *R. indica*, especially at low prey densities. However, further studies are needed to investigate whether *R. indica* supports the development of immature stages of *N. barkeri*.

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Keywords: Biological control; Mites; Invasive pest; Predatory mite.

Introduction

Raoiella indica Hirst (Acari: Tenuipalpidae), known as the red palm mite, is an invasive pest that has emerged as a threat to agriculture in several countries, especially in the Americas (FLECHTMANN; ETIENNE, 2004; PEÑA *et al.*, 2012; NAVIA *et al.*, 2015). The arrival of this pest in new areas has enabled it not only to expand in population size and spread quickly, but also extend its host range (COCCO; HOY, 2009; CARRILLO *et al.*, 2012a). To date, it has been found attacking several plant species, including species

¹FILGUEIRAS, Rosenya Michely Cintra *et al.* Prey stage preference and functional and numerical responses of *Neoseiulus barkeri* Hughes (Acari: Phytoseiidae) to eggs of *Raoiella indica* Hirst (Acari: Tenuipalpidae). **Systematic and Applied Acarology**, v. 25, n. 6, p. 1147-1157, 2020.

in Arecaceae, Cannaceae, Cycadaceae, Heliconiaceae, Musaceae, Strelitziaceae and Zingiberaceae families (COCCO; HOY, 2009; NAVIA *et al.*, 2011; CARRILLO *et al.*, 2012a). Coconut (*Cocos nucifera* L.; Arecaceae) and bananas (*Musa* spp.; Musaceae) seem to be the host plants most threatened by *R. indica* (RODRIGUES *et al.*, 2007; MORAIS *et al.*, 2011; GONDIM *et al.*, 2012; RODRIGUES; IRISH, 2012).

Raoiella indica colonies are located on the underside of the leaves (Nusantara *et al.* 2017). Feeding of this pest usually causes localized yellowing of the leaves followed by large and chlorotic spot or tissue necrosis (Peña *et al.* 2009; Carrillo *et al.* 2012a). The information on crop loss due to *R. indica* attacks is scarce. In Trinidad and Tobago, growers have reported 70% losses to coconut production (unpublished information of Philippe Agostine, President of Trinidad and Tobago Growers Association, reported by Roda *et al.* 2012).

In areas where *R. indica* has already been introduced, control methods such as chemical controls (RODRIGUES; PEÑA, 2012; DE ASSIS *et al.*, 2013) and biological controls (PEÑA *et al.*, 2009; CARRILLO *et al.*, 2010; 2012b; MENDES *et al.*, 2018) have been explored. However, the size of the host plants (such as coconut palms and other palms) and the cost involved with chemical control (the purchase of equipment and supplies) may impair implementation of this method for low-yield farmers (NAVIA *et al.*, 2015). In most countries, low-yield farmers are the main coconut (PORDESMO; NOBLE, 1990) and banana producers (FAO, 2017). In such systems, biological control becomes an economically viable option for small producers. Currently, particular attention has been paid to identifying a predator that can be effective against *R. indica*. Among the commercialized predatory mites, only the predatory mite *Neoseiulus barkeri* Hughes (Acari: Phytoseiidae) has been tested against *R. indica* (FILGUEIRAS *et al.*, 2020).

Neoseiulus barkeri has been reported in all zoogeographic regions (XIN, 1988; ZHANG, 2003; DE MORAES *et al.*, 2004; WU *et al.*, 2014; DEMITE *et al.*, 2019). It is able to develop and reproduce on a wide range of prey species, including not only mites of different families but also small insects (e.g. thrips, whiteflies and cochineals) and nematodes (FAN; PETITT, 1994a, FAN; PETITT, 1994b; JAROSIK; PLIVA, 1995; PEÑA; OSBORNE, 1996; GERSON *et al.*, 2003; LI *et al.*, 2017; RODRÍGUEZ-CRUZ *et al.*, 2017; LI *et al.*, 2018).

A recent study also has suggested the potential of *N. barkeri* as a biological control agent of *R. indica* (FILGUEIRAS *et al.*, 2020). In the laboratory, this predator showed superior performance (consumption and oviposition) to that observed for *Amblyseius largoensis* (Muma) (Acari: Phytoseiidae), a predatory mite species frequently found in association with *R. indica*

populations in the field (PEÑA *et al.*, 2009; GONDIM *et al.*, 2012). In the field, releases of *N. barkeri* at densities of 900 and 600 predators/plant resulted in significant reductions in *R. indica* populations.

An assessment of the functional and numerical responses of this phytoseiid against *R. indica* is a critical first step in determining its ability to regulate the prey. The functional response describes the relationship between an individual's consumption rate and food density (SOLOMON, 1949) while the numerical response relates the changes in predator to prey densities (HASSELL, 1978). Therefore, a set of experiments were designed to provide insight into the potential efficiency of this predatory mite for managing *R. indica*. The objectives of this study were to determine the prey stage preference of *N. barkeri* when offered different stages of *R. indica* and to evaluate the functional and numerical response of *N. barkeri* over its preferred prey stage.

Material and methods

Collection and rearing of the predatory mite N. barkeri

The *N. barkeri* colony used in this study was obtained from bell peppers (*Capsicum annuum* L.) cultivated in the municipality of Icapuí (Ceará, Brazil, 4°51' S, 37°21' W) and multiplied by Topbio-Insumos Biológicos Indústria e Comércio Ltda. About 1,000 mites were supplied to the Mite and Insect Management Laboratory (LAMAI) of the Federal University of Ceará (UFC) for the establishment of stock rearing. In the laboratory, these predators were kept in rearing units. Each rearing unit consisted of plastic pots (approximately 60 cm in height and 25 cm in diameter, with a capacity of 7 L) with two lateral orifices (7 cm diameter) sealed with *voile* fabric to allow gas exchange. Inside the pot was added 1.5 L rice husk and 1.5 L vermiculite as substrate for mite multiplication. *Tyrophagus putrescentiae* (Schrank) (Acari: Acaridae) populations obtained from stock rearing were used as food source for *N. barkeri*. *Tyrophagus putrescentiae* rearing was kept in pots similar to those described for *N. barkeri* rearing. In each pot, 3 L wheat bran moistened with distilled water were kept as a food source for these mites. Approximately 500 mL *T. putrescentiae* infested wheat bran was added weekly to the *N. barkeri* rearing pots. The rearing units were kept at $25 \pm 1^\circ\text{C}$, $70 \pm 10\%$ relative humidity (RH) and 12-h photoperiod.

All experiments were conducted with fertilized *N. barkeri* females approximately 4 days-old and kept in food deprivation for a period of 4 h before their use in the experiments. For this, each female was transferred from stock rearing to one cell of the bioassay tray (128

cells, Bio-Serv, Frenchtown, NJ, USA) which was then closed by transparent adhesive lids (hereinafter referred to experimental units).

Prey stage preference of Neoseiulus barkeri to Raoiella indica

The preferences of *N. barkeri* to *R. indica* developmental stages were determined by choice tests. The following combinations were offered to *N. barkeri* females: 40 eggs + 40 immatures (larvae + protonymphs + deutonymphs) of *R. indica*, 40 eggs + 40 adults of *R. indica* and 40 immatures (larvae + protonymphs + deutonymphs) + 40 adults of *R. indica*. Eggs, immatures and adults of *R. indica* used in the experiments were collected on naturally infested coconut leaflets and were transferred to experimental units. For each combination, 20 females were used, where each female corresponded to one replicate. After a period of 24 h, the number of each stage consumed was recorded. The prey-stage preference was quantified based on the index β proposed by Manly (1974):

$$\beta_1 = \frac{\text{Log} \left(\frac{e_1}{A_1} \right)}{\text{Log} \left(\frac{e_1}{A_1} \right) + \text{Log} \left(\frac{e_2}{A_2} \right)}$$

Where variables A_1 and A_2 indicate the total number of each prey-stage offered to the predator and variables e_1 and e_2 corresponding to the number of each prey-stage remaining after the experiment. The index β assigns preference values from 0 to 1. If the preference index is closer to 1, the predator prefers prey-stage1, and if closer to 0 the predator prefers prey-stage2. An index close to 0.5 indicates that there is no preference between prey-stages (COCK, 1978; SHERRATT; HARVEY, 1993). Mean β -values were considered significant when 95% confidence intervals did not overlap with $\beta = 0.5$.

