

POTENTIAL OF FLORIDA NATURAL ENEMIES TO CONTROL THE INVASIVE
SPECIES *RAOIELLA INDICA* (ACARI: TENUIPALPIDAE)

By

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To my family and friends, whose patience and encouragement have helped me throughout the process of writing this dissertation

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Abstract of Dissertation Presented to the Graduate School
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POTENTIAL OF FLORIDA NATURAL ENEMIES TO CONTROL THE INVASIVE
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The central issue of this dissertation is the red palm mite, *Raoiella indica*, a phytophagous tenuipalpid mite that recently invaded the Western Hemisphere. Efforts have been made to identify potential biological control agents of *R. indica*. A predatory mite *Amblyseius largoensis* (Acari: Phytoseiidae) has been found associated with *R. indica* in Florida. A series of studies evaluated *A. largoensis* for potential control of *R. indica* by determining (1) the development and reproduction of *A. largoensis* feeding on *R. indica*, other potential prey and pollen; (2) the prey-stage preferences and functional and numerical responses of *A. largoensis* to *R. indica* (3) the variability in response of four populations of *A. largoensis* with contrasting feeding histories to *R. indica* and *Tetranychus gloveri* Banks (Acari: Tetranychidae); (4) the effect of *A. largoensis* on *R. indica* using predator exclusion and predator release techniques. The results of these studies showed that: (1) Predators fed on a diet of *R. indica* had a faster development and a greater intrinsic rate increase than those fed on the other prey tested or pollen; (2) *A. largoensis* had a marked preference for *R. indica* eggs, followed by larvae, over other developmental stages. Predation and oviposition of *A. largoensis* increased as a function of increasing *R. indica* population densities. *Amblyseius largoensis* exhibited a type II functional response; (3) all predators, including those with no previous exposure to *R. indica*, had a high likelihood of

consuming *R. indica* eggs over eggs and larvae of *T. gloveri*. However, populations of *A. largoensis* varied in their consumption of *R. indica* motile stages. Predators with previous exposure to the invasive species were more likely than naïve predators to consume *R. indica* larvae and finally; (4) a predator release rate of 1 *A. largoensis*/ 10 *R. indica* reduced *R. indica* densities by 80% over the untreated control and contributed to reduce damage on coconut leaves caused by the phytophagous mite. Overall, results of this research suggest that *A. largoensis* is an important mortality factor of *R. indica* and should be considered as a key biological control agent in integrated pest management programs targeting *R. indica*.

CHAPTER 1 INTRODUCTORY REMARKS

The introduction of non-indigenous species to an ecosystem sometimes results in serious harm to the environment. In such cases, immigrant species are referred to as invasive species. Invasive species can cause serious alterations to plant and animal communities resulting in high economic losses, calculated in several billions of dollars in the USA (Pimentel et al. 2005) and trillions worldwide (Ricciardi et al. 2011). During the last few centuries, the opportunities for unintentional introductions of organisms (i.e., animals, microbes, plants) to new areas have greatly increased due to an expansion in trade among nations, human movement and population growth (Elton 1958). Therefore, extensive efforts have been made to prevent further introductions and to manage the species that have already established in various ecosystems.

This dissertation focuses on a phytophagous mite species that has several traits shared by most invasive species. In general, phytophagous mites are *r*-strategists, or species with small size, rapid development and high reproductive rates that enables a high instantaneous rate of increase. Most phytophagous mites can reproduce parthenogenetically; therefore, a single female has the ability to start a new population. Mites are difficult to detect because they are often hidden on their host plants and the symptoms of mite infestation usually appear after populations are large and unmanageable. Moreover, several dispersal mechanisms allow phytophagous mites to colonize distant plants, including aerial dispersal through specific behaviors (silking or ballooning) or as aerial plankton, and phoresy (attaching themselves to insects or other hosts). All these traits increase the likelihood of establishment into new areas and the probability they will become invasive species.

Documented examples of phytophagous mites as invasive species are scarce compared to invasive insects or plants. However, many mite species have been moved around the world

inadvertently (Navia et al. 2010). The lack of mite taxonomic and biogeographical studies, and lack of experts in those areas, may have contributed to the failure of detecting and mitigating the effects of those invasions in a timely manner. Perhaps the best documented case was the introduction and spread of the cassava green mite *Mononychellus tanajoa* (Bondar) (Acari: Tetranychidae) into Africa (Yaninek and Herren 1988). This mite, of Neotropical origin, was discovered on cassava in Uganda in the 1970s and spread to other African countries thereafter causing significant damage to this important food crop. Several researchers, agencies and institutions made extensive efforts to identify mortality factors and practices for managing this species [Bellotti et al. 1999, Centro Internacional de Agricultura Tropical (CIAT-Colombia), International Institute of Biological Control (IIBC UK-Benin and Kenya), International Institute of Tropical Agriculture (IITA-Nigeria), Empresa Brasileira de Pesquisa Agropecuaria (EMBRAPA-Brazil)].

The central issue of this dissertation is the red palm mite, *Raoiella indica*, a tenuipalpid mite that recently invaded the Western Hemisphere. *Raoiella indica* is a polyphagous species with a wide host-plant range, mostly within the Arecaceae (palms), but it also attacks some plants within the Pandanaceae, Musaceae, Heliconiaceae, Zingiberaceae and Strelitziaceae (Appendix). Its major host is coconut *Cocos nucifera* L. *Raoiella indica* is highly destructive to coconut sometimes reaching 4,000 individuals/ leaflet (Peña et al. 2009) (Figure 1-2D). Infestations found on other host plants are lower in numbers but still problematic because of their importance as ornamental or native plants.

Before its arrival in the New World *R. indica* was found only in tropical and subtropical areas of Africa and Asia (Figure 1-1). In 2004, this mite was detected in Martinique (Flechtmann and Etienne 2004) and subsequently spread to multiple islands of the Caribbean. In 2007, it was

found in West Palm Beach, Florida (USA), and in the state of Sucre, Venezuela. More recently, the mite was reported in the northern state of Roraima in Brazil, in the north coast of Colombia and in Isla Mujeres and Cancun, Mexico (Figure 1-1).

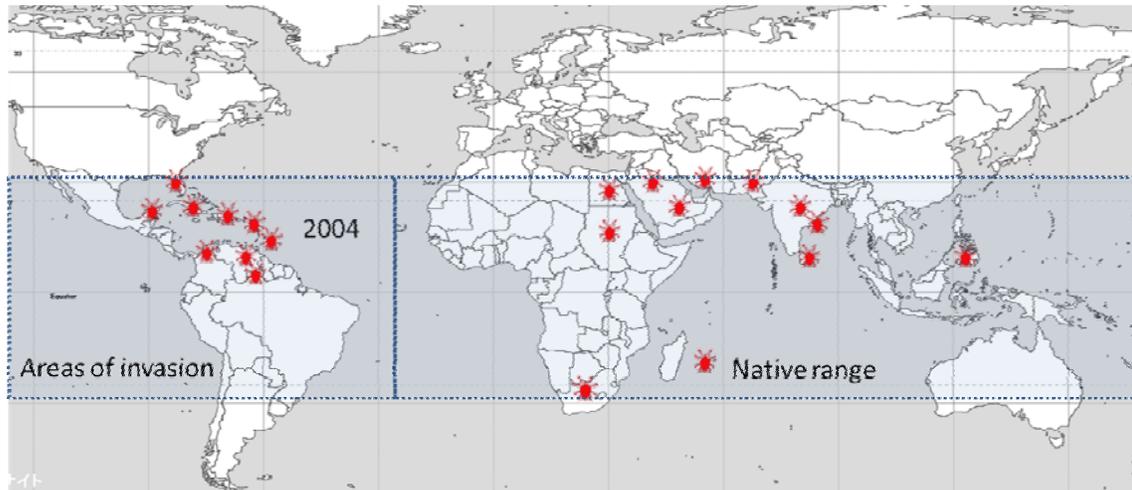


Figure 1-1. Reports of the presence of *Raoiella indica*. 1. Native range in the Eastern Hemisphere [Egypt (Zayed 1942), India (Puttarudriah and ChannaBasavanna 1956), Mauritius (Moutia 1958), Pakistan (Chaudri 1974), Israel (Gerson et al. 1983), Oman (Elwan 2000), Iran (Askari et al. 2002), Phillipines (Gallego et al. 2003), and Benin and Tanzania (Zannou et al. 2010)]. 2.Areas of recent invasion in the New World [Martinique (Flechtmann and Etienne 2004), St. Lucia and Dominica (Kane et al. 2005), Guadeloupe and Saint Martin (Etienne and Flechtmann 2006), Jamaica (RADA 2007), Puerto Rico and Culebra Island (Rodrigues et al. 2007), Florida USA (FDACS 2007), Trinidad and Tobago (Roda et al. 2008), Cuba (de la Torre et al. 2010), Venezuela (Vásquez et al. 2008), Brazil (Marsaro Jr. et al. 2009), Mexico (NAPPO 2009 and Colombia (Carrillo et al. 2011b)].

The establishment of *R. indica* in the Caribbean has caused serious economic harm to coconut production with over 50% yield reductions at some locations (CARDI 2010). In Florida, infestations have spread to six counties in the southern part of the peninsula (FDACS 2011). Quarantine-mandatory acaricide sprays before shipping *R. indica* hosts have been adopted in order to prevent *R. indica*'s rapid dissemination (TDA 2008, FDACS 2011). However, this mite

continues to spread throughout the Neotropical region where a great diversity of plants could potentially be affected (Appendix).

The host-plant range and dispersal of *R. indica* throughout natural, agricultural, recreational and residential areas suggest that large-scale mitigation programs are required for managing this species. Chemical control, host-plant resistance and cultural-control tactics could be used to manage local populations; however, the most promising approach is to find a practical, long-lasting solution in the form of biological control. One of the factors contributing to the aggressiveness of invasive species is that they often arrive in new areas without their specific natural enemies (Herren and Neuenschwander 1991). Thus, one of the most common approaches to suppress invasive species is to search for natural enemies in their site of origin in order to reunite pest and natural enemies through importation of the latter (classical biological control) (Van Driesche and Bellows 1996). In many cases, indigenous natural enemies may provide some suppression of the invasive pest. In addition, biological control might be achieved with species that have not experienced close prior relationship with the target organism (Hokanen and Pimentel 1984).

Raoiella indica became the target of classical biological control after its arrival in the New World (Hoy 2011; Taylor et al. 2011). In parallel, efforts were made to identify the natural enemy fauna in areas of invasion in the Western Hemisphere (Peña et al. 2009). Interestingly, surveys for natural enemies conducted in India, Mauritius, and those made in the New World all found *Amblyseius largoensis* Muma (Acari: Phytoseiidae) as the most abundant predator of *R. indica*. Bowman (2010) hypothesized that, as seen in other phytoseiids (Noronha and Moraes 2004), biotypes or cryptic species could exist within the taxonomic group classified as *A. largoensis*. However, the existence of *A. largoensis* in Florida would likely impede the release of

predators under the same taxonomic identity unless it is proven that they will not interbreed with or have deleterious effects on the local populations (Hoy, pers. comm.).

The actual lack of other candidates for classical biological control and the observed association of various predators with *R. indica* (a complete review of the natural enemies of *R. indica* is presented in chapter 2) suggest that Florida native natural enemies merit a more thorough investigation. Moreover, the conspicuous association between *A. largoensis* and *R. indica* in Florida suggested the need for detailed studies to better understand their predator-prey interactions. These issues are the main focus of this dissertation.

Research Objectives

The aims of this dissertation were to identify the natural enemy complex of *R. indica* in Florida and to conduct analyses of the predator-prey interactions between *A. largoensis* and *R. indica*.

Structure of the Dissertation

This dissertation is presented as five separate papers (Chapters 2-6) in publication styles ready for submission to scientific journals. Each paper is therefore a stand-alone document, addressing different aspects of biological control of *R. indica*. Preceding these articles, Chapter 1 provides an introduction to the research problem and research objectives, and a brief literature review on the taxonomy and biology of *R. indica*, including information on interactions with its major host. Following the articles, Chapter 7 presents the main conclusions of the dissertation and explores future directions for research focused on biological control of *R. indica*. In addition, an Appendix entitled “Host plant range of *Raoiella indica* Hirst (Acari: Tenuipalpidae) in areas of invasion of the New World” was included to complement the information on the biology and potential effects of *R. indica* in the Neotropics. This article was submitted for publication in the journal *Experimental and Applied Acarology* as the result of a collaborative project between

researchers from the Ministry of Agriculture, Land and Marine Resources of Trinidad and Tobago, USDA-APHIS, and the University of Florida TREC. Divina Amalin, Farzan Hosein, Amy Roda, Rita Duncan and Jorge E. Peña are coauthors.

The first article (Chapter 2) entitled “A review of the natural enemies of the red palm mite, *Raoiella indica* (Acari: Tenuipalpidae)” presents a compendium of all the available information about the natural enemies reported in association with the red palm mite both in the Eastern Hemisphere and in areas of recent invasion in the New World. This review outlines which natural enemies are more promising candidates for classical biological control programs of *R. indica* according to the available literature, and which have shown more potential among the natural enemies found in areas of recent invasion by *R. indica*, and thus, were selected for experimental evaluations in Florida. This article was submitted to *Experimental and Applied Acarology*. Drs. J. Howard Frank, Jose Carlos V. Rodrigues and Jorge E. Peña are coauthors.

The second article (Chapter 3) entitled “Development and reproduction of *Amblyseius largoensis* (Acari: Phytoseiidae) feeding on pollen, *Raoiella indica* (Acari: Tenuipalpidae), and other micro-arthropods inhabiting Coconuts in Florida” presents a study on the basic biology of *A. largoensis*. The study was designed to determine how feeding on *R. indica* could affect the life-table parameters of *A. largoensis* when compared to feeding on other potential prey with a longer history of inhabiting coconuts in Florida. This article was published in *Experimental and Applied Acarology* (2010, 52: 119-129). Drs. Jorge E. Peña, Marjorie A. Hoy and J. Howard Frank are coauthors.