Functional and numerical responses

Raoiella indica eggs were obtained from coconut plants naturally infested with *R. indica* and spray-free for more than 5 years. The eggs were carefully removed from the leaves and transferred to black PVC plates (\varnothing 1 cm) with the aid of a soft bristle brush. The amount of eggs transferred was that required to make the following densities: 5, 10, 20, 40, 60, 80, 100, 120 and 140 eggs. Each PVC plate was transferred to experimental units.

After 24 hours of introduction of the predator, the number of eggs consumed (functional response) and the number of eggs deposited (numerical response) by each *N. barkeri* female were counted. The number of eggs consumed was obtained by subtracting the total offered by

the total remaining in the arena. For each egg density 20 predators were tested, each predator corresponded to one repetition.

Statistical Analysis

The densities where 100% eggs were consumed were not considered for functional response. This procedure was used to avoid limitations associated with the investigation of the type of existing functional response, since the type III functional response is characterized by a more than linear increase in prey consumption at lower densities (OKUYAMA, 2013; SOUSA NETO *et al.*, 2019). The type of functional response of *N. barkeri* was determined using logistic regressions between the proportion of eggs consumed and the density of eggs offered, Holling disc equation. The Holling discequation is:

where N_e is the number of prey eaten, N_0 is the initial prey density and P_0 , P_1 , P_2 and P_3 are the parameters to be estimated. To solve that equation, the protocol described by Juliano (2001) was used (Proc CATMOD; SAS INSTITUTE, 2002). From the obtained equation, the significance of the regression coefficients and the sign of the linear coefficient were observed, thus determining the type of functional response (HOLLING, 1959; 1961). The three possibilities are: 1) Linear coefficient is not significant, indicating a Type I response, characterized by a linear increase in prey consumption as prey density increases. 2) Linear coefficient is significant and negative, indicating a Type II functional response, in which the number of preys consumed by the predator increases rapidly due to greater prey availability and gradually decreases until plateau stabilization (predator satiation). 3) Linear coefficient is significant and positive, indicating a Type III response, which resembles a Type II response except that at low prey densities an accelerated phase of prey consumption is observed, which leads to a more than linear increase in the rate of prey consumption (sigmoid response).

The parameters of the functional response (handling time T_h and attack rate a) were estimated using nonlinear least-squares regressions of the number of preys eaten versus prey density. Given consumed prey was not replaced (*i.e.*, prey depletion), the random predator equation of Rogers (1972) was used for Type II functional responses. The equation is as follows: $N_e = N_0 \{1 - \exp [a (T_h N_e - T)]\}$ In this equation, N_e corresponds to the number of prey consumed by each predator during the time T (24 h), N_0 corresponds to the initial prey

density, a corresponds to the attack rate coefficient (proportion of prey captured by each predator per time unit) and T_h corresponds to the handling time (time spent by the predator to identify, stalk, kill, consume and digest a prey).

Numerical response (variation in oviposition rate as a function of prey density) were assessed by regression analysis using the TableCurve 2D program (Systat, San Jose, CA, USA). The selection of the models was based on simplicity, parsimony and high values of F and R².

Results

Prey stage preference of Neoseiulus barkeri to Raoiella indica

Neoseiulus barkeri showed preference for eggs over others prey-stages of *R. indica*, and preference for immatures over adults (Figure 1). These results were confirmed by the very high (>0.65) preference index β recorded and the 95% confidence intervals did not include $\beta = 0.5$.

Functional and numerical responses

The consumption of *R. indica* eggs by *N. barkeri* rises with prey density, but decelerates reaching a plateau (about 70 eggs) at which consumption remains constant (Fig. 2A). In contrast, the proportion of prey consumed decreased with increasing eggs densities offered, being close to 0.9 (90%) for the lowest density (60 eggs) and about 0.5 (50%) for the highest density (140 eggs) (Fig. 2B).

The regression analysis used to determine the shape of the functional response resulted in the linear coefficient (L) significant and negative (Table 1), indicating that *N. barkeri* exhibited a Type II functional response. The values of the attack rate (a') and handling time (Th) for *N. barkeri* females over eggs of *R. indica* was $0.12 \pm 0.02 \text{ h}^{-1}$ (95% CI = 0.08 – 0.16) and $0.0136 \pm 0.005 \text{ h}$ (95% CI = 0.013 – 0.014), respectively.

The prey density needed to start the oviposition was 10 *R. indica* eggs. The number of eggs laid by *N. barkeri* females increased with an increase in the prey density and tended to stabilize when prey availability was greater than 80 *R. indica* eggs (with average oviposition of 2 eggs/female) (Fig. 3). The relationship between the density of *R. indica* eggs and the number of eggs laid by *N. barkeri* females was expressed by the following equation: $y = -1.24 + 0.65\ln x$; ($R^2 = 0.95$; $P < 0.001$), where “y” is the rate of oviposition, and “x” is the prey density.

Discussion

The results of the present study demonstrated that *N. barkeri* prefers eggs of *R. indica* over other stages and that the predation and oviposition of *N. barkeri* increased as a function of

an increase in the density of *R. indica* eggs. The functional response of *N. barkeri* to eggs of *R. indica* followed the Type II model. In this model, the number of prey items consumed increased as a function of an increase in the prey densities but began to stabilize when a maximum point was reached. It was also demonstrated that the handling time of *N. barkeri* was short when compared to its attack rate.

The preference for eggs of *R. indica* exhibited by *N. barkeri* females could be explained by the following reasons: (i) an innate trend to feed on smaller prey; (ii) the lower biomass of eggs when compared to other stages and thus the predators need to feed on a higher number of eggs to get the same amount of nutrients; and/or (iii) the ease to handle and subdue eggs. Although the findings should be interpreted with caution once that the prey-stage preference may change by the prey species, predator hunger level, prey defense mechanisms, and/or the nutritional value of prey individuals (SANDNESS; MCMURTRY, 1970; BLACKWOOD *et al.*, 2001; BADII *et al.*, 2004), similar results were found to *A. largoensis* by Carrillo and Peña (2012). A marked preference for eggs may be a main factor from a pest management point of view as this predator will kill eggs before they hatch and begin to feed.

Several studies indicate that the Type II functional response is very common among phytoseiid species, including predatory mites that have been successfully released as biocontrol agents (MORI; CHANT, 1966; SANDNESS; MCMURTRY, 1970; LAING; OSBORN, 1974; SANTOS, 1975; RYOO, 1986; REIS *et al.*, 2003; BADII *et al.*, 2004; XIAO; FADAMIRO; 2010; ZHU *et al.*, 2019). The Type II response was also detected for *N. barkeri* on different pest species, for instance, on *T. urticae* (FAN; PETITT, 1994A; JAFARI *et al.*, 2012), on *Eotetranychus kankitus* Ehara (Acari: Tetranychidae) (LI *et al.*, 2017), on *Polyphagotarsonemus latus* (Banks) (Acari: Tarsonemidae) (FAN; PETITT, 1994B), on *Tarsonemus confusus* Ewing (Acari: Tarsonemidae) (LI *et al.*, 2018), and on *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae) (WU *et al.*, 2015). This type of response suggests that this predator could be more efficient at low or moderate prey densities (KOEHLER, 1999).

The handling time (*Th*) of *N. barkeri* was relatively short when compared to its attack rate (*a'*). This behavior is typically observed in generalist predators. Due to the short time to handle a prey item that has already been discovered the predator can almost immediately start a new search. Similar results were obtained by Carrillo and Peña (2012) for *A. largoensis* over eggs of *R. indica*. According to McMurtry *et al.* (2013) both species are classified as generalist predators. Predatory mite Type III feed on a wide range of prey species, including mites of

different families as well as small insects such as thrips, whiteflies, cochineals and nematodes (MCMURTRY *et al.*, 2013).

The number of eggs laid/day by *N. barkeri* females increased curvilinearly as a function of prey killed. At the highest densities (120 and 140 eggs), there was a trend to stabilization in the number of eggs laid/day by *N. barkeri* females (about 2eggs/day/female). Carrillo and Peña (2012) observed similar results for *A. largoensis* fed *R. indica* eggs. The number of eggs laid/day by *N. barkeri* females at the highest densities can be considered reasonable for this predator (NEGM *et al.*, 2014; RODRÍGUEZ-CRUZ *et al.*, 2017). According to Janssen and Sabelis (1992) there is a correlation between oviposition rate and intrinsic growth rate of phytoseiid mites when feeding on tetranychid mites. Thus, if this can be extrapolated to other prey mites, the average numbers found here will indicated the growth rate of *N. barkeri* as well as its ability to control *R. indica*.

Our study suggests that *N. barkeri* has the potential to reduce populations of *R. indica*, especially at low prey densities. However, further studies are needed to determine whether *R. indica* supports the development of immature stages of *N. barkeri* and its reproduction. It is also important to consider that the results described here were obtained under controlled conditions, thus more studies are needed to determine the performance of this predator under greenhouse and/or field conditions and to obtain more realistic efficiency results.

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Disclosure

The authors declare no conflicts of interest.

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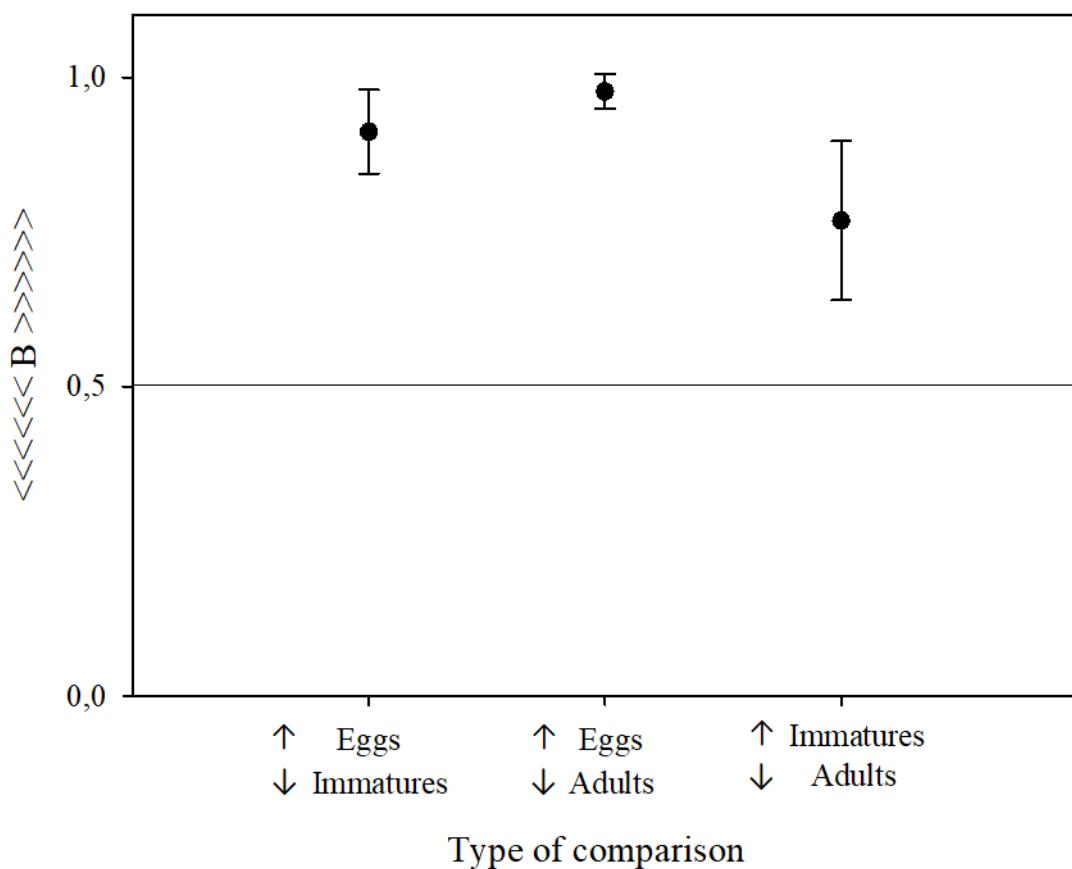


Figure 1. Preference of *Neoseiulus barkeri* females for *Raoiella indica* stages in a choice condition. Paired comparisons among *R. indica* stages. Mean β -values we considered significant when 95% confidence intervals (error bars) based on the t-distribution did not overlap with $\beta = 0.5$