The third article (Chapter 4) entitled “Prey-stage preferences and functional and numerical responses of *Amblyseius largoensis* (Acari: Phytoseiidae) to *Raoiella indica* (Acari: Tenuipalpidae)” presents a series of experiments designed to determine whether the predator

showed differences in consumption or preferences among *R. indica* stages, and how the predator could respond to changes in the population density of *R. indica* in terms of its predation and reproductive rates. This article was submitted to *Experimental and Applied Acarology*. Dr. Jorge E. Peña is coauthor.

The fourth article (Chapter 5) entitled “Variability in response of four populations of *Amblyseius largoensis* (Acari: Phytoseiidae) to *Raoiella indica* (Acari: Tenuipalpidae) and *Tetranychus gloveri* (Acari: Tetranychidae) eggs and larvae” presents a study designed to determine how populations of predators with disparate previous exposure to *R. indica* could differ in their likelihood of feeding on *R. indica*. This article was submitted to *Biological Control*. Drs. Martha E. de Coss, Marjorie A. Hoy and Jorge E. Peña are coauthors. The fifth article (Chapter 6) entitled “Effect of *Amblyseius largoensis* (Acari: Phytoseiidae) on *Raoiella indica* (Acari: Tenuipalpidae) using predator exclusion and predator release techniques” presents quantitative evaluations of the efficacy of *A. largoensis*. The target journal for this article has not been defined yet. Drs. Josep A. Jacas and Jorge E. Peña are coauthors.

Literature Review

This section presents a brief review on the taxonomy and biology of *R. indica*, including information on mite-plant interactions with its major host plant, *C. nucifera*.

Taxonomy of *Raoiella indica*

The superfamily Tetranychoidae groups several families of obligate plant-feeding mites (Krantz 2009). The Tetranychidae (spider mites) are the most recognized and studied mites within the superfamily because this family includes many species considered as important pests around the world. The Tenuipalpidae, commonly known as flat mites or false spider mites, also has a worldwide distribution with several species considered as important pests. Most of the tenuipalpid species that cause economic damage to cultivated plants (by direct feeding or

vectoring plant viruses, Rodrigues et al. 2003) belong to the two largest genera, *Brevipalpus* Donnadieu and *Tenuipalpus* Donnadieu (Mesa et al. 2009). However, the genus *Raoiella* has gained importance after *R. indica* started to spread aggressively in the Neotropical region.

The genus *Raoiella* was first described and placed in the Tetranychidae by Hirst (1924), but later transferred to the Tenuipalpidae by Sayed (1950). Twelve species described from the Indomalayan (8), Afrotropical (2), Australasian (1) and Western Palearctic (1) zoogeographic regions compose the genus (Table 1-1); however, seven are suspected to be junior synonyms of the type species *R. indica* (Mesa et al. 2009). Moreover, the genera *Rarosiella* Rimando 1996 and *Neoraoiella* Mohanasundaram 1996 were synonymized with *Raoiella* Hirst 1924.

Biology of *Raoiella indica*

Raoiella indica is usually found forming large multigenerational colonies on the abaxial surface of the leaflets (pinnae) of their host plants (Figure 1-2C). Eggs are oblong, smooth, glossy, red in color, and measure 0.12 mm in length and 0.09 mm in breadth, with a stipe 0.15 long (Moutia 1958) (Figures 1-2A and 1-3). A single droplet of an unknown substance is sometimes found at the tip of the stipe. Upon hatching, larvae are slow in their movements and start feeding on leaf tissue near the site of emergence. Development of *R. indica* passes through two nymphal stages with a quiescent period before each molt. The immature stages of *R. indica* are red with blackish marks in the dorsum and develop near the site of oviposition. The duration of the immature development varies from approximately 22 to 34 d depending on the host plant and environmental conditions (Table 1-2) (Figure 1-3).

Males are triangular in form, develop faster and are smaller than females. They actively seek out females and settle closely behind a quiescent deutonymph or adult female in a “guarding state” until mating (Figure 1-2B). The preoviposition period ranges from 3 to 7 d

(Moutia 1958, Flores et al. 2010). The type of reproduction in *R. indica* is unusual because mated females (sexual reproduction) produce progeny consisting solely of females and unfertilized

Table 1-1. Species of the genus *Raoiella* following the classification by Mesa et al. (2009).

Species name and descriptor	Country of collection	Host plant
<i>australica</i> Womersley	Australia	<i>Eucalyptus</i> spp. (Myrtaceae)
<i>eugenia</i> (Mohanasundaram 1996)	India	<i>Eugenia</i> sp. (Myrtaceae)
<i>shimapana</i> Meyer	South Africa	<i>Cassine transvaalensis</i> (Celastraceae)
<i>macfarlanei</i> Pritchard & Baker	Greece	olive (Oleaceae)
<i>indica</i> Hirst	India	<i>Cocos nucifera</i> (Arecaceae)
<i>camur</i> Chaudhri & Akbar *	Pakistan	<i>Phoenix dactylifera</i> (Arecaceae)
<i>empedos</i> Chaudhri & Akbar *	Pakistan	<i>Phoenix dactylifera</i> (Arecaceae)
<i>neotericus</i> Chaudhri & Akbar *	Pakistan	<i>Phoenix dactylifera</i> (Arecaceae)
<i>obelias</i> Hasan & Akbar *	Pakistan	<i>Phoenix dactylifera</i> (Arecaceae)
<i>rahii</i> Akbar & Chaudhri *	Pakistan	<i>Phoenix dactylifera</i> (Arecaceae)
<i>phoenica</i> Meyer *	Sudan	<i>Phoenix dactylifera</i> (Arecaceae)
<i>pandanae</i> Mohanasundaram *	India	<i>Pandanus</i> sp. (Pandanaeae)

*Species suspected to be junior synonyms of the type species *R. indica*.

females (asexual reproduction) produce only males (NageshaChandra and Channabasavanna 1984). The species is reported to have four and two chromosomes (females and males, respectively) which suggests that its reproduction is arrhenotokous (Helle et al. 1980). According to NageshaChandra and Channabasavanna (1984) mated females produce at an average of 22 ± 4.41 (females/female) and unmated females produce 18.4 ± 1.95 (males/female) during their adult life on coconuts. Moutia (1958) reported a similar average fecundity (~ 28.1 eggs/female) but other studies with other hosts reported substantially lower fecundities (12.6 ± 4.3 and 7.8 ± 2.6 eggs/female on coconut and banana leaves, respectively (Gonzales and Ramos 2010), and 7.0 ± 3.5 eggs per female on Areca palms (Flores-Galano et al. 2010). According to NageshaChandra and Channabasavanna (1984), longevity is greater in females (50.9 ± 11.4 days) than in males (21.6 ± 1.95 days), but this can also be influenced by the host plant (Gonzales and Ramos 2010).

Adults are more active and are responsible for dispersion within the leaf, to colonize new leaves or new plants.

Table 1-2. Developmental times of immature stages of *R. indica* females reported by various studies using variations of the leaf flotation method under laboratory conditions.

Host Plant	Temp.(°C)	Rel. Hum. (%)	Develop. time (d)	Reference
<i>Cocos nucifera</i>	24.2	*	22	Moutia 1958
<i>Cocos nucifera</i>	17.9	*	33	Moutia 1958
<i>Phoenix dactylifera</i>	26.1	57.90	21.4	Zaher et al. 1969
<i>Cocos nucifera</i>	23.9-25.7	59.85	24.5± 1.92	NageshaChandra and Channabasavanna 1984
<i>Cocos nucifera</i>	26.3± 1.26	74.8± 4.3	31.4± 3.31	Gonzales and Ramos 2010
<i>Musa acuminata</i>	26.3± 1.26	74.8± 4.3	33.4± 4.76	Gonzales and Ramos 2010
<i>Areca catechu</i>	25.4± 1.20	57.5± 6.5	31.0± 4.11	Flores-Galeano et al. 2010

*information not provided

Mite and Host Plant Interactions

Host plants could have an important influence in the population dynamics of *R. indica*. However, other than reports on the suitability or not of plants as hosts for *R. indica* (a complete list of the host plants *R. indica* is presented in Appendix), very little is known about the influence of plant characteristics on their quality as hosts for *R. indica*. Sakar and Somchoudhury (1989) studied possible resistance mechanisms to *R. indica* in 8 coconut varieties based on morphological (pinnae length and width, leaf thickness, depth of midrib groove, and intervial distance) and biochemical (crude protein, moisture, nitrogen and mineral content) characteristics. The study concluded that there was no relationship between mite populations and the physical (morphological) characteristics of the coconut varieties but the chemical characteristics had an influence on mite densities. Varieties which contained higher amounts of nitrogen and crude protein showed higher incidence and higher mite population densities.

More recently, *R. indica* was reported as the first mite species observed feeding through the stomata of their host plants (Kane et al. 2005). Later it was shown that feeding via stomatal

aperture occurred among several *Raoiella* species (Ochoa et al. 2011). Further studies are needed to understand the implications of the feeding habits of *R. indica*. Through this specialized feeding habit, *R. indica* probably interferes with the photosynthesis and respiration processes of their host plants. Moreover, the type of stomata and their densities could determine the suitability of a plant as host for *R. indica*. For instance, coconut varieties can be divided in two main groups, tall and dwarf varieties. Tall palms may attain heights of 30 m (approximately) while dwarfs have shorter internodes and thus are much shorter (Gomes and Prado 2007). Tall varieties are greatly influenced by wind currents that remove the humidity from the leaf surface and thus have more efficient osmoregulation mechanisms than dwarf varieties (Gomes and Prado 2007). Dwarf varieties have a greater number of stomata per leaf area and lower wax content on the leaf surface compared to the tall varieties (Rajagopal et al. 1990). These differences among coconut palm varieties could be influential on their suitability as hosts for *R. indica* which merits investigation.

Detailed studies are needed to determine the suitability of banana cultivars as hosts of *R. indica*. Cocco and Hoy (2009) reported a poor establishment and reproduction of *R. indica* on leaf disks and young plants of several banana and plantain cultivars. *Raoiella indica* failed to establish on seedlings and young banana plants of the Grand Nain cultivar despite multiple inoculations with mites from field-infested bananas and coconuts (Carrillo, unpublished data). However, high populations of *R. indica* were found infesting banana trees of unknown cultivars in Big Pine Key (Carrillo, pers. obs.) and Lake Worth (Cocco and Hoy 2009) Florida. Further studies are needed to determine why some banana trees can sustain large populations of *R. indica* but others seem to be non-hosts of this mite.

Influence of Abiotic Factors on *Raoiella indica*

Few studies have examined the effects of abiotic factors on *R. indica*. Moutia (1958) reported that population build up was positively related with temperature and sunshine hours, but negatively related with rainfall and relative humidity. In agreement, Nagesha and Channabasavanna (1983b) reported an increase of mite populations associated with periods of lower relative humidity, and Taylor et al. (2011) found that when conditions were hotter and drier, *R. indica* densities were significantly higher. Sakar and Somchoudhury (1989) reported that mite density had a significant positive relation with temperature and that *R. indica* densities declined with the onset of the rainy season in India. The available literature suggests that this mite adapts well to tropical climatic conditions; however, prolonged dry conditions could favor *R. indica* population growth.

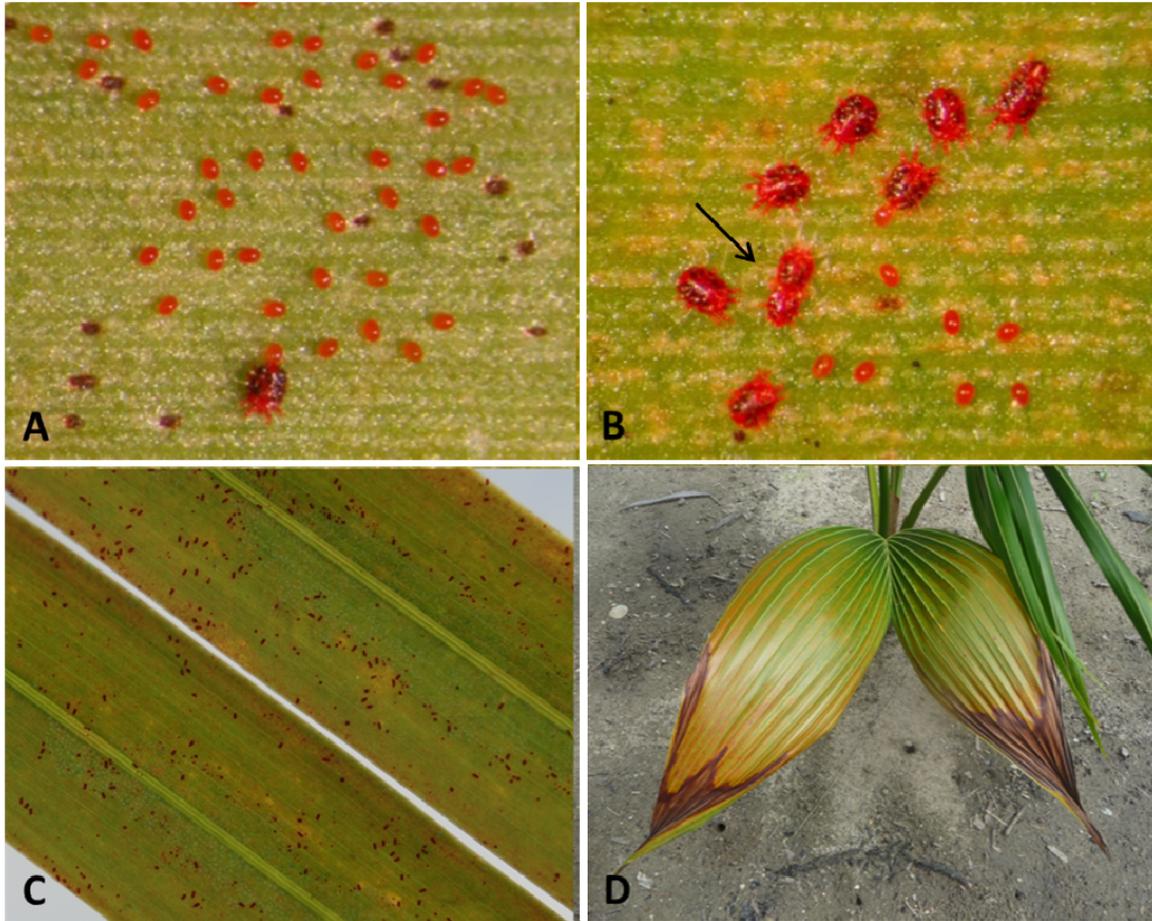


Figure 1-2. *Raiella indica* on coconut. A. Oviposition by *R. indica*. B. *R. indica* colony, the arrow shows a male closely behind a female deutonymph in a “guarding state”. C. *R. indica* colonies on the underside of coconut leaves. D. Damage on coconut leaves caused by *R. indica*.

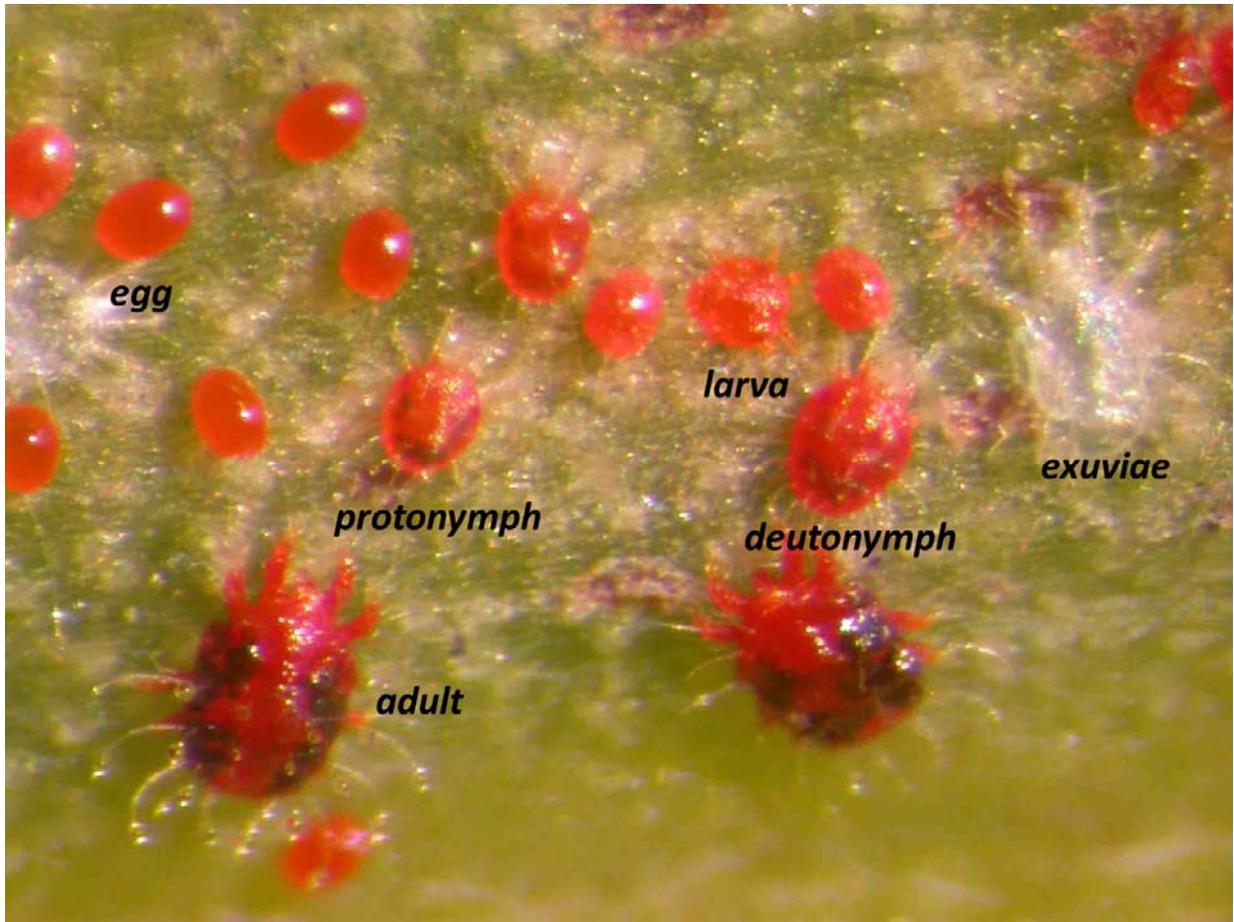


Figure 1-3. *Raiiella indica* forming multigenerational colonies on the abaxial surface of coconut leaflets (pinnae).

CHAPTER 2
A REVIEW OF THE NATURAL ENEMIES OF THE RED PALM MITE, *RAOIELLA INDICA*
(ACARI: TENUIPALPIDAE)¹

Summary

A review of all the available information about the natural enemies reported in association with the red palm mite, *Raoiella indica* is presented. Twenty-eight species of predatory arthropods, including mites and insects, have been reported in association with *R. indica* in Asia, Africa and the Neotropics. The available literature indicates that each site has a different natural enemy complex with only one predator species, *Amblyseius largoensis* (Acari: Phytoseiidae), present in all the geographical areas. The phytoseiids, *Amblyseius caudatus* Berlese, *Amblyseius channabasavanni* Gupta and *A. largoensis*, were regarded as important natural enemies of *R. indica*, and their predatory efficiency studied in some detail. Among the predatory insects the coccinellids *Stethorus keralicus* Kapur and *Telsimia ephippiger* Chapin were reported as major predators of *R. indica*. In addition, pathogenic fungi associated with *R. indica* in the Caribbean have been reported. The known distribution, abundance and relative importance of each species reported in association with *R. indica* are discussed. Key words: *Raoiella indica*, natural enemies, predatory mites, predatory insects, *Amblyseius largoensis*.

Introduction

The red palm mite, *Raoiella indica* Hirst (Acari: Tenuipalpidae), is an invasive phytophagous pest with a large host range of plants within the Arecaceae, Musaceae, Strelitziaceae, Heliconiaceae and Zingiberaceae (Carrillo et al. 2011a). During 2004, *R. indica* was detected in Martinique, and in a few years it spread through the Caribbean and reached North America (United States of America, Mexico) and northern South America (Venezuela,

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Colombia, Brazil), now threatening the entire Neotropical region. The host-plant range and dispersal of *R. indica* throughout natural, agricultural, recreational and residential areas suggest that large-scale mitigation programs are required for managing this species. Chemical control, host-plant resistance and cultural control tactics could be used to manage local populations; however, only biological control has the potential to regulate populations of this species on a large scale. The objective of this review is to analyze all the available information about the natural enemies, within various taxonomic groups, that have been reported in association with *R. indica*.

Natural Enemies of *Raoiella indica*

Altogether 28 species of predatory arthropods, including mites and insects, have been reported in association with *R. indica* in various parts of the world (Table 2-1). In addition, three species of pathogenic fungi found infecting *R. indica* in Puerto Rico have been reported.

Predatory Mites

Sixteen predacious mite species belonging to six families in two orders have been reported.

Phytoseiidae (Mesostigmata). Among the families of predatory mites the Phytoseiidae has the most species reported in association with *R. indica*. Moutia (1958) reported *Amblyseius caudatus* Berlese as the main predator of *R. indica* on coconut in Mauritius. Predation by *A. caudatus* was recorded both in the field and in the laboratory showing a marked preference for *R. indica* eggs, a high numerical response, and population growth coinciding with peak populations of the pest. The species *Amblyseius channabasavanni* Gupta and Daniel was reported by Daniel (1981) feeding upon *R. indica* on Areca (*Areca catechu* L.) leaves in the field and the laboratory. The author determined the biology and habits of *A. channabasavanni* preying on *R. indica* infesting Areca leaves in Kerala, India. Daniel (1981) referred to this species as a potentially

effective predator because of its short generation time relative to that of the pest. In the field *A. channabasavanni* densities peaked coinciding with *R. indica* highest populations. When *R. indica* was not present *A. channabasavanni* were able to survive on alternate food sources including *Tetranychus fijiensis* Hirst (Acari: Tetranychidae), eggs and crawlers of scale insects and mealybugs.

According to the available literature, *A. caudatus* and *A. channabasavanni* were promising natural enemies of *R. indica* in the Old World. After *R. indica* gained importance as an invasive pest in the New World, surveys for natural enemies were conducted in Mauritius, India, Tanzania and Benin (Zannou et al. 2010; Bowman and Hoy 2011; Taylor et al. 2011). These surveys did not encounter *A. channabasavanni* nor *A. caudatus*, but rather predatory mites identified as *Amblyseius largoensis* Muma. *Amblyseius largoensis* was also found on coconut palms infested with *R. indica* in the Phillipines (Gallego et al. 2003). In the New World, the association between *R. indica* and *A. largoensis* was noticed soon after the pest was found in the Caribbean and Florida (USA) (Peña et al. 2009). This species was the most abundant predator associated with *R. indica* on coconut leaves in Trinidad and Tobago and Puerto Rico. In both sites, populations of *A. largoensis* increased in response to the arrival of *R. indica*. In Florida, *A. largoensis* was the most abundant predator associated with *R. indica*, and the only one found continuously throughout the year. The interaction between *A. largoensis* and *R. indica* in Florida was further investigated (Carrillo et al. 2010; Carrillo and Peña 2011, see also Chapters 3 and 4, respectively). An initial study demonstrated that *A. largoensis* was able to feed, develop and reproduce on a diet consisting solely of *R. indica* (Figures 2-1 and 2-2). The intrinsic rate of natural increase of the predator was significantly higher when fed on *R. indica* than on other diets, including *Tetranychus gloveri* Banks (Acari: Tetranychidae), *Aonidiella orientalis*

(Newstead) (Hemiptera: Diaspididae), *Nipaecoccus nipae* (Maskell) (Hemiptera: Pseudococcidae) and live oak (*Quercus virginiana* Mill.) pollen. Further studies showed that *A. largoensis* had a marked preference for *R. indica* eggs over other stages of the pest. In addition, the predator showed a Type II functional response and a positive numerical response to increasing densities of *R. indica* which could explain the population increase observed in areas of recent invasion. *Amblyseius largoensis* was also recorded as the only phytoseiid species in association with *R. indica* in Cuba and Colombia (Ramos et al. 2010, Carrillo et al. 2011b).

The species *Neoseiulus longispinosus* Evans was regarded as a potential predator of a large number of mite pests (Gupta 1998, 2001). The author reported an association between this predator and *R. indica*, citing a study conducted by Nangia and ChannaBasavanna (1989) in Karnataka, India. However, that study was conducted with the species *Typhlodromips tetranchivorus* Gupta, not with *N. longispinosus*. It is unclear whether predation by *N. longispinosus* upon *R. indica* has ever been recorded in the Eastern Hemisphere. Nevertheless, *N. longispinosus*, which has primarily an Asian distribution, was recorded in Martinique (Moraes et al. 2000), the same region where *R. indica* was first found in the Caribbean a few years later. Interestingly, *N. longispinosus* was observed feeding on *R. indica* in the field in Saint Lucia (Ochoa, pers. comm.). The predator is found in other islands of the Caribbean (Hastie et al. 2010) and its potential as a biological control agent of *R. indica* should be further investigated.

Other phytoseiid mites reported in association with *R. indica* include *T. tetranchivorus*, *Amblyseius raoiellus*, and two unspecified species in the genera *Amblyseius* and *Phytoseius*. The life history of *T. tetranchivorus* was studied in the laboratory using *R. indica* as a host (Jagadish and Nageshachandra 1981; Nangia and ChannaBasavanna 1989). The predator was able to develop and reproduce feeding exclusively on *R. indica*, showing a preference to feed on eggs.

However, no records of association of the two species are available despite the fact that both are found in Karnataka, India (Chinnamade-Gowda and Mallik 2010) (Table 2-1). In contrast, *A. raoiellus* was reported preying on *R. indica* in the same region (Denmark and Muma 1989). Finally, populations of *Amblyseius* sp. and *Phytoseius* sp. were reported in West Bengal (India) having negative and positive correlations with the populations of *R. indica*, respectively (Somchoudhury and Sarkar 1987). No further information is available for either species about their association with *R. indica*.

Ascidae (Mesostigmata). The feeding potential of *Lasioseius* sp. upon all stages of *R. indica* was determined in the laboratory (Sheeja and Ramani 2009). These authors reported that all stages of *Lasioseius* sp. preyed on all stages of *R. indica*. However, there are no reports of association of *Lasioseius* sp. and *R. indica* under natural conditions in Karnataka (India). This predator is naturally associated with the species *Aceria ailanthae* Mohanasundaram (Acari: Eriophyidae), which feeds on *Euodia lunu-ankenda* (Rutaceae) plants (Sheeja and Ramani 2009). Further studies are necessary to substantiate the potential of this species as a biological control agent of *R. indica*.

Bdellidae (Trombidiformes). Two snout mite species, *Bdella* sp. and *Bdella distincta* (Barker and Bullock), were reported in association with *R. indica* in Trinidad and in Florida, respectively (Peña et al. 2009). Further investigations in Florida found *B. distincta* feeding on *R. indica* (Figure 2-3), but also upon *A. largoensis* eggs (Carrillo, pers. obs.). The bdellid from Trinidad was also observed feeding on *A. largoensis* (Roda, pers. comm.). The low abundance observed in the field, and the intraguild-predation upon *A. largoensis*, makes these Bdellidae species unlikely to be promising biocontrol agents of *R. indica*.