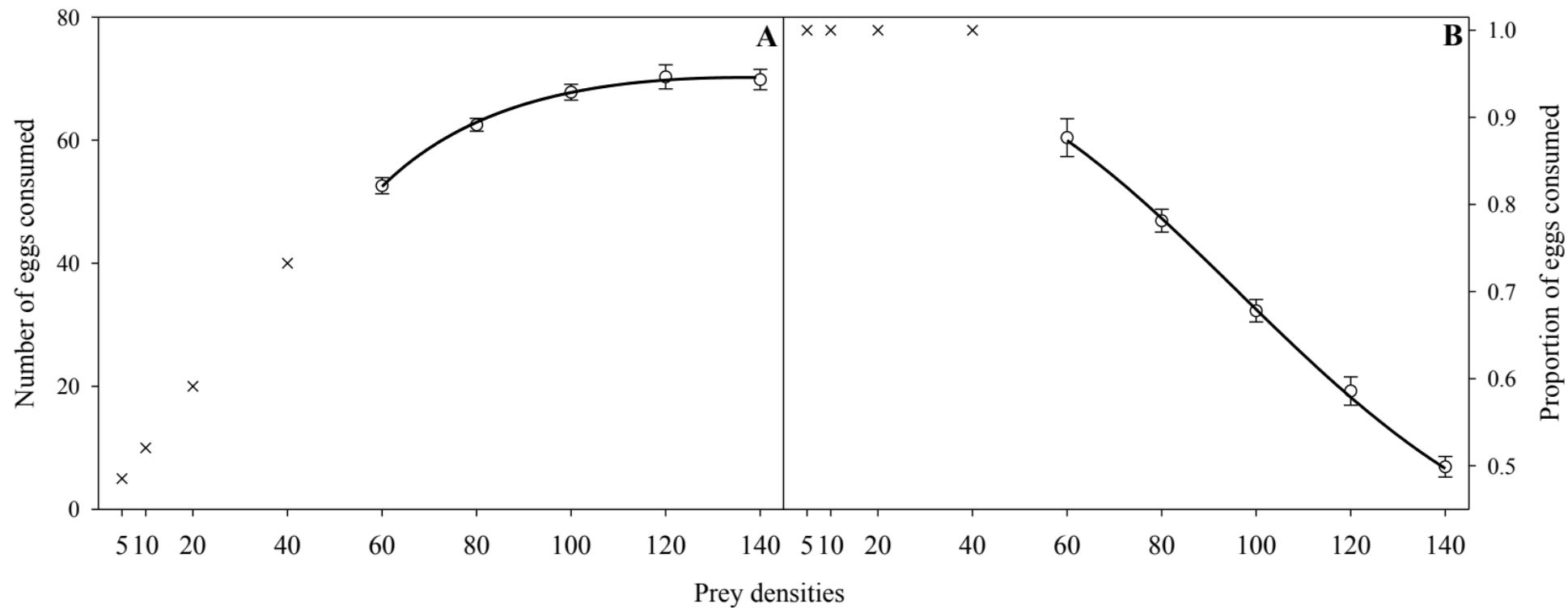


Figure 2. Functional response curves of *N. barkeri* preying on increasing densities of eggs of *R. indica* (A) and proportions of egg consumption by the predator (B). In the panel A the solid line represents the predicted values of Roger's type II functional response, while in the panel B the solid lines represent the predicted values of Holling's type II disc equation. X symbols represent densities where 100% eggs were consumed, excluded in the analysis.

Table 1. Holling's equation and Type of functional response in *Neoseiulus barkeri* fed with *Raoiella indica* eggs

Species	Holling Disc Equation	χ^2	GL	P	Logistic regression coefficient			Type ⁴
					I ¹ (P)	L ² (P)	Q ³ (P)	
<i>N. barkeri</i>	$y = \frac{\exp(0.000130x^2) - (0.0503x) + 4.4814}{1 + \exp[(0.000130x^2) - (0.0503x) + 4.4814]}$	237.53	97	<.0001	4.4814 (<.0001)	-0.0503 (<.0001)	0.000130 (<.0001)	II

¹Intercept; ²Linear; ³Quadratic; ⁴Type of functional response

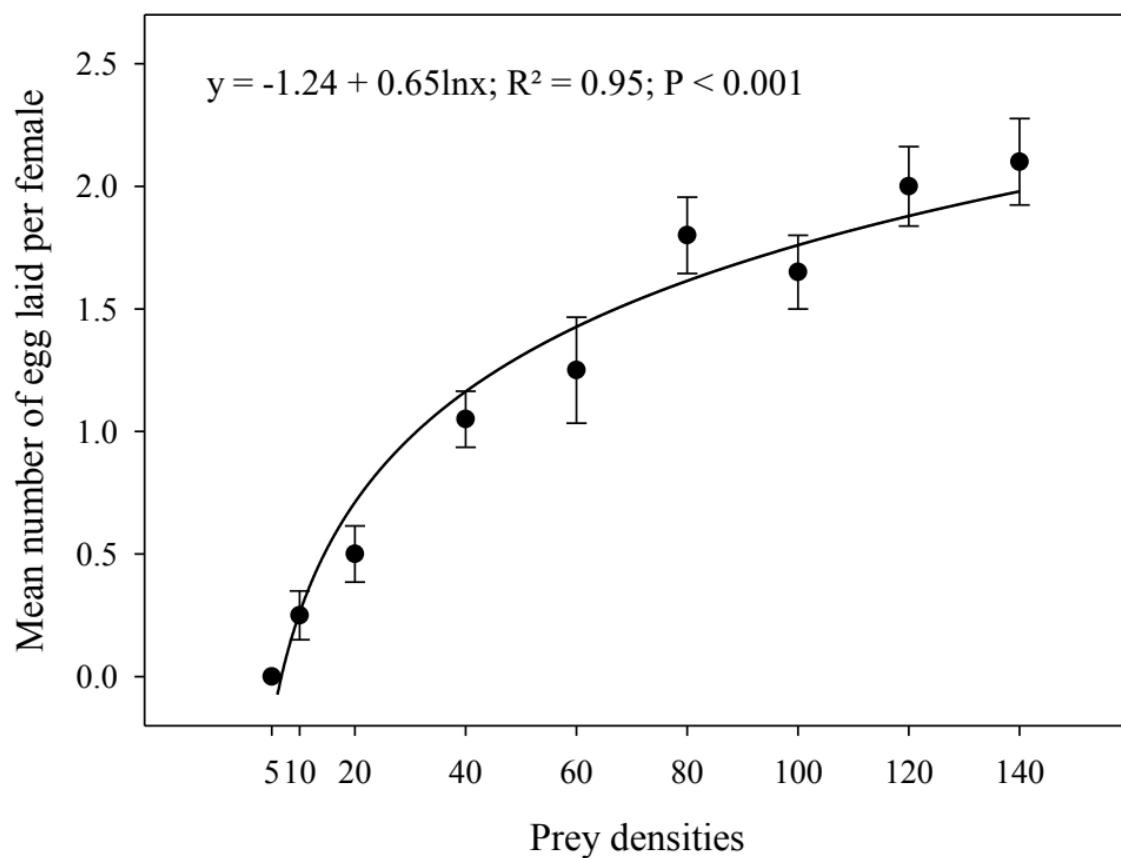


Figure 3. Daily oviposition of *N. barkeri* females as a function of the number of *R. indica* eggs offered. Error bars represent standard error of the mean.

4 ARTIGO DA TESE 3

Can the prey species *Raoiella indica* Hirst (Acari: Tenuipalpidae) support the development and reproduction of *Neoseiulus barkeri* Hughes (Acari: Phytoseiidae)?¹

Rosenya Michely Cintra Filgueiras², Bruna Weida Rabelo da Silva², Eduardo Pereira de Sousa Neto², Jairo de Almeida Mendes², José Wagner da Silva Melo²

²Departamento de Fitotecnia, Universidade Federal do Ceará, Pici, 60455-760, Fortaleza, CE, Brasil.

Abstract

The potential of *Neoseiulus barkeri* Hughes (Acari: Phytoseiidae) as a biological control agent has been widely studied and demonstrated against several pest species, mainly against pest mites. However, its potential has not been assessed with tenuipalpid mites, which emerged as a threat to agriculture, especially in coconut and banana crops. The potential of natural enemies can be predicted through life history tests; thus, we conducted experiments to determine the life history parameters of *N. barkeri* that fed on tenuipalpid mite *Raoiella indica* Hirst (Acari: Tenuipalpidae) individuals of different developmental stages. *Neoseiulus barkeri* successfully completed its development by feeding on *R. indica* of all developmental stages; there were no differences in survivorship during the entire immature period (always above 95%) irrespective of the developmental stages of the *R. indica* used. The average oviposition period was 27 days with an average fecundity of 56.2 eggs/female. Positive r_i values obtained for all prey developmental stages indicated that *R. indica* of all developmental stages is suitable to support the population growth of *N. barkeri*. Additionally, the life table parameters of *N. barkeri* recorded in this study [net reproductive rate ($R_0 = 37.9$ female/female), intrinsic rate of increase ($r_m = 0.22$ female/female/day), finite rate of increase ($\lambda = 1.24$ female/female/day), mean generation time ($T = 16.67$ days), and population doubling time ($DT = 3.18$ days)] show that *N. barkeri* performed excellently when feeding on *R. indica* eggs. Our results show that the prey species *R. indica* is a suitable food source for the predatory species *N. barkeri*.

Keywords: Biological control. Phytoseiid. Predatory mites. Life table. Invasive mite pest.

Introduction

The development and reproduction of a predatory mite are directly correlated with the quantity and quality of the food consumed (JANSSEN; SABELIS, 1992; SABELIS, 1992). A generalist predatory mite kills several different prey species in the course of its lifetime and uses them as food for maintenance, growth, or reproduction (SABELIS, 1992). Thus, the

¹FILGUEIRAS, Rosenya Michely Cintra *et al.* Can the prey species *Raoiella indica* Hirst (Acari: Tenuipalpidae) support the development and reproduction of *Neoseiulus barkeri* Hughes (Acari: Phytoseiidae)? **Systematic and Applied Acarology**, v. 25, n. 8, p. 1485-1494, 2020.

biology of a predatory mite is dependent on the prey species it consumes.

According to McMurtry *et al.* (2013), the predatory mite *Neoseiulus barkeri* Hughes (Acari: Phytoseiidae) is a type III generalist predator. This type of predator feeds on a wide range of prey species, including mites of different families, nematodes, and small insects such as thrips, whiteflies, and cochineals (MCMURTRY *et al.* 2013). *Neoseiulus barkeri* is as much capable of feeding on them as it is feeding on pollen (BONDE, 1989). This predatory mite has been widely studied owing to its value as a biological control agent. Its potential has been demonstrated or suggested against several pest species, such as *Tetranychus urticae* Koch (Acari: Tetranychidae) (FAN; PETITT, 1994A; HEKMAT *et al.*, 2017; JAFARI *et al.*, 2012), *Eotetranychus kankitus* Ehara (Acari: Tetranychidae) (LI *et al.*, 2017), *Polyphagotarsonemus latus* (Banks) (Acari: Tarsonemidae) (FAN; PETITT, 1994B), *Steneotarsonemus laticeps* (Halbert) (Acari: Tarsonemidae) (MESSELINK; VAN HOLSTEIN-SAJ, 2006), *Aleuroglyphus ovatus* (Troupeau) (Acari: Acaridae) (XIA *et al.*, 2012), *Oligonychus afrasiaticus* (McGregor) (Acari: Tetranychidae) (NEGM *et al.*, 2014), *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) (NOMIKOU *et al.*, 2001), *Thrips tabaci* Lindeman (Thysanoptera: Thripidae) (BONDE, 1989; HANSEN, 1988; JAFARI *et al.*, 2013; WU *et al.*, 2014; WU *et al.*, 2015), and *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae) (WU *et al.*, 2014).

Despite its potential as a biological agent, especially against pest mites, *N. barkeri* has not been subjected to life history tests with tenuipalpid mites as prey. The family Tenuipalpidae has been neglected as a family of economic importance (JEPPSON *et al.*, 1975). However, some tenuipalpid species, such as *Raoiella indica* Hirst (Acari: Tenuipalpidae), have emerged as threats to agriculture in several countries of the western hemisphere (CARRILLO *et al.*, 2012; FLECHTMANN; ETIENNE, 2004; GONDIM *et al.*, 2012; KANE *et al.*, 2012; MELO *et al.*, 2018). Coconut and banana crops appear to be the most threatened among those infested by *R. indica* (NAVIA *et al.*, 2013; PEÑA *et al.*, 2010). Thus, the objective of the present study was to determine the life history parameters of *N. barkeri* when supplied with *R. indica* of different developmental stages as prey. These parameters provide important information about the ability of a predator to develop, reproduce, establish, and/or persist with given prey types, while it is also possible to predict its numerical response and impact on pest populations (BELLOWS *et al.*, 1992; MCMURTRY, 1983).

Material and methods

Collection and mass rearing of mites

Tyrophagus putrescentiae (Schrank) (Acari: Acaridae) specimens were obtained from a stock colony maintained in the laboratory. The rearing unit consisted of a 7-L plastic pot (approximately 60 cm high × 25 cm in diameter) with two holes on the upper half (\varnothing 7 cm) that were closed with *voile* (mesh of 0.2 mm) to allow gas exchange. *Tyrophagus putrescentiae* specimens fed on wheat bran. In each pot, 3 L of wheat bran moistened with distilled water was kept as a food source for these mites.

Neoseiulus barkeri specimens were obtained from bell peppers (*Capsicum annuum* L.) cultivated in the municipality of Icapuí, Ceará, Brazil ($4^{\circ} 51' S$, $37^{\circ} 21' W$). In the laboratory, these predators were kept in rearing units similar to those of *T. putrescentiae* described above. In each pot, we added 1.5 L of rice husk and 1.5 L of vermiculite, and about 8,000 predators were introduced into each rearing unit. Every week, we added approximately 1 L of wheat bran infested with *T. putrescentiae* into each unit. All the rearing units were kept at $25 \pm 1^{\circ}C$, $70 \pm 10\%$ relative humidity (RH), and a 12-h photoperiod.

The *R. indica* mites used in the experiments were obtained from naturally infested coconut plants found at the Federal University of Ceará (UFC).

Development and survival of immature N. barkeri

The experiment was conducted under the same temperature, relative humidity, and photoperiod conditions used for rearing the predatory mites. The development and survival of predators were evaluated using *R. indica* of different developmental stages as food sources (eggs, larvae, protonymphs + deutonymphs, and adult females). Initially, 100 fertilized *N. barkeri* females were confined for 24 h in experimental unit to produce an egg wave. This unit consisted of rectangular plastic tray (18 cm × 10 cm × 3.5 cm tall) containing a polyethylene sponges (18 cm × 10 cm × 1 cm thick), on which a square of black polyvinyl chloride (PVC) (10 x 10 cm) was placed. To prevent mites from escaping, the PVC margins were covered with hydrophilic cotton, and both sponge and cotton, were kept wet with distilled water on a regular basis.

After 24 h, *N. barkeri* females were removed and the eggs (of a maximum age of 24 h) were maintained. The neonate larvae were carefully transferred into individual experimental units and separated into four groups fed with *R. indica* eggs, larvae, nymphs (protonymphs and deutonymphs), and adults, respectively. The experimental units consisted of petri dishes (1.5 cm high, \varnothing 9 cm) containing polyethylene sponge (1-cm thick, \varnothing 8 cm), overlaid with filter paper discs (\varnothing 7 cm) and a square black PVC (4 × 4 cm). The PVC margins were covered by a

layer of hydrophilic cotton moistened with distilled water to prevent the escape of mites. Each experimental unit was observed every 12 h under a stereomicroscope to determine the duration and survival of the immature *N. barkeri*. Daily, 80 *R. indica* specimens (eggs, larvae, nymphs, or adult females) were offered to each *N. barkeri* individual as food sources. As the developmental time (from egg to adulthood) and juvenile survival (proportion of eggs reaching adulthood) data did not satisfy the normality assumption, the non-parametric Kruskal-Wallis test was used (SAS Institute 2002).

Longevity, reproduction and life table parameters of N. barkeri feeding on R. indica eggs

The immature individuals (female and male) that fed exclusively on *R. indica* eggs were sexed when reaching adulthood. Approximately 100 *R. indica* eggs were supplied daily to each experimental unit. The experimental units were inspected daily for oviposition and adult mortality. Whenever a male died, it was replaced by another male from the rearing units. The pre-oviposition, oviposition, and post-oviposition periods, the number of eggs per female per day, and longevity were determined with 27 replicates (i.e., *N. barkeri* females). Population parameters were estimated using a fertility life table. The fertility life table was elaborated according to Silveira Neto *et al.* (1976), using data on age (x), mean oviposition (mx), and survival (lx). The following parameters were estimated: net reproduction rate ($R_0 = \sum lxmx$), mean generation time ($T = \frac{\sum xlxmx}{\sum lxmx}$), intrinsic population growth rate ($r_m = \frac{Ln(R_0)}{T}$), finite population growth rate ($\lambda = e^{r_m}$), and population doubling time ($DT = \frac{Ln(R_0)}{r_m}$).