Cheyletidae (Trombidiformes). Three Cheyletidae species have been observed associated with *R. indica* in areas of recent invasion by this pest. *Cheletomimus* sp. and *Mexecheles* sp. were found in association with *R. indica* in Trinidad (Peña et al. 2009; Welbourn, pers. comm.). The species *Hemicheyletia bakeri* Ehara was observed feeding upon all stages of *R. indica* and *A. largoensis* in Florida (Carrillo, pers. obs.). According to Muma (1975), *H. bakeri* feeds and reproduces readily on various tetranychid, phytoseiid and acarid mites found on citrus plants in Florida, and is relatively common during winter and spring.

Cunaxidae (Trombidiformes). The species *Armascirus taurus* Kramer was reported as a predator of *R. indica* infesting coconut palms in Camiguin, northern Mindanao, Philippines (Gallego et al. 2003); however, no further information is available regarding this species and its relationship with *R. indica*.

Xenocaligonellidae (Trombidiformes). *Xenocaligonellidus* sp. was observed once in association with *R. indica* in Trinidad and Tobago (Welbourn, pers. comm.). No additional information is available to substantiate its potential as biocontrol agent of *R. indica*.

Predatory Insects

Altogether 12 predacious insect species belonging to five families in four orders have been reported in association with *R. indica* (Table 2-1).

Coccinellidae (Coleoptera): Several species belonging to the genus *Stethorus* Weise, composed of specialist mite predators (Biddinger et al. 2009), have been reported in association with *R. indica*. The species *Stethorus keralicus* Kapur was described from specimens collected on areca palm leaves infested with *R. indica* (Kapur 1961). Laboratory studies revealed that both their larval and adult stages fed in large quantities upon all stages of *R. indica* (Puttaswamy and Rangaswamy 1976). Predators also showed a high reproductive potential and were present throughout the year on coconut and areca palms. Daniel (1981) considered *S. keralicus* the most

important predator of *R. indica* in Kerala, India. The predator was also reported feeding on *Raoiella macfarlanei* Pritchard and Baker (Acari: Tenuipalpidae) infesting roseapple (*Syzygium jambos* L.) (Nageshachandra and Channabasavanna 1983a). The authors suggested that *S. keralicus* could be specific on mites of the genus *Raoiella*.

Apart from *S. keralicus*, three other *Stethorus* species have been recorded associated with *R. indica* on Areca plants in the state of Karnataka. *Stethorus tetranychii* Kapur and *Stethorus parcampunctatus* Kapur were recorded in the region of Mysore, whereas *Stethorus pauperculus* Weise was found in Shimoga (Puttarudriah and ChannaBassavana 1956; Yadav-Babu and Manjunatha 2007). However, feeding upon *R. indica* was observed only in *S. tetranychii* and no other information is available for the other species indicating their potential as biological control agents.

Because of the reports of *Stethorus* spp. preying on *R. indica* in India, the potential of a Florida native species, *Stethorus utilis* Horn, was addressed. Before *R. indica* arrived in Florida *S. utilis* was reported as a common predator found associated with *Tetranychus gloveri* Banks (Acari: Tetranychidae) and other spider mite species on coconut leaves (Peña et al. 2009). A simple bioassay using field-collected adult beetles was designed to determine whether *S. utilis* could feed on *R. indica* (Carrillo, unpublished data). Twenty adult beetles were placed individually in Petri dishes and starved for 8 hrs before the beginning of the bioassay. The feeding test was conducted under no-choice conditions by introducing coconut leaf rectangles infested with *R. indica* in half of the petri dishes and with *T. gloveri* in the other half. A few minutes after introducing the prey items, predators were actively preying upon *T. gloveri* but not on *R. indica*. The situation did not change over time. While most predators offered *T. gloveri* consumed all their prey and oviposited on the coconut leaves, those offered *R. indica* spent most

of their time wandering on the petri dish walls, refused to feed on *R. indica*, and ultimately died of starvation after approximately 48 hr. A similar assessment was used to test feeding on *R. indica* by *Stethorus punctillum* Weise, a species that is mass-produced and commercially available. Adult beetles provided by the United States Department of Agriculture (USDA)-National Biological Control Laboratory (Stoneville, Mississippi, USA) showed a similar response when offered *R. indica* and *T. gloveri*. The lack of feeding on *R. indica* by *S. punctillum* was surprising because this species is known to feed upon various food items in absence of their common tetranychid mite prey (Biddinger et al. 2009). However, results of feeding tests on *S. utilis* and *S. punctillum* suggest they are not promising biocontrol agents of *R. indica*.

Apart from *Stethorus*, three Coccinellidae species have also been recorded in association with *R. indica*. *Telsimia ephippiger* Chapin was found preying on *R. indica* on coconut leaves in the Phillipines (Gallego et al. 2003). Further studies determined that this species could complete its life cycle and reproduce feeding exclusively on *R. indica*, showing high consumption rates (Gallego and Batomalaque-Galazar 2004). *Jauravia soror* (Weise) and *Chilorus cacti* L. were collected from *R. indica*-infested areca palms in Mysore (Karnataka, India) and coconut palms in Florida, respectively (Puttarudriah and ChannaBassavana 1956; Peña, pers. obs.). However, there is no further information available to substantiate their importance as predators of *R. indica*.

Staphylinidae (Coleoptera): Rove beetles belonging to the genus *Oligota* have been reported in association with *R. indica* in India. Somchoudhury and Sarkar (1987) reported *Oligota* sp. as the dominant predator of *R. indica* on coconut in West Bengal, India, where predator-prey populations showed a positive correlation. In Shimoga, Karnataka, *Oligota* sp. was found associated with peak population of *R. indica* on Arecas (Yadav-Babu and Manjunatha

2007). Additional sampling to identify the rove beetle species observed in association with *R. indica* is required to explore their potential as biocontrol agents of this pest.

Chrysopidae (Neuroptera): Peña et al. (2009) reported Chrysopidae species associated with *R. indica* in Trinidad and Tobago, Puerto Rico, and Florida on coconut palms. Follow-up studies in Florida that involved collecting and rearing lacewing larvae observed feeding on *R. indica* in coconut fields, identified two lacewing species, *Ceraeochrysa claveri* (Navás) and *Chrysopodes collaris* (Scheider) (Carrillo unpublished data). *Ceraeochrysa claveri* was more common and repeatedly found feeding upon *R. indica* and ovipositing on infested coconut leaves (Figure 2-4). Studies on the development and reproduction of *C. claveri* feeding on three phytophagous arthropods commonly found inhabiting coconuts in Florida [*R. indica*, *Nipaeococcus nipae* Maskell (Hemiptera: Pseudococcidae) and *Aonidiella orientalis* (Newstead) (Hemiptera: Diaspididae)] showed these lacewings active preyed upon *R. indica* during the first two instars and had similar developmental times compared to that observed with the two other diets (Figure 2-4 C and D). Carrillo (unpublished data) also observed that third-instar development, survivorship and reproduction by adults reared on the *R. indica* diet were poor compared to these attributes for lacewings reared on the other two diets. Results suggested that *C. claveri* can use *R. indica* as an alternate prey, especially during the first two instars, but it depends on the presence of larger prey to complete its life cycle and reproduce successfully.

Cecidomyiidae (Diptera): Predacious midge larvae have occasionally been observed feeding on *R. indica* in Trinidad and Tobago and Florida. Few larvae were reared into adults on a *R. indica* diet in Florida (Figure 2-5). Two females were obtained and identified as *Arthrocnodax* sp. (by Dr. R. J. Gagné, USDA Systematic Entomology Laboratory, Washington D.C.), a cosmopolitan genus of about 50 species known to feed mainly on the Eriophyidae (Gagné 2004).

Efforts will continue to complete the identification of the species for which males are needed; however, their relatively low abundance in the field makes them unlikely to be promising mortality factors of *R. indica*.

Phlaeothripidae (Thysanoptera): The predatory thrips *Aleurodothrips fasciapennis* (Franklin) was reported in association with *R. indica* in Trinidad and Tobago and Florida (Peña et al. 2009). The available literature on this species (Watson et al. 1998, 2000), and results from the surveys in Trinidad, Puerto Rico and Florida suggest that the life history of *A. fasciapennis* depends on diaspidid insect prey (Figure 2-6). This predator has been occasionally observed feeding upon *R. indica* but so far has not shown potential as a biological control agent of this pest.

Pathogens

There are no studies known of pathogens associated or infecting *R. indica* in the literature. Pronounced reductions of the pest were observed in various sites in Puerto Rico (Rodrigues and Colon, unpublished). The reduction coincided with rainfall increases and consequent higher moisture, which leads to the suggestion that rainfall could directly play an important role on the mite population density. Similar understandings were reported in Mauritius (Moutia 1958). However, a close look on the mites showed that great numbers were dying from an infection. In order to isolate the potential infectious microorganisms associated with the mite populations, individual mites showing symptoms of infection were placed in Petri dishes with selective media for isolation of bacteria, fungi and actinomycetes. Isolated fungi were morphologically identified and had a fragment of ITS gene sequenced (Rodrigues and Colon, unpublished). Four isolates of fungi were found to be pathogenic to the mites. Three species, *Simplicillium* sp., *Lecanicillium lecanii*, and *Hirsutella thompsonii*, were isolated and identified. Studies were carried out in leaf

arenas and in greenhouse conditions to demonstrate infection of *R. indica* (Rodrigues and Colon, unpublished).

Discussion

Altogether 28 species of predators have been reported in association with *R. indica* in various regions of Asia, Africa, the Caribbean basin, and North and South America (Table 2-1). Most records were made in India, with 13 species from the southwestern states of Karnataka, Kerala, and fewer from West Bengal. Apart from India, the only other reports from Asia were from the Philippines. In Africa, two species were reported in Mauritius and one in Benin and Tanzania. In the New World five predatory species have been recorded in the Caribbean (Trinidad, Puerto Rico, Cuba and St. Lucia), nine species in Florida (USA), and one both in Colombia and Mexico. According to the available literature, each site has a different natural enemy complex with only one predator species, *A. largoensis*, present in all the geographical areas.

Of the sixteen predatory mites species recorded in association with *R. indica* in various parts of the world, only nine were observed feeding on *R. indica* in the field. Within those eight species, only three Phytoseiidae species have been studied in some detail, including *A. caudatus*, *A. channabasavanni* and *A. largoensis*. Bioassays conducted with these species determined that they were able to complete development and reproduce when feeding solely on *R. indica* (Moutia 1958; Daniel 1981; Carrillo et al. 2010, chapter 3). The three *Amblyseius* species showed preference for eggs over other developmental stages of the pest; in addition, *A. channabasavanni* also preferred quiescent larvae and nymphs. The three species have shorter developmental times (approximately one week) than *R. indica* and a positive numerical response to increasing populations of the pest. Until now there is no information suggesting that the other Phytoseiidae species found associated with *R. indica* could play an important role regulating this

pest. The predatory mites of the families Bdellidae, Cheyletidae and Cunixidae were found at significantly lower densities and sometimes preying on phytoseiids. There is no information suggesting that they could be of importance as biological control agents of *R. indica*. The available evidence suggests that within the Phytoseiidae *A. caudatus*, *A. channabassavani* and *A. largoensis* have the most potential as biological control agents of *R. indica*. The first two species were regarded as major predators of *R. indica* in Mauritius and the state of Kerala (India), respectively. However, no other records are available of the association between these predators and *R. indica* since they were reported in 1958 and 1981, respectively. Since then, *R. indica* gained importance as an invasive pest in the Neotropics and surveys for natural enemies were conducted in several places of the world (Roda et al. 2008; Peña et al. 2009; Ramos et al. 2010; Zannou et al. 2010; Carrillo et al. 2011b) including Mauritius and Kerala (Bowman and Hoy 2011; Taylor et al. 2011). Through these surveys, *A. largoensis* was identified as the most abundant predator and often as the only phytoseiid species associated with *R. indica*. With the exception of a single observation of *N. longispinosus* feeding on *R. indica* recorded by Ochoa in Saint Lucia, to this day no other phytoseiid mite has been reported feeding upon *R. indica* in the multiple surveys.

This particular situation raises the question of whether *A. largoensis* is truly a single species or a group of morphologically similar species. The species *A. largoensis* was first described from specimens collected in Key Largo, Florida by Muma (1955) under the combination *Amblyseiopsis largoensis*, and later moved to the genus *Amblyseius*. However, *A. largoensis* has a cosmopolitan distribution (McMurtry and Moraes 1984; Moraes et al. 2004). This species is part of a group of nine closely related species referred collectively as the Largoensis group (McMurtry and Moraes 1984). Except for *A. largoensis*, *A. herbicolus* and *A.*

eharai, the other six species of the group have been recorded only in the Australian biogeographic realm, suggesting that the *largoensis* group originated there (McMurtry and Moraes 1984). The authors hypothesized that *A. largoensis* was dispersed through movement of plant material. Observations made in Florida suggest that *A. largoensis* has an important ability to colonize various plants. For instance, in surveys conducted at the Fairchild Tropical Botanic Garden in Florida *R. indica* was found on 36 palm species and *A. largoensis* was found on half of them (Appendix). This predator was also repeatedly reported on citrus and other plants (Muma 1955; Muma 1975; Daneshvar 1980; Galvao et al. 2007). It has been hypothesized that the classification of *A. largoensis*, based on morphological characters, could obscure the fact that these are multiple cryptic species. Using molecular techniques Bowman (2010) compared populations of *A. largoensis* from Florida and Mauritius. Their analysis found differences between the populations but was not conclusive as to whether the Florida and Mauritius populations were biotypes or cryptic species of *A. largoensis*. Therefore, it should be determined whether all the ‘species’ identified as *A. largoensis* are equally efficient natural enemies of *R. indica*. Only two studies have addressed the efficiency of *A. largoensis*. In an initial approach Peña et al. (2009) concluded that *A. largoensis* was unable to suppress the large populations of *R. indica* observed in Trinidad and Puerto Rico during the early stages of the invasion by this pest. In Florida, the functional and numerical responses of the *A. largoensis* to *R. indica* were determined in the laboratory and their efficacy tested using exclusion and release techniques (see chapter 6). Results of those studies suggested that *A. largoensis* could be efficient at regulating low prey population densities. It will be important to design strategies that can allow realistic comparisons of the efficiency of predators of *R. indica* in various parts of the world. While these questions remain unresolved, it is clear that predatory mites identified as *A. largoensis* have

shown a conspicuous association with *R. indica* and represent the most important biotic factor known to be acting over *R. indica* in the different places where this pest is present.

Among the 12 predatory insects reported in association with *R. indica* the family Coccinellidae is represented by 7 species. Four *Stethorus* species were reported in the regions of Karnataka and Kerala in India. Among these the species *S. keralicus* showed great potential and was reported as a voracious predator of all stages of *R. indica*, was found throughout the year and showed a degree of specificity towards *Raoiella* species. However, the latest record of the association between the two species was made in 1976 despite the increasing interest in identifying possible classical biological control agents for *R. indica*. There is no information available regarding the other three *Stethorus* spp. reported in association with *R. indica*. Simple bioassays, such as those conducted in Florida with *S. utilis* and *S. punctillum*, could be useful to determine whether they feed on *R. indica*. Studies on *S. keralicus* and other *Stethorus* species previously reported in association with *R. indica* are needed as they could represent an important tool in managing this invasive species in the Neotropics and other areas of the world. Apart from *Stethorus*, the other ladybeetle species showing some potential as a biological control agent of *R. indica* is *T. ephippiger*. The species was regarded as a voracious predator of *R. indica* in the Philippines (Gallego et al. 2003). The authors reported *T. ephippiger* as predator of *Rarosiella cocosae* Rimando, a synonym of *R. indica* (Mesa et al. 2009), which could create confusion for researchers interested in the literature about natural enemies of *R. indica*. There is no information suggesting that the other two coccinellids (*J. soror* and *C. cacti*) could be important as biological control agents of *R. indica*. The predacious beetle *Oligota* sp. was regarded as an important predator of *R. indica* in West Bengal (India) (Somchoudhury and Sakar 1987). The genus *Oligota* contains multiple species that are specialist mite predators (Frank et al. 1992). It would

be useful to investigate the occurrence of *Oligota* species in West Bengal and in other areas where *R. indica* is present. The other insect predators include predacious lacewings, thrips, and midges. All of them were reported in areas of recent invasion in the Neotropics and were observed feeding on *R. indica*. However, the available evidence suggests that their life history is linked to other prey. Their prey preferences and low abundance observed in the field make these species unlikely to be promising biocontrol agents of *R. indica*.

The observation and isolation of arthropod-pathogens associated with *R. indica* is quite recent (Rodrigues and Colon, unpublished). Four acaropathogenic fungi were reported associated with epizootics in *R. indica* populations in Puerto Rico. Fungi could be particularly important in reducing mite densities in humid regions, which are mostly found in coconut and banana-growing areas in Central America, the Caribbean and parts of South America. These pathogens, rather than direct impact of the rain as suggested by Moutia (1958), could be the cause of significant reductions in mite population densities observed in some sites in Puerto Rico (Rodrigues et al. unpublished). Further studies to evaluate the efficiency of single or complex species of pathogens to control *R. indica* should be undertaken to determine their potential use in commercial settings.

In conclusion, the available literature indicates that *A. largoensis* is the most abundant predator of *R. indica* where this pest is present. It will be important to determine which populations of *A. largoensis* are more efficient in preying upon *R. indica* and why other phytoseiid species, such as *A. caudatus* and *A. channabasavanni*, have not been recorded in recent surveys for natural enemies. Due to the marked preference that *A. largoensis* exhibits for *R. indica* eggs, it would be desirable to find natural enemies that could prey on or parasitize other stages of the pest. For instance, the recent finding of acaropathogenic fungi attacking *R. indica* in

Puerto Rico could be very useful for managing this pest (Rodrigues et al. unpublished). At a local level some predatory species (*S. keralicus* and *T. ephippiger*) were reported as important predators but remain unexplored as potential biological control agents of *R. indica*. It is likely that other mortality factors will be required to effectively suppress the populations of this invasive pest. Search for effective natural enemies should be intensified and ways to improve the levels of control by the existing natural enemies (i.e. provision of alternative food sources) should be further investigated.

Table 2-1. Predatory arthropods reported in association with *R. indica*.

Scientific name (synonyms)	Order: Family	Place of report	Reference
<i>Amblyseius caudatus</i> Berlese 1914 (= <i>Typhlodromus caudatus</i>)	(Acari: Phytoseiidae)	Mauritius	Moutia 1958
<i>Amblyseius channabasavanni</i> Gupta & Daniel (= <i>Amblyseius channabasavannai</i>)	(Acari: Phytoseiidae)	Karnataka and Kerala, India	Daniel 1981, Gupta 2001
<i>Amblyseius largoensis</i> (Muma 1955)	(Acari: Phytoseiidae)	Benin; Tanzania Mauritius; Kerala, India; Philippines; Trinidad; Puerto Rico; Florida, USA; Colombia;Cuba	Zannou et al. 2010 Bowman and Hoy 2011 Taylor et al. 2011 Gallego et al. 2003 Peña et al. 2009; Roda et al. 2008 Peña et al. 2009 Carrillo et al. 2011b; Ramos et al. 2010
<i>Amblyseius raoiellus</i> Denmark & Muma 1989	(Acari: Phytoseiidae)	Karnataka, India	Denmark and Muma 1989
<i>Neoseiulus longispinosus</i> (Evans 1952) (= <i>A. longispinosus</i>)	(Acari: Phytoseiidae)	St. Lucia	Roda et al. 2008
<i>Typhlodromips tetranychivorus</i> Gupta 1978 (= <i>Amblyseius tetranychivorus</i> = <i>Transeius tetranychivorus</i>)	(Acari: Phytoseiidae)	Bangalore and Karnataka, India	Jagadish and Nageshachandra 1981, Nangia and Channabasavanna 1989
<i>Amblyseius</i> sp.	(Acari: Phytoseiidae)	West Bengal, India	Somchoudhury and Sakar 1987
<i>Phytoseius</i> sp.	(Acari: Phytoseiidae)	West Bengal, India	Somchoudhury and Sakar 1987
<i>Lasioseius</i> sp.	(Acari: Ascidae)	Karnataka, India	Sheeja and Ramani 2009
<i>Bdella distincta</i> (Barker and Bullock)	(Acari: Bdellidae)	Florida, USA	Peña et al. 2009
<i>Bdella</i> sp.	(Acari: Bdellidae)	Trinidad	Roda et al. 2008; Peña et al. 2009
<i>Cheletomimus</i> sp.	(Acari: Cheyletidae)	Trinidad	Roda et al. 2008; Peña et al. 2009
<i>Hemicheyletia bakeri</i> (Ehara)	(Acari: Cheyletidae)	Florida, USA	Carrillo, pers. obs.
<i>Mexecheles</i> sp.	(Acari: Cheyletidae)	Trinidad	Welbourn, pers. obs.
<i>Armascirus taurus</i> Kramer	(Acari: Cunaxidae)	Philippines	Gallego et al. 2003
<i>Xenocaligonellidus</i> sp.	(Acari: Xenocaligonellidae)	Trinidad	Welbourn, pers. obs.
<i>Stethorus keralicus</i> Kapur	(Coleoptera: Coccinellidae)	Kerala, India	Kapur 1961; Puttaswamy and Rangaswamy. 1976
<i>Stethorus parcempunctatus</i> Kapur	(Coleoptera: Coccinellidae)	Karnataka, India	Puttarudriah and ChannaBassavana 1956
<i>Stethorus pauperculus</i> Weise	(Coleoptera: Coccinellidae)	Karnataka, India	Yadav-Babu and Manjunatha 2007
<i>Stethorus tetranychchi</i> Kapur	(Coleoptera: Coccinellidae)	Kerala, India	Puttarudriah and ChannaBassavana 1956

Table 2-1. Continuation

Scientific name	Order: Family	Place of report	Reference
<i>Jauravia soror</i> (Weise)	(Coleoptera: Coccinellidae)	Karnataka, India	Puttarudriah and ChannaBassavana 1956
<i>Telsimia ephippiger</i> Chapin	(Coleoptera: Coccinellidae)	Phillipines	Gallego et al. 2003
<i>Chilorus cacti</i> L.	(Coleoptera: Coccinellidae)	Florida, USA	Peña, pers. obs.
<i>Oligota</i> sp.	(Coleoptera: Staphylinidae)	Karnataka and West Bengal, India	Yadav-Babu and Manjunatha 2007; Somchoudhury and Sakar 1987
<i>Ceraeochrysa claveri</i> (Navás)	(Neuroptera: Chrysopiidae)	Florida, USA	Carrillo, pers. obs.
<i>Chrysopodes collaris</i> (Scheider)	(Neuroptera: Chrysopiidae)	Florida, USA	Carrillo, pers. obs.
<i>Aleurodothrips fasciapennis</i> (Franklin 1908)	(Thysanoptera: Phlaeothripidae)	Florida, USA; Trinidad	Peña et al. 2009; Roda et al. 2008
<i>Arthrocnodax</i> sp.	(Diptera: Cecidomyiidae)	Florida, USA	Carrillo, pers. obs.

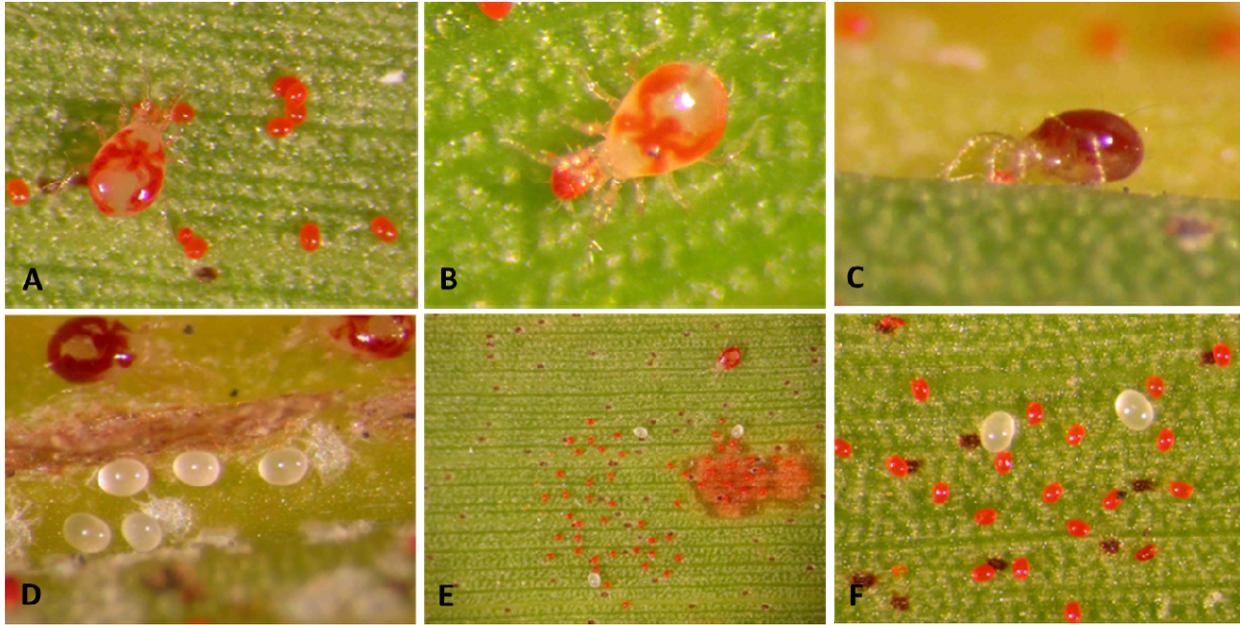


Figure 2-1. *Amblyseius largoensis* feeding upon *R. indica* and ovipositing on infested coconut leaves in Florida. A. *A. largoensis* female feeding on *R. indica* eggs. B. *A. largoensis* female feeding on *R. indica* larva, dorsal view. C. *A. largoensis* female feeding on *R. indica*, lateral view. D. *A. largoensis* eggs on the midvein of a coconut pinna. E. *A. largoensis* oviposition near *R. indica* eggs. F. *A. largoensis* and *R. indica* eggs.

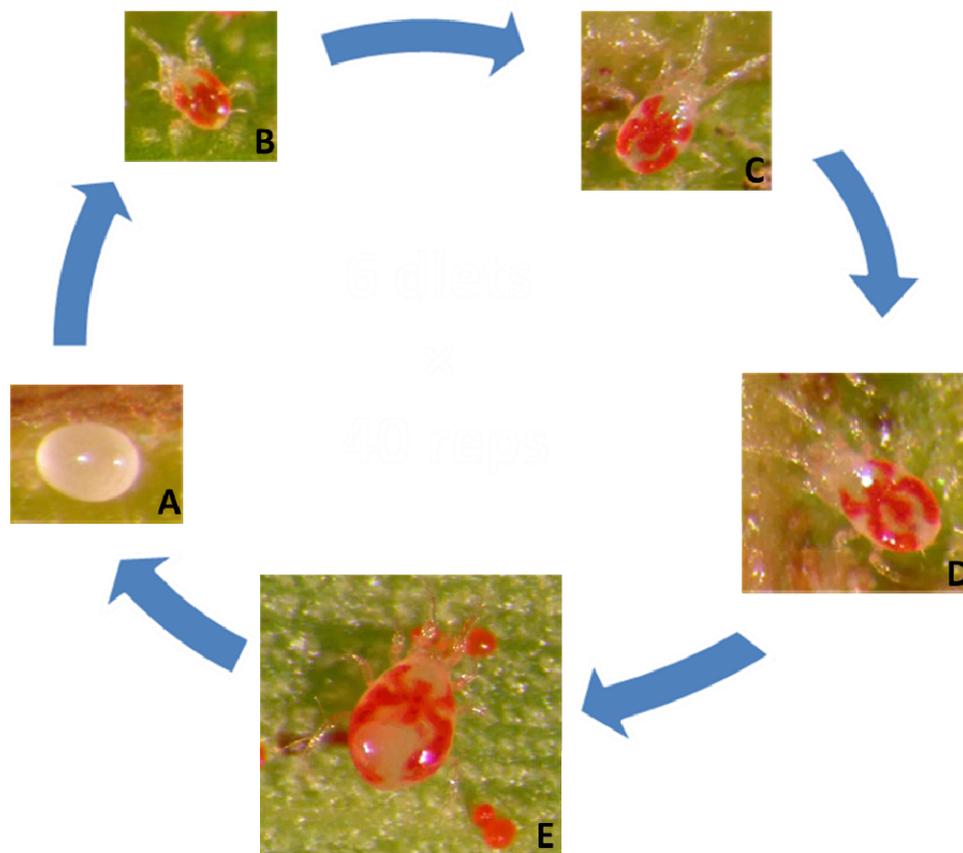


Figure 2-2. Life cycle of *A. largoensis* feeding upon *R. indica* in Florida. A. Egg. B. Larva C. Protonymph D. Deutonymph. E. Adult female.