Instantaneous rate of N. barkeri population increase across the R. indica developmental stages

The instantaneous rate of population increase (r_i) was calculated for *N. barkeri* across the developmental stages of *R. indica*. The experimental units used were similar to those used to determine the duration and survival of immature *N. barkeri*. Each experimental unit received one gravid *N. barkeri* female (~ 5 days old). Each *N. barkeri* female represented one replicate and 27 replicates (i.e., females) were used for each prey stage. One hundred *R. indica* specimens (eggs, larvae, protonymphs + deutonymphs, or adult females) were offered daily to *N. barkeri* females in each unit. The assessments were performed after 5 days. The instantaneous rate of increase (r_i) was estimated as: $r_i = ln(N_t/N_0)/(\Delta t)$, where N_0 is the initial number of individuals in the population, N_t is the number of individuals in the population at the end of the time interval, and Δt is the interval (the number of days) (STARK *et al.*, 1997). 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. As the data did not satisfy the normality assumption, the confidence intervals (95%) were estimated to compare the instantaneous rate of increase (r_i) across the developmental stages of *R. indica*.

Results

Development and survival of immature N. barkeri

The four groups of *N. barkeri* immatures, fed on *R. indica* of different developmental stages respectively, all completed their development successfully. The duration and the survivorship during each immature stage and the entire immature period (egg-adult) are presented in Table 1. No statistically significant differences in survivorship ($\chi^2 \geq 0.59$, d.f. = 3, $P \geq 0.9$) during the entire immature period were observed (always above 95% regardless of the developmental stages of the *R. indica* tested). However, the duration of the entire immature period (egg-adult) of *N. barkeri* fed with *R. indica* eggs was slightly, but significantly ($\chi^2 \geq 16.7$, d.f. = 3, $P \geq 0.001$), shorter than that of those fed with *R. indica* adults or larvae, while intermediate values were recorded for those fed with *R. indica* nymphs (Table 1).

Longevity, reproduction and life table parameters of N. barkeri fed with R. indica eggs

Neoseiulus barkeri females survived for a maximum of 50 days (Figure 1A), while the mean survival time was 42 days. From days 4–25 of the oviposition period, the daily oviposition rate of *N. barkeri* was always high (up to 2 eggs/female/day) (Figure 1B), while the mean oviposition rate was 2.1 eggs/female/day. The longevity, reproduction, and life table parameters of *N. barkeri* that fed on *R. indica* eggs are presented in Table 2.

Instantaneous rate of N. barkeri population increase across the R. indica developmental stages

The different *R. indica* developmental stages affected significantly ($\chi^2 \geq 16.5$, d.f. = 3, $P \geq 0.001$) the instantaneous rate of population increase (r_i) of *N. barkeri*. The highest, intermediate, and lowest r_i -values were obtained for *N. barkeri* when females fed with *R. indica* nymphs, eggs and adults, and larvae, respectively (Figure 2). Despite these differences, positive r_i values were obtained for all the developmental stages of the prey, thus indicating the suitability of *R. indica* of different developmental stages to be used as a food source and support the population growth of *N. barkeri*.

Discussion

All developmental stages of *R. indica* allowed survival (up to 95%) and development of the immature form of *N. barkeri*. The high survivorship rate obtained not only suggest that *R. indica* was a suitable prey species for *N. barkeri*, but also that the number of specimens offered per immature *N. barkeri* was adequate. Dietary restrictions (low-quality prey or reduced food supply) during the development of immature phytoseiids, result in high mortality (EVELEIGH; CHANT, 1982). The average developmental time from the egg to the adult stage varied from 5.6 to 6 days (at 25 °C) when *R. indica* of different developmental stages were used as food. Similar results were observed when immature *N. barkeri* fed on *T. urticae* nymphs (6.3 days at 25 °C) (JAFARI *et al.*, 2012) or *T. tabaci* larvae (5.98 days at 25 °C) (BEGLYAROV; SUCHALKIN, 1983). However, the developmental time of *N. barkeri* that fed on *A. ovatus* (7.8 days at 24 °C) (XIA *et al.*, 2012) and *O. afrasiaticus* (9.6 days at 25 °C) (Negm *et al.* 2014) was longer. These results suggest that *R. indica* of different developmental stages provide immature *N. barkeri* with nutrition of similar quality to that of *T. urticae* and *T. tabaci*.

Prey quality can influence the fecundity and longevity of phytoseiid mites. Rasmy *et al.* (2000) revealed that the type of food used affects significantly the longevity and fecundity of female phytoseiid mites *Amblyseius deleoni* Muma & Denmark (Acari: Phytoseiidae). Even when fed with the same amounts of different prey species, the fecundity of predatory mites differs, which suggests a different energetic supply from different prey species (JANSSEN; SABELIS, 1992; SABER, 2013). In the present study, the daily oviposition rate (2.1 ± 0.04 eggs/female/day), total fecundity (56.2 ± 1.1 eggs/female), and oviposition period (27 ± 0.5 days) of *N. barkeri* females that fed on *R. indica* eggs, were higher than the corresponding values obtained when this predator fed on other prey species. For example, in contrast to our findings (Table 2), *N. barkeri* females that fed on *O. afrasiaticus* (NEGM *et al.*, 2014) and *T. urticae* (JAFARI *et al.*, 2010) showed lower values in daily oviposition rate (1.0 ± 0.07 eggs/female/day and 1.97 eggs/female/day, respectively), total fecundity (18.80 ± 1.39 eggs/female and 36.80 ± 0.86 eggs/female, respectively), and oviposition period (25.92 ± 0.71 days and 18.7 ± 0.44 days, respectively). Thus, our results demonstrated that *N. barkeri* was very efficient in converting the *R. indica* consumed into egg biomass. Phytoseiids that have this high capacity to convert prey into egg mass can be considered as promising biocontrol agents (SABELIS, 1985).

Additionally, the age of the females influences egg production (Momen 1994). We observed that the daily oviposition rate of *N. barkeri* began decreasing when the females were

25 days old. The longevity of females was also influenced by the food consumed. A longer longevity was obtained when *N. barkeri* females fed on *R. indica* (40.1 days) than when they fed on *O. afrasiaticus* (35.6 days, NEGM *et al.*, 2014) and *A. ovatus* (34.6 days, XIA *et al.*, 2012) (Table 2). This is an important result for biological control, as it suggests that this predator could survive and reproduce for a considerable amount of time by preying on the *R. indica* pest mite.

Life table parameters allow for the assessment of a predator's growth, reproduction, and longevity potential. Prey nutrient composition influences the biological characteristics of predatory mites (FERRERO *et al.*, 2014; HUANG *et al.*, 2013). Indeed, different life table parameters have been recorded for *N. barkeri* feeding on different prey species. For example, the R_0 , r_m , λ , T, and DT parameters of *N. barkeri* had different values when *T. tabaci* (27.78, 0.22, 1.25, 19.10, and 3.15, respectively) (BONDE, 1989), *A. ovatus* (20.14, 0.14, 1.15, 22.1, and 5.1, respectively) (XIA *et al.*, 2012), and *T. urticae* (22.02, 0.22, 1.25, 13.95, and 3.13, respectively) (JAFARI *et al.*, 2010) were used as prey species (Table 2). The fact that in the present study these values were 37.9, 0.22, 1.24, 16.67, and 3.18, respectively, indicates that *N. barkeri* performed better on *R. indica*.