Figure 2-3. *Bdella distincta* found feeding upon *R. indica* in Florida.



Figure 2-4. *Ceraeochrysa claveri* feeding upon *R. indica* and ovipositing on infested coconut leaves in Florida. A. *C. claveri* eggs B. *C. claveri* neonate larvae C. *C. claveri* first instar larva feeding on *R. indica* nymphs D. *C. claveri* second instar larvae feeding on *R. indica*.

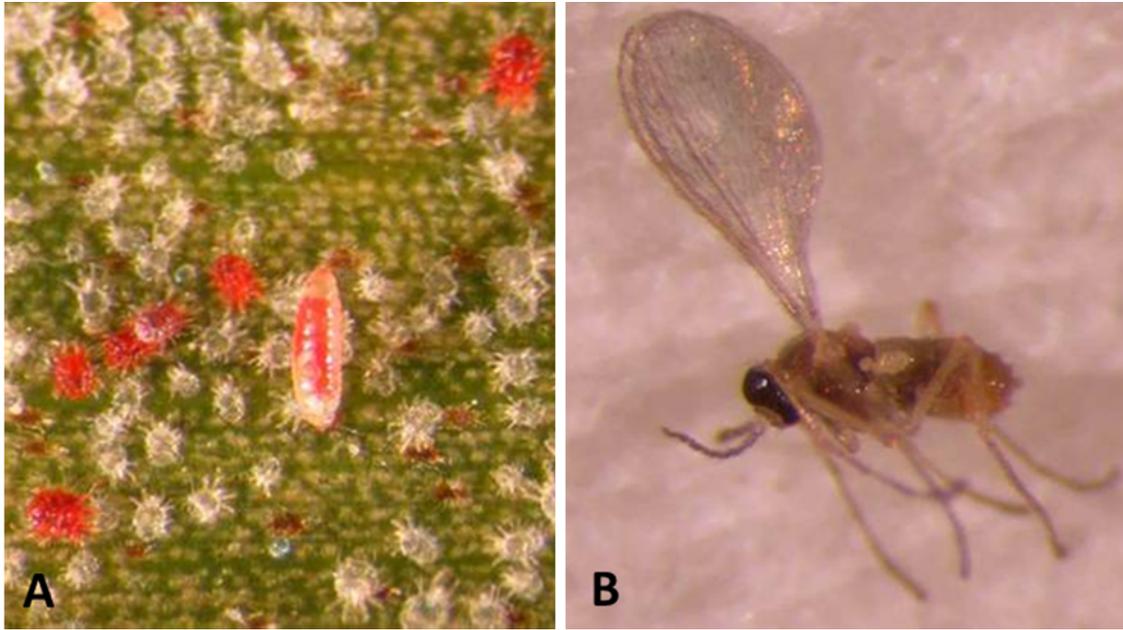


Figure 2-5. *Arthrocnodax* sp. observed feeding on *R. indica* in Florida. A. Predacious midge larvae B. Adult female.



Figure 2-6. *Aleurodothrips fasciapennis* observed feeding upon *R. indica* in Florida. A. Adult in lateral view. B. *A. fasciapennis* feeding upon *R. indica* C. *A. fasciapennis* feeding upon on diaspidid insect prey.

CHAPTER 3
DEVELOPMENT AND REPRODUCTION OF *AMBLYSEIUS LARGOENSIS* (ACARI:
PHYTOSEIIDAE) FEEDING ON POLLEN, *RAOIELLA INDICA* (ACARI:
TENUIPALPIDAE), AND OTHER MICROARTHROPODS INHABITING COCONUTS IN
FLORIDA²

Summary

The red palm mite, *Raoiella indica* (Acari: Tenuipalpidae), is an important pest of palms (Arecaceae) and other species within the Zingiberaceae, Musaceae and Strelitziaceae families. *Raoiella indica* was discovered in the United States of America (Palm Beach and Broward counties, Florida) late in 2007, and it subsequently spread to other Florida counties. The predatory mite *Amblyseius largoensis* (Acari: Phytoseiidae) has been found associated with *R. indica* in Florida. In order to verify whether *A. largoensis* can develop and reproduce when feeding exclusively on *R. indica*, the biology of this predator was evaluated on contrasting food sources, including *R. indica*. Five diets [*R. indica*, *Tetranychus gloveri*, *Aonidiella orientalis*, *Nipaecoccus nipae*, oak (*Quercus virginiana*) pollen] and a no-food control were tested to determine the predator's development, survivorship, oviposition rate, sex ratio and longevity at $26.5 \pm 1^\circ\text{C}$, RH $70 \pm 5\%$ under a 12:12 L:D photophase. *Amblyseius largoensis* was able to complete its life cycle and reproduce when fed exclusively on *R. indica*. The development of immature stages of *A. largoensis* was faster and fecundity and survivorship were higher when fed on *R. indica* or *T. gloveri* compared to the other food sources. The intrinsic rate of natural increase of *A. largoensis* was significantly higher when fed on *R. indica* than on other diets. These results suggest that, despite earlier assessments, *A. largoensis* can play a role in controlling *R. indica*. Key words: mites, invasive species, biological control, *Cocos nucifera*, *Raoiella indica*, *Amblyseius largoensis*.

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Introduction

Raoiella indica Hirst (Acari: Tenuipalpidae) is an important pest of coconut, *Cocos nucifera* L. (Hirst 1924), areca palm, *Dyopsis lutescens* (H.Wendland) (Kane et al. 2005), date palm, *Phoenix dactylifera* L. (Sayed 1942; Zaher et al. 1969), canary palm, *Phoenix canariensis* hort. ex Chabaud (Etienne and Flechtmann 2006), other species within the Areaceae, bananas and plantains (*Musa* spp.) (Kane et al. 2005; Etienne and Flechtmann 2006) within the Musaceae, gingers *Zingiber* spp. (Peña et al. 2006) within the Zingiberaceae, and bird of paradise, *Strelitzia* spp. (Etienne and Flechtmann 2006), within the Strelitziaceae. Until recently, this mite was found only in the Eastern Hemisphere, probably widespread throughout tropical and subtropical regions (Nageshachandra and Channabasavanna 1983b) of India, the Philippines, Mauritius, Reunion, Malaysia, Israel and Egypt. During 2004, *R. indica* was detected in Martinique and St. Lucia in the Western Hemisphere, and rapidly expanded its geographical range to other Caribbean islands, where high populations have severely affected the coconut, ornamental palm and banana industries (Kane et al. 2005; Etienne and Flechtmann 2006; Rodrigues et al. 2007). More recently, *R. indica* was discovered in North and South America; an infestation was reported in the state of Sucre in Venezuela in 2007 (Gutiérrez et al. 2007; Vásquez et al. 2008), and in December of the same year it was detected in the West Palm Beach area of south Florida (FDACS 2007). Since then, *R. indica* has spread to six counties in Florida, being detected in at least 439 individual sites on 25 host plant species (Welbourn 2007). Populations of this tenuipalpid have increased dramatically, sometimes reaching 4,000 individuals per leaflet on coconuts (Peña et al. 2009), the most common host plant in Florida. On coconuts, *R. indica* is found in very large numbers on palm fronds with all stages located on the abaxial surface of the leaflets (pinnae). Recent studies indicate that some Florida native palms, such as the saw palmetto, cabbage palm, dwarf palmetto, and sable palmetto, are unsuitable hosts

for *R. indica* (Cocco and Hoy 2009; Amalin, pers. comm.). Nevertheless, *R. indica* has been found attacking the endangered native Florida thatch palm (*Thrinax radiata* Lodd. ex J. A. and J. H.) and several ornamental palms (Ward et al. 2003; Amalin, pers. comm.). Furthermore, this is a quarantined pest for some countries (EPPO 2009). A management plan to mitigate the damage that this invasive species is causing to Florida's palms and commercial ornamental palm industry is urgently needed.

Biological control is one of the most important components of integrated pest management with the potential to regulate populations of invasive species. Several predators have been found associated with *R. indica* in the Old World. Moutia (1958) reported that *Amblyseius caudatus* Berlese (= *Typhlodromus caudatus* Chant) (Acari: Phytoseiidae) was the main predator of *R. indica* in Mauritius. Daniel (1981) reported that the phytoseiid mite *Amblyseius channabasavanni* Gupta and the lady beetle *Stethorus keralicus* Kapur (Coleoptera: Coccinellidae) were the most important predators of *R. indica* in India. In Florida, pre- and post-infestation surveys identified some predatory natural enemies. On coconuts, *Amblyseius largoensis* (Muma) (Acari: Phytoseiidae), *Stethorus utilis* (Horn) (Coleoptera: Coccinellidae), and *Ceraeochrysa claveri* Navas (Neuroptera: Chrysopidae) were the most common predators followed by *Bdella distincta* (Barker and Bullock) (Acari: Bdellidae) and *Aleurodothrips fasciapennis* (Franklin) (Thysanoptera: Phlaeothripidae) (Peña et al. 2009). Among these, *A. largoensis* was the most abundant predator and its populations increased in numbers after the arrival of *R. indica* in south Florida (Peña et al. 2009). *Amblyseius largoensis* was also found in Trinidad and Tobago, Puerto Rico and Dominica associated with *R. indica* on coconut (Rodrigues et al. 2007; Peña et al. 2009; Hoy, pers. comm.; Roda, pers. comm.). Based on these

observations it became apparent that a detailed study on the biological control potential of *A. largoensis* against *R. indica* was needed.

This study was designed to determine whether *A. largoensis* can: 1) develop and reproduce when feeding solely on *R. indica*, 2) develop and reproduce on other prey or food sources, and 3) compare developmental and reproductive parameters of *A. largoensis* when feeding on *R. indica*, other potential prey or pollen.

Materials and Methods

The development and reproduction of *A. largoensis* was evaluated on five diets. The diets were selected based on two surveys made in south Florida to determine the most common phytophagous arthropods inhabiting coconuts (Peña et al. 2009). In addition, pollen was included as a food source that might play a role on sustaining the populations of *A. largoensis* (Yue and Tsai 1996). These diets were *R. indica*, *Tetranychus gloveri* Banks (Acari: Tetranychidae), *Aonidiella orientalis* (Newstead) (Hemiptera: Diaspididae), *Nipaecoccus nipae* (Maskell) (Hemiptera: Psudococcidae) and live oak (*Quercus virginiana* Mill) pollen. A no-food treatment was included as a negative control.

Source of *A. largoensis*, *R. indica*, *T. gloveri*, *A. orientalis*, *N. nipae* and *Q. virginiana* Pollen.

Stock colony of *A. largoensis*

Foliage samples containing large numbers of *R. indica* and *A. largoensis* were collected from a pesticide-free Malayan dwarf coconut palm plantation located in Broward County (26°02.925 N 080°09.822 W) in January of 2009, and taken to the Tropical Fruit Entomology Laboratory at the Tropical Research and Education Center (TREC) of the University of Florida, Homestead. The samples were carefully inspected under a dissecting microscope (50×) to isolate and transfer 150 *A. largoensis* females into 3 rearing arenas (50 females per arena) that were kept at $26.5 \pm 1^\circ\text{C}$, RH $70 \pm 5\%$ and 12:12 L:D photophase. The rearing arenas were square (8×8

cm) black card stock (acid and lignin free) coated with paraffin and placed on water-soaked cotton (10 × 10 × 3 cm); paper strips (Kimwipe[®], Kimberly–Clark Corporation, Roswell, GA) were placed along the edges of the cardboard to keep the mites within the arena. Each rearing unit was placed in a plastic tray (12 × 12 cm) and water was added daily to keep the cotton wet. The predatory mites were provided *R. indica* (all stages brushed onto the arena), oak (*Quercus virginiana*) pollen and honey-water solution (10 %) as food sources three times a week. The colonies were subcultured by transferring a minimum of 50 adult female predators to a new arena every three weeks.

Stock colony of *R. indica*

Additional foliage samples from the same site as above were used to collect about 500 *R. indica* females to infest 1-year-old potted Malayan dwarf coconut palms (about 1.5 m in height). Five palms were carefully cleaned with a brush to remove all other arthropods. A yellow “tagging” tape coated with Tanglefoot[®] was tied around the base of the palms to exclude any crawling arthropods. On each palm, 100 *R. indica* females were placed on the abaxial surface of the frond and the palms were placed inside an isolated rearing room kept at 30 ± 1°C, RH 60 ± 5 % under a 12:12 L:D photophase. The palms were left undisturbed for 45 days; then *R. indica* females were collected from 1 palm and used as stock to infest a new palm and also to feed the *A. largoensis* colonies. The procedure was repeated every week.