Positive instantaneous rate of increase (r_i) values were obtained for all the developmental stages of the prey, thus indicating that *R. indica* eggs, larvae, nymphs, and adults are suitable food sources for *N. barkeri*. In addition, Filgueiras *et al.* (2020) had demonstrated that the consumption of *R. indica* by *N. barkeri* was inversely related to the life stage of the former, and individuals of all developmental stages enabled the reproduction of *N. barkeri*. According to Cuellar *et al.* (2001), the development, survival, and reproduction of predators are influenced by the abundance and developmental stages of their prey, which vary widely in their nutritional quality. The slight differences among the r_i of *N. barkeri* that fed on *R. indica* of different developmental stages could possibly be related to the nutritional quality of the latter. However, whether *R. indica* of different developmental stages differ greatly in nutritional composition remains an important question to be addressed in future.

Before *N. barkeri* can be considered as a biological agent against *R. indica*, it is advisable to determine this predator's potential of establishment on aerial plant parts and evaluate the interaction of *N. barkeri* with other predatory mites, such as *Amblyseius largoensis* (Muma) (Acari: Phytoseiidae), which are commonly found in plants infested by *R. indica*.

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Disclosure

The authors declare no conflicts of interest.

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Table 1. Duration of immature stages (mean \pm SE) and survival (mean \pm SE) of *Neoseiulus barkeri* that fed on *Raoiella indica* of different life stages

Food (<i>R. indica</i> stage)	Biological parameters	N° of immatures	Immature stages (<i>N. barkeri</i>)				
			Egg	Larva	Protonymph	Deutonymph	Egg-Adult
Egg	Duration (days)	40	1.5 \pm 0.05	0.8 \pm 0.04	1.5 \pm 0.04	1.5 \pm 0.05	5.6 \pm 0.06 b
	Survival (%)		100	99.98 \pm 0.03	99.95 \pm 0.04	99.97 \pm 0.03	99.96 \pm 0.00 A
Larva	Duration (days)	30	1.5 \pm 0.08	0.8 \pm 0.05	1.9 \pm 0.04	1.8 \pm 0,04	6.0 \pm 0.06 a
	Survival (%)		100	99.97 \pm 0.03	99,97 \pm 0.03	99,96 \pm 0.03	99.90 \pm 0.03 A
Nymph	Duration (days)	40	1.4 \pm 0.06	0.9 \pm 0.04	1.8 \pm 0.04	1.8 \pm 0.04	5.9 \pm 0.08 ab
	Survival (%)		100	99.98 \pm 0.03	99.95 \pm 0.04	99.95 \pm 0.04	99.87 \pm 0.05 A
Adult	Duration (days)	33	1.4 \pm 0.07	0.8 \pm 0.05	1.9 \pm 0.07	1.9 \pm 0.06	6.0 \pm 0.08 a
	Survival (%)		100	99.97 \pm 0.03	99.97 \pm 0.03	99.91 \pm 0.05	99.85 \pm 0.00 A

Means followed by same letters uppercase or lowercase in the same column are not significantly different by the Kruskal-Wallis test ($P > 0.05$).

Table 2. Summary of the reported reproduction, longevity, and life table parameter values of *Neoseiulus barkeri* that fed on *Raoiella indica* eggs

Prey (mean \pm SE and/or CI)	<i>Raoiella indica</i>	<i>Oligonychus afrasiaticus</i>	<i>Aleuroglyphus ovatus</i>	<i>Tetranychus urticae</i>	<i>Thrips tabaci</i>
Preoviposition period (days)	2 \pm 0.1	4.17 \pm 0.17	2.68 \pm 0.25	2.47 \pm 0.09	2.1 \pm 0.21
Oviposition period (days)	27 \pm 0.5	25.92 \pm 0.71	20.39 \pm 1.13	18.7 \pm 0.44	20.3 \pm 1.29
Postoviposition period (days)	11.1 \pm 0.8	5.58 \pm 0.47	11.50 \pm 0.70	11.11 \pm 0.91	1.3 \pm 0.69
Daily fecundity (eggs/female/day)	2.1 \pm 0.04	1.00 \pm 0.07	1.53 \pm 0.04	1.97	2.3 \pm 0.08
Total fecundity (eggs/female)	56.2 \pm 1.1	18.80 \pm 1.39	30.64 \pm 1.67	36.80 \pm 0.86	47.1 \pm 3.26
Longevity (days)	40.1 \pm 0.9	35.67 \pm 0.91	34.57 \pm 1.53	21.73 \pm 0.10	23.4
Net reproductive rate (R_0) (female/female)	37.9 (36.35 – 39.5)	10.19	20.14	22.02 \pm 0.52	27.78
Intrinsic rate of increase (r_m) (female/female/day)	0.22 (0.21 – 0.22)	0.13	0.14	0.22 \pm 0.003	0.22
Finite rate of increase (λ) (female/female/day)	1.24 (1.24 – 1.25)	1.12	1.15	1.25 \pm 0.003	1.25
Doubling time (DT) (day)	3.18 (3.11 – 3.25)	5.33	5.1	3.13 \pm 0.04	3.15
Mean generation time (T) (day)	16.67 (16.25 – 17.09)	17.83	22.1	13.95 \pm 0.18	19.1
Temperature (°C)	25	25	24	25	25
References	Present study	Negm <i>et al.</i> 2014	Xia <i>et al.</i> 2012	Jafari <i>et al.</i> 2010	Bonde 1989