Stock colony of *T. gloveri*

Tetranychus gloveri was collected and reared similarly to *R. indica*. The *T. gloveri*-infested palms were placed in a separate glass-greenhouse (30 ± 10°C, 60 ± 30 % RH and natural photophase), and regularly inspected with a 10 × hand lens to remove all other arthropods. *Tetranychus gloveri* and *A. largoensis* were identified by Dr. Cal Welbourn, Florida Department of Agriculture and Consumer Services, Division of Plant Industry, Gainesville, Florida.

Stock colonies of *A. orientalis* and *N. nipae*

The oriental scale (*A. orientalis*) and the coconut mealybug (*N. nipae*) were obtained from existing colonies reared for several generations at TREC. *Aonidiella orientalis* was reared on butternut squash, *Cucurbita moschata* (Duchesne ex Lam.), as described by Elder and Smith (1995). *Nipaecoccus nipae* was reared on sprouted potatoes (*Solanum tuberosum* sub. *tuberosum*) inside an incubator kept at $25 \pm 1^\circ\text{C}$, RH $60 \pm 5\%$ in complete darkness, as described by Meyerdirk et al. (1988). Coconut palms were cleaned as described previously and infested by transferring either scale or mealybug crawlers to the abaxial surface of the fronds. Infested palms were transferred to separate greenhouses ($30 \pm 10^\circ\text{C}$, $60 \pm 30\%$ RH and natural photophase) and inspected regularly to remove all other arthropods.

Source of pollen

The pollen used in this study was collected from flowering live oak (*Quercus virginiana* Mill) trees in Homestead, FL ($25^\circ 32.39\text{ N } 80^\circ 28.26\text{ W}$) during February 2009. Inflorescences were cut and placed in paper bags and sealed for a week until the pollen was released. The pollen was then sifted through a 3 sieve set (U.S.A. Standard testing sieves, Nos. 50, 100 and 325; 0.297, 0.149 and 0.043 mm openings respectively; Fisher Scientific Company) to remove any impurities, collected and placed in a sealed container and kept frozen at 0°C until used.

Feeding Experiments with *A. largoensis*

Experimental arenas

The experimental arenas consisted of squares ($4 \times 4\text{ cm}$) cut from mature coconut leaves that were previously infested with the different arthropod species considered. Each leaf square was inspected under a dissecting microscope to remove any other arthropod and to standardize prey density in the treatments that had prey as a food source. Treatments containing either *R. indica* or *T. gloveri* had at least 30 individuals including a minimum of 10 eggs, 10 nymphs, and

10 adults. Treatments containing either *A. orientalis* or *N. nipae* had 3 gravid adult females and at least 30 crawlers (mobile first instars). The treatment containing pollen was constructed by placing approximately 0.2 g of *Q. virginiana* pollen on a clean leaf square, and the no-food treatment consisted of a clean leaf square. The coconut leaf squares corresponding to the different treatments were placed with the abaxial surface up on cotton squares ($6 \times 6 \times 2$ cm) saturated with water in a plastic tray (hexagonal polystyrene weighing dishes 12.7/ 8.9 cm, Fisherbrand cat. No. 02-202-103). Paper strips (Kimwipe[®], Kimberly –Clark Corporation, Roswell, GA) were placed along the edges of the leaf squares to prevent the arthropods used in the assay from escaping. A small plastic square (0.5×0.5 cm) cut from Ziplock[®] bags was placed on top of each arena as a shelter for *A. largoensis*.

Experimental setup

About 300 *A. largoensis* females from the colonies were transferred to 3 experimental arenas (100 individuals per arena) with no food for 24 h at $26.5 \pm 1^\circ\text{C}$, RH 70 ± 5 % and 12:12 L:D photophase. Afterwards females were removed from the arenas and the newly deposited eggs kept at the same environmental conditions. The arenas were observed every 2 h for egg eclosion. Emerged larvae were transferred to experimental arenas individually and observed every 12 h to determine the survivorship and duration of the immature stages on each diet. The diets in each arena were replenished daily and arenas were replaced every three days. After completing development, sex was determined and a male from the colony was transferred into each arena where an adult female had emerged. Introduced males were replaced if they died. Adults were observed every 24 h to determine their preoviposition and oviposition periods, and the longevity of both sexes. Eggs were removed and transferred to new experimental arenas to determine the sex ratio of the offspring in each treatment.

Statistical Analysis

The experimental design was completely randomized with six treatments (5 diets and a no-food control) and 40 replicates per treatment. ANOVAs (SAS Institute, Inc. 2003) were used to detect the effect of diet on the duration of the immature stages, preoviposition and oviposition period, daily and total oviposition, and the longevity of *A. largoensis*. Survivorship of immature stages and the sex ratios of the progeny were compared using chi-square (χ^2) tests (SAS Institute, Inc. 2003). Fertility life table parameters, including the net reproductive rate (R_o), generation time (T), and intrinsic rate of increase (r_m) were estimated using the Jackknife procedure as described by Maia et al. (2000). We tested differences in the fertility life table parameters between diets using Student Newman-Keuls multiple range test (SAS Institute, Inc. 2003).

Results

The development and survivorship of immature stages of *A. largoensis* were influenced by the type of diet consumed. Larvae of *A. largoensis* developed into protonymphs feeding on *R. indica*, *T. gloveri*, *A. orientalis*, *N. nipae* or *Q. virginiana* pollen, but also without food. Larval developmental times of *A. largoensis* were not influenced by the type or presence of food ($F = 1.21$; $df = 5, 212$; $P = 0.300$); however, larval survival was significantly higher with food than without [$\chi^2 (5, N=240) = 55.96, P < 0.001$] (Table 3-1). In the absence of food, the protonymphs of *A. largoensis* failed to develop into the deutonymph. In the presence of food, protonymphal development was influenced by the type of diet ($F = 26.35$; $df = 5, 133$; $P < 0.001$). Protonymphal development of *A. largoensis* was significantly faster when feeding on *R. indica* or *T. gloveri* than on *A. orientalis*, *N. nipae* or *Q. virginiana* pollen (Table 3-1). Survival rates were similar on protonymphs fed with *R. indica*, *T. gloveri*, *A. orientalis* or *Q. virginiana* pollen, but significantly lower on those fed with *N. nipae* [$\chi^2 (5, N=213) = 89.93, P < 0.001$]. Deutonymphal development of *A. largoensis* was also influenced by the type of diet ($F = 25.4$; df

= 4, 104; $P < 0.001$), being significantly shorter on the *R. indica* or *T. gloveri* than on *A. orientalis*, *N. nipae* or *Q. virginiana* pollen diets (Table 3-1). Survival rates of this stage were high in individuals fed with *R. indica*, *T. gloveri* or *Q. virginiana* pollen but significantly lower on those fed with *A. orientalis* or *N. nipae* [χ^2 (4, N=134) = 36.23, $P < 0.001$]. In summary, the immature development of *A. largoensis* was significantly faster when fed either *R. indica* or *T. gloveri* than on other food sources ($F = 44.02$; $df = 5, 104$; $P < 0.001$) (Table 3-1). In addition, more *A. largoensis* individuals reached the adult stage when fed on *R. indica*, *T. gloveri* or *Q. virginiana* pollen [χ^2 (5, N=240) = 103.94, $P < 0.001$]. Survival rates of *A. largoensis* were significantly lower when fed on *A. orientalis* and lowest when fed on *N. nipae*.

The type of diet influenced the reproductive rates of *A. largoensis*. For instance, females fed with *A. orientalis* or *N. nipae* did not reproduce. For this reason, *A. largoensis* females fed with these two diets were excluded from the fertility and life table analyses. The diet significantly influenced the duration of the pre-oviposition period ($F = 23.91$; $df = 5, 52$; $P < 0.001$). Females fed with *R. indica* or *T. gloveri* had a significantly shorter preoviposition periods compared to those fed with *Q. virginiana* pollen (Table 3-2). However, the oviposition period was similar among those three diets ($F = 1.34$; $df = 5, 52$; $P = 0.267$). Female longevity was not influenced by the type of diet, but males fed with *R. indica* and *T. gloveri* lived significantly longer compared to those fed with *Q. virginiana* pollen ($F = 2.72$; $df = 5, 52$; $P = 0.276$ and $F = 7.36$ $df = 5, 52$; $P < 0.001$ for female and male longevities respectively) (Figure 3-2). The daily oviposition rate of *A. largoensis* females fed exclusively with *R. indica* was significantly higher than those fed with *T. gloveri* or *Q. virginiana* pollen ($F = 25.88$; $df = 5, 52$; $P < 0.001$) (Figure 3-1). In addition, the total number of eggs deposited was significantly higher in females fed *R. indica* or *T. gloveri* compared to those fed *Q. virginiana* pollen ($F = 9.54$; $df = 5, 52$; $P < 0.001$)

(Table 3-2, Figure 3-1). The proportion of females in the progeny ranged from 0.63 to 0.73 among the three diets. No effects of the type of diet on the sex ratio of the offspring were detected [χ^2 (2, N=682) = 5.64 P = 0.059] (Table 3-2). The net reproductive rate (R_o) of *A. largoensis* fed with *R. indica* was significantly higher than those fed with *T. gloveri* or *Q. virginiana* pollen (F = 18.32; df = 2, 58; P < 0.001). The mean generation time (T) was significantly shorter in *A. largoensis* fed on *R. indica* or *T. gloveri* compared to those fed with *Q. virginiana* pollen (F = 12.36; df = 2, 58; P < 0.001). Finally, the intrinsic rate of increase (r_m) of *A. largoensis* fed with *R. indica* was significantly higher than those fed with *T. gloveri* or *Q. virginiana* pollen (F = 34.18; df = 2, 58; P < 0.001) (Table 3-2).

Discussion

The results of this study suggest that larvae of *A. largoensis* are facultative feeders. We found no differences among developmental times of larvae with or without food. These results contrast with those reported by Schausberger and Croft (1999) who found that larvae of 13 other phytoseiid mite species developed slower without food than those with food, regardless of the feeding type (non, facultative, or obligatory feeding types). However, we found that survival of *A. largoensis* larvae was higher with food than without, regardless of the type of diet. Direct observations of feeding and indirect indications of feeding (coloration of the alimentary tract) suggested that when food is present *A. largoensis* larvae tend to feed.

The type of prey influenced the development and survivorship of succeeding immature stages and the reproduction of adult *A. largoensis*. In this study we tested four arthropod species that are commonly found inhabiting coconut leaves in Florida (Peña et al. 2009). Our results suggest that hemipteran prey alone (*A. orientalis* and *N. nipae*) do not provide enough resources to sustain *A. largoensis* populations. We found high mortality rates and longer developmental times of immature stages, and no reproduction of *A. largoensis* when feeding on either of these

prey species. In contrast, *A. largoensis* had shorter developmental times and higher survival rates of immature stages, and the highest reproductive parameters, when feeding on the two mite species (*R. indica* and *T. gloveri*). Our results suggest that mites are better prey-resources for population growth of *A. largoensis* than the other prey used in this experiment. However, it is important to point out that, under natural conditions, *A. largoensis* could potentially feed on various prey and alternative food sources. Based on studies conducted using *T. urticae*, McMurtry and Croft (1997) considered *A. largoensis* a type III generalist predator. This group of predators usually has a broad prey range including several mite and insect species. Potential mite prey includes tetranychids, eriophyids, tydeids, tenuipalpids and acaridids. Potential insect prey includes thrips, whiteflies, mealybugs, and scale crawlers (McMurtry and Croft 1997). Lawson-Balagbo et al. (2008) reported that *A. largoensis* was the most abundant predator found on coconut leaflets in several regions of Brazil, where it was found associated with varied potential prey including several prostigmatid (*Tetranychus mexicanus* (McGregor), *T. neocaledonicus* André, *Oligonychus* sp., *Tenuipalpus* sp., *Brevipalpus phoenicis* (Geijkes), *Retracrus johnstonii* (Keifer), *Notostrix nasutiformes* Gondim Jr, Flechtmann, Moraes, and *Lorryia formosa* Cooreman) and oribatid mites. Moreover, Kamburov (1971) found that *A. largoensis* can develop and reproduce readily feeding on three mite species (*B. phoenicis*, *Eutetranychus orientalis* (Klein) and *Tetranychus cinnabarinus* (Boisduval)) showing developmental times and oviposition rates similar to those found in our study. That same study reported poor development and reproduction of *A. largoensis* when fed on scale crawlers of *Aonidiella aurantii* (Maskell) and *Chrysomphalus aunidum* (L.) (Hemiptera Diaspididae), similar to the results obtained here. A study conducted by Rodriguez and Ramos (2004) showed that *A. largoensis* can develop and reproduce feeding solely on the broad mite *Polyphagotarsonemus latus* (Banks) (Acari:

Tenuipalpidae). In addition to having a broad prey range it is possible that *A. largoensis*, as seen in other phytoseiid species (Kamburov 1971; Van Rijn and Tanigoshi 1999), could use non-prey food sources. Some phytoseiids may use plant exudates as well as honeydew as food supplements, which may increase their reproductive potential in the presence of prey (McMurtry 1992). Results of this study, and those reported by Yue and Tsai (1996), suggest that *A. largoensis* is not an obligatory predator because it can develop and reproduce on a pollen diet. Galvao et al. (2007) evaluated the survival of *A. largoensis* feeding on *Aceria guerreronis* Keifer (Acari: Eriophyidae) and other non-prey food sources (pollen and honey) and determined that a mixed diet of *A. guerreronis* or *T. urticae*, plus either pollen or honey, improved its demographic parameters as compared to a diet of *A. guerreronis* alone. The broad food range of *A. largoensis* could be considered a desirable attribute in terms of its potential control of *R. indica* because it suggests that *A. largoensis* can persist when *R. indica* densities are low or absent.