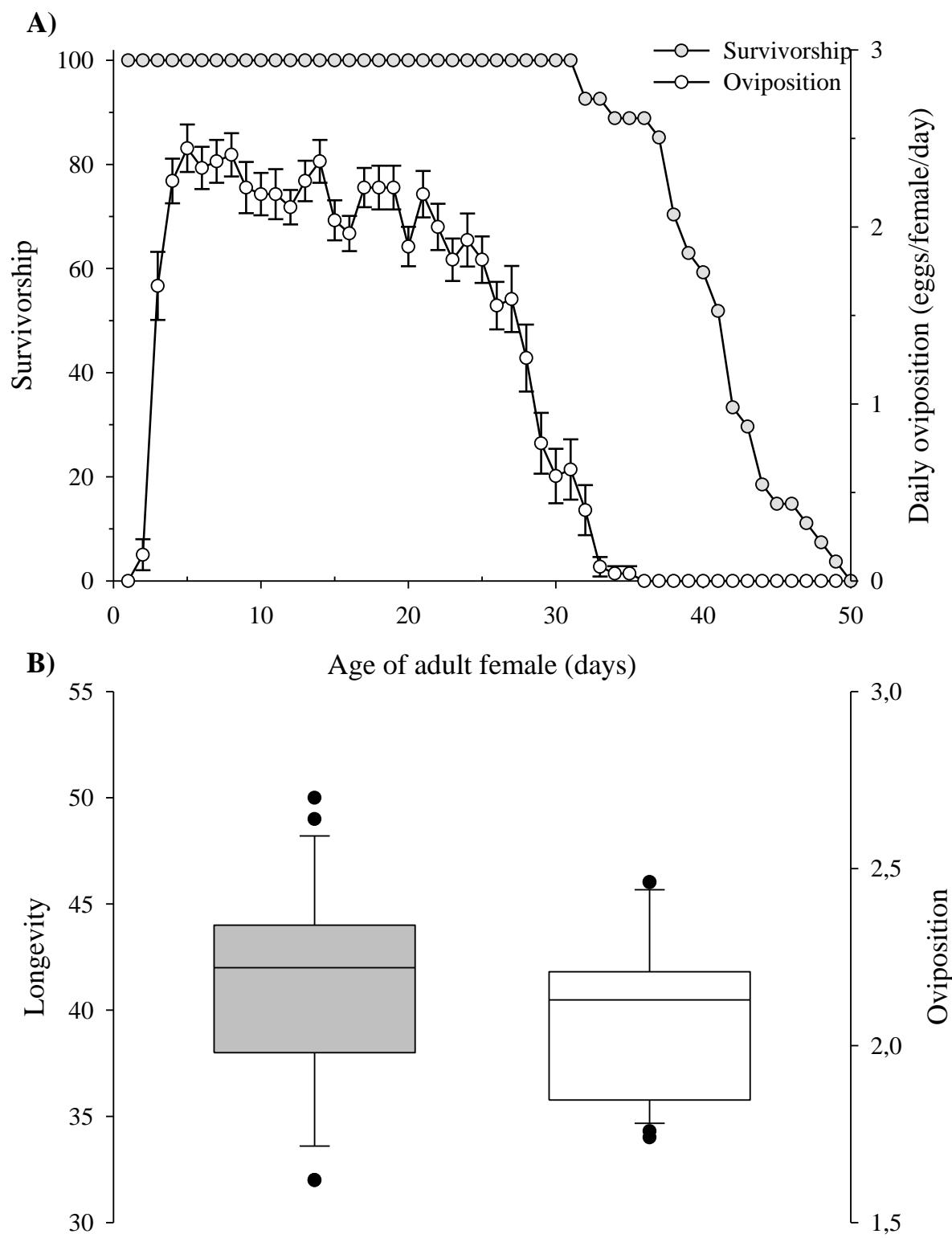


Figure 1. Survivorship and daily oviposition (mean \pm SE) (A) and average female longevity and oviposition (B) of *Neoseiulus barkeri* fed with *Raoiella indica* eggs (temp. 25 °C, 70 \pm 10 % RH and a 12h photoperiod)

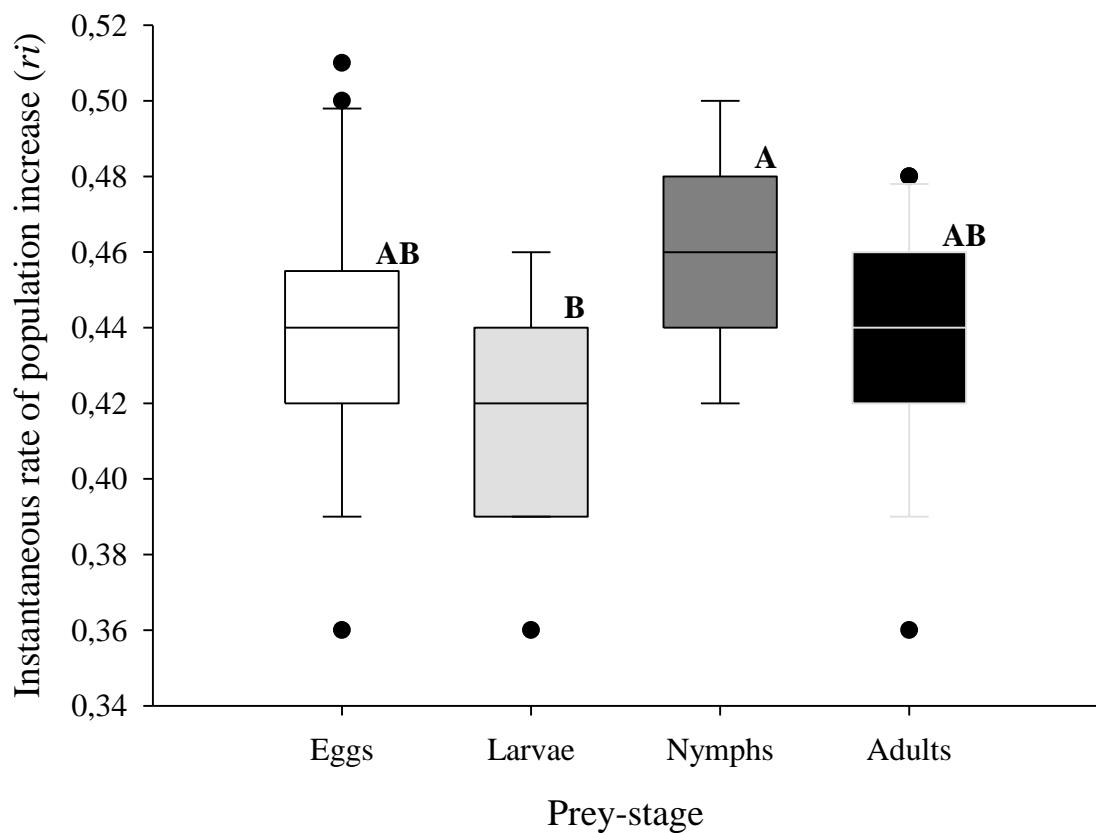


Figure 2. The instantaneous rate of population increase (r_i) of *Neoseiulus barkeri* that fed on *Raoiella indica* of different life stages.

5 CONSIDERAÇÕES FINAIS

Esta pesquisa contribui significativamente para o avanço do controle biológico de pragas, pois, este é o primeiro estudo a sugerir o potencial de *N. barkeri* como agente de controle biológico de *R. indica*. Isso tem grande importância no cenário atual porque muitas vezes não existem produtos químicos registrados para o controle de determinadas pragas. Além disso, existe crescente exigência por parte dos consumidores em adquirir produtos de elevada qualidade nutricional e livre dos riscos de contaminantes.

A partir desse estudo foi possível descobrir que *N. barkeri* prefere se alimentar de ovos em dentramento dos demais estágios de desenvolvimento de *R. indica* e que a predação e oviposição de *N. barkeri* aumentaram em função do aumento da densidade de ovos de *R. indica* que foram ofertados. Além disso, observou-se que todos os estágios de desenvolvimento de *R. indica* permitiram a sobrevivência (até 95%) e o desenvolvimento da forma imatura de *N. barkeri*. Ademais, nossos resultados demonstraram que *N. barkeri* foi muito eficiente na conversão da presa consumida (*R. indica*) em biomassa de ovos depositados. Também foi obtida maior longevidade quando as fêmeas de *N. barkeri* se alimentaram de *R. indica* o que sugere que esse predador poderia sobreviver e se reproduzir por um período considerável de tempo predando o ácaro-praga *R. indica*.

Quando liberado no campo em densidades de 900 e 600 predadores por planta, *N. barkeri* foi capaz de reduzir significativamente o número médio de formas móveis de *R. indica*. É importante ressaltar ainda que esses testes incluíram populações altas de *R. indica* (surtos). É possível que em áreas recentemente infestadas (ou aquelas com densidades menores do que as avaliadas, provavelmente encontrada em viveiros de mudas), *N. barkeri* pode ser ainda mais eficaz no controle de *R. indica*.

No entanto, antes que *N. barkeri* seja considerado um agente de controle biológico outros aspectos devem ser avaliados como: (i) a preferência alimentar de *N. barkeri*, pois em condições de campo múltiplas presas espécies podem estar presentes e se *R. indica* não é sua presa preferida, sua liberação pode não ser eficaz; (ii) se *N. barkeri* pode se estabelecer nas partes aéreas das plantas; e (iii) se o liberação desse predador poderia deslocar outras espécies presentes nos folíolos do coqueiro, como *Euseius alatus* De Leon (Acari: Phytoseiidae) e *A. largoensis*, importantes para o controle de Aceria guerreronis Keifer (Acari: Eriophyidae), outra praga chave do cultivo de coco (Melo *et al.* 2015).

Outrossim, as informações geradas neste trabalho são pertinentes e de valia para os grandes e pequenos produtores, desta forma, é importante que exista divulgação das mesmas.

Nesse contexto, a parceria entre empresas agrícolas e o meio científico/acadêmico se fazem essenciais, uma vez que estes podem levar mais facilmente as informações obtidas aos agricultores, que muitas vezes não tem acesso aos textos científicos, dessa forma, não utilizando técnicas que podem auxiliar seu cultivo por não terem conhecimento a respeito.

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