The importance of this study lies in addressing the biological control potential of an invasive species by *A. largoensis*. Our results show that *A. largoensis* has some desirable attributes in terms of its potential as biological control agent of *R. indica*. *Amblyseius largoensis* showed high survival rates, shorter developmental times and high reproductive rates when fed solely on *R. indica* compared with single other food sources. However, it is important to point out that the colony used as source of *A. largoensis* was fed on *R. indica* and pollen, which could have resulted in lab selection affecting the ability of this predator to use other food. Nevertheless, *A. largoensis* developed and reproduced feeding on *T. gloveri*, showing some demographic parameters similar to those found when feeding on *R. indica*. This suggests that *A. largoensis* retained the ability to use *T. gloveri* as prey despite any possible lab selection. Our findings suggest that *A. largoensis* has a high numerical response when feeding on *R. indica*, which could

explain previous reports showing that populations of *A. largoensis* increased in numbers on coconut leaves after the arrival of *R. indica* to south Florida (Peña et al. 2009). A similar situation was observed in Puerto Rico and Trinidad and Tobago (Peña et al. 2009; Roda, pers. comm.). Another consideration, in terms of the potential control of *R. indica* is that *A. largoensis* has a shorter developmental period than *R. indica*. Our results suggest that *A. largoensis* can complete its immature development in less than a week (5.92 ± 0.67 d) at $26.5 \pm 1^\circ\text{C}$, a much shorter developmental period than *R. indica* (24.5 d) at $23.9\text{-}25.7^\circ\text{C}$ (Nageshachandra and Channabasavanna 1984). However, despite exhibiting attributes that are vital in terms of population growth, further studies are needed to determine the effectiveness of *A. largoensis*. We are currently investigating the functional response and prey preference of *A. largoensis*. It is possible that *A. largoensis* will be effective only at certain prey population densities, playing a complementary role to other mortality factors (other predators, acaropathogens, weather) that could act as regulators of *R. indica* populations. Moreover, *A. largoensis* could increase its reproductive potential in the presence of alternative foods. These aspects of *A. largoensis* biology are part of an on-going investigation because they might furnish a key to improving the control of *R. indica*.

Table 3-1. Duration and survivorship of immature stages of *A. largoensis* fed on five diets and a no-food control treatment (26.5 ± 1°C, 70 ± 5 % RH and 12:12 L:D photophase).

Immature stage		<i>R. indica</i>	<i>T. gloveri</i>	<i>A. orientalis</i>	<i>N. nipae</i>	Pollen	No food
Larva	Duration (d)*	1.56 ± 0.40 a	1.73 ± 0.51 a	1.73 ± 0.48 a	1.82 ± 0.56 a	1.77 ± 0.48 a	1.77 ± 0.55 a
	Survival (%)**	97.5 a	97.5 a	98 a	92.5 a	92.5 a	55 b
Protonymph	Duration (d)	2.30 ± 0.65 a	2.98 ± 0.88 a	4.65 ± 1.58 b	4.62 ± 1.50 b	4.61 ± 1.37 b	0
	Survival (%)	90 a	82 a	75 a	22 b	91 a	0
Deutonymph	Duration (d)	2.04 ± 0.43 a	2.33 ± 0.51 a	5.2 ± 2.65 b	4 ± 0.00 b	5.07 ± 1.74 b	0
	Survival (%)	97a	85a	52 b	25 b	94 a	0
Egg-Adult	Duration (d)	5.92 ± 0.67 a	7.11 ± 0.94 a	11.43 ± 3.27 b	13.5 ± 0.00 b	11.51 ± 2.11 b	0
	Survival (%)	85a	68a	38b	5c	70a	0

*(means ± SEM), means times (d) within a row followed by the same letter are not significantly different ($P < 0.05$; ANOVA, Tukey's studentized range test).

** Percentage of surviving individuals within a row followed by the same letter are not significantly different, ($P < 0.05$; χ^2 test).

Table 3-2. Fertility life table parameters and other reproductive parameters (duration of preoviposition and oviposition periods, total number of ovipositions, daily rate of oviposition, female and male longevity, sex ratios) of *A. largoensis* fed on three diets ($26.5 \pm 1^\circ\text{C}$, $70 \pm 5\%$ RH and 12:12 L:D photophase).

	<i>R. indica</i>	<i>T. gloveri</i>	Pollen
Pre-oviposition period (d)*	3.18 ± 1.74 a	3.38 ± 1.50 a	9.12 ± 2.62 b
Oviposition period (d)*	14.09 ± 8.30 a	8.69 ± 5.05 a	10.12 ± 9.00 a
Total number of eggs deposited *	19.96 ± 14.6 a	6.82 ± 6.05 a	3.00 ± 2.86 b
Daily rate of oviposition (eggs / d)*	1.63 ± 0.27 a	1.13 ± 0.39 b	0.54 ± 0.32 c
Female longevity (d)*	23.70 ± 13.4 a	15.67 ± 8.20 a	22.76 ± 11.9 a
Male longevity (d)*	16.44 ± 6.65 a	9.00 ± 4.18 a	8.72 ± 4.52 b
Female proportion of the progeny **	0.73 ± 0.19 a	0.63 ± 0.23 a	0.67 ± 0.22 a
Net reproductive rate (females per female) R_o ***	12.59 ± 1.46 a	5.66 ± 0.78 b	3.90 ± 0.30 b
Generation time (d) T ***	19.95 ± 1.55 b	17.01 ± 1.19 b	27.74 ± 1.54 a
Intrinsic rate of increase (day^{-1}) r_m ***	0.127 ± 0.008 a	0.102 ± 0.06 b	0.049 ± 0.002 c

Means \pm SEM within a row followed by the same letter are not significantly different

*($P < 0.05$; Tukey's studentized range test).

**($P < 0.05$; χ^2 -test)

***($P < 0.05$; Student Newman-Keuls multiple range test)

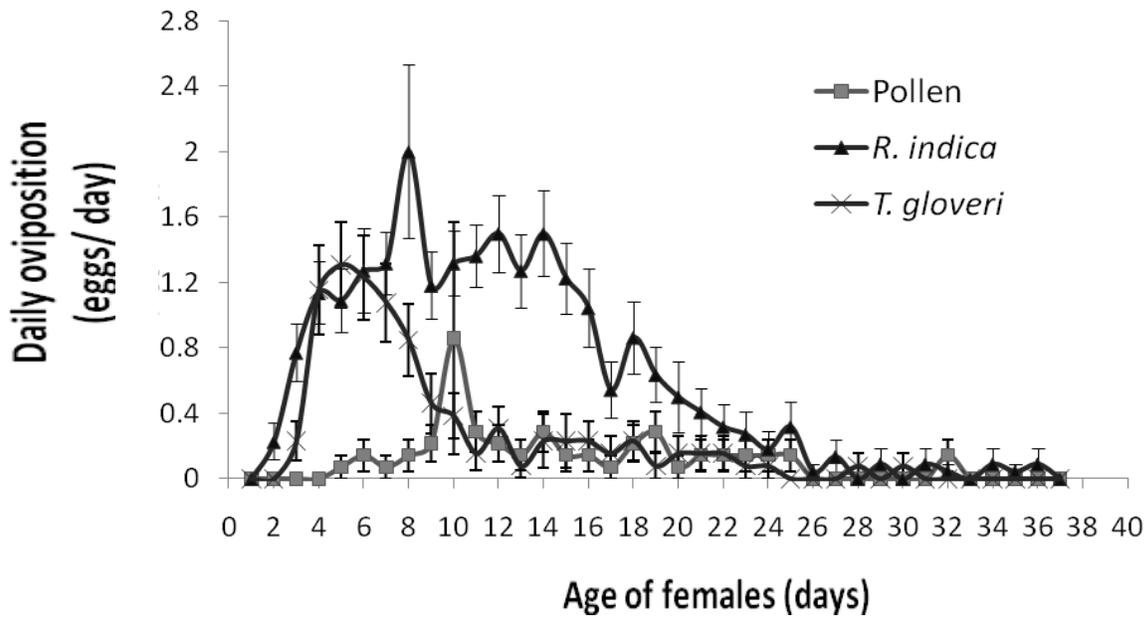


Figure 3-1. Daily oviposition rate of *A. largoensis* females fed on either *R. indica*, *T. gloveri* or *Q. virginiana* pollen at $26.5 \pm 1^\circ\text{C}$, $70 \pm 5\%$ RH and 12:12 L:D photophase. Error bars represent the standard error of the mean.

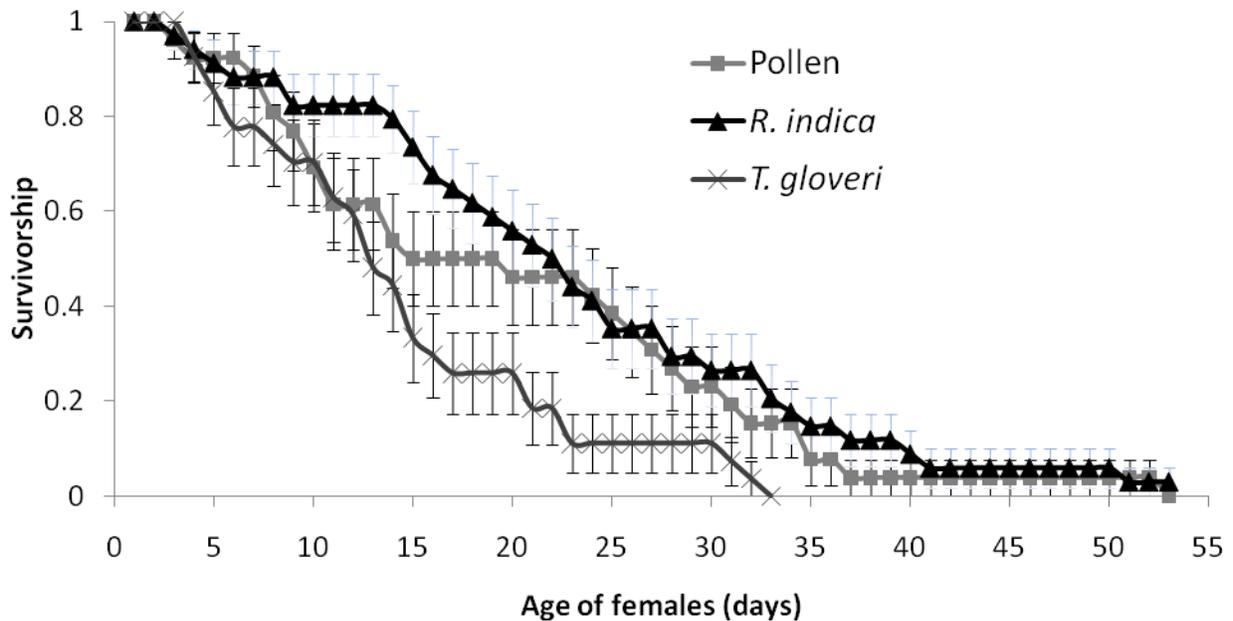


Figure 3-2. Survivorship of *A. largoensis* females fed on either *R. indica*, *T. gloveri* or *Q. virginiana* pollen at $26.5 \pm 1^\circ\text{C}$, $70 \pm 5\%$ RH and 12:12 L:D photophase. Error bars represent standard error of the mean.

CHAPTER 4
PREY-STAGE PREFERENCES AND FUNCTIONAL AND NUMERICAL RESPONSES OF
AMBLYSEIUS LARGOENSIS (ACARI: PHYTOSEIIDAE) TO *RAOIELLA INDICA* (ACARI:
TENUIPALPIDAE)³

Summary

Raoiella indica Hirst (Acari: Tenuipalpidae) is a phytophagous mite that recently invaded the Western Hemisphere. This mite is a multivoltine and gregarious species that can reach very high population densities and cause significant damage to various palm species (Arecaceae). The predatory mite *Amblyseius largoensis* (Muma) (Acari: Phytoseiidae) has been found associated with *R. indica* in Florida. This study evaluated *A. largoensis* for potential to control *R. indica* by (1) determining predator preferences among developmental stages of *R. indica*, and (2) estimating predator functional and numerical responses to varying densities of its most preferred prey-stage. Under no-choice conditions *A. largoensis* consumed significantly more eggs than other stages of *R. indica*. In choice tests *A. largoensis* showed a preference for *R. indica* eggs over all other prey stages ($P < 0.01$). *Amblyseius largoensis* displayed a type II functional response showing an increase in number of prey killed with an increase in prey population density. Consumption of prey stabilized at approximately 45 eggs/day, the level at which oviposition by the predator was maximized (2.36 ± 0.11 eggs /day; mean \pm SEM). Results of this study suggest that *A. largoensis* can play a role in controlling *R. indica* populations, particularly when prey densities are low.

Key words: mites, invasive species, *Cocos nucifera*, *Raoiella indica*, *Amblyseius largoensis*, prey-stage preference, functional response, numerical response.

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Introduction

Raoiella indica Hirst (Acari: Tenuipalpidae), the red palm mite, is a phytophagous mite that recently invaded the Western Hemisphere. This mite is a multivoltine and gregarious species that can reach very high population densities and cause significant damage to various plant species, especially palms (Arecaceae). *Raoiella indica* was detected in Martinique and St. Lucia in 2004, and rapidly expanded its geographical range throughout the Caribbean (Kane et al. 2005; Etienne and Flechtmann 2006;Rodrigues et al. 2007). In December 2007, *R. indica* was detected in Florida in Palm Beach County (FDACS 2007) and since has spread to six other Florida counties. *Raoiella indica* also has spread to South America, reported from Venezuela (Gutiérrez et al. 2007; Vásquez et al. 2008), Brazil (Marsaro et al. 2009) and Colombia (Carrillo et al. 2011b). More recently, the mite was found in Mexico (NAPPO 2009). Establishment of this exotic species in the Neotropical region has given rise to concerns about its potential effect on a vast number of economically and ecologically important plants.

Efforts have been made to identify and evaluate the potential of native predators to control *R. indica* in areas of invasion in the Western Hemisphere (Peña et al. 2009; chapter 2). A predatory mite, *Amblyseius largoensis* Muma (Acari: Phytoseiidae), has been found as the most abundant predator associated with *R. indica* infesting coconuts in Florida, Puerto Rico, and Trinidad and Tobago. Pre- and post- infestation surveys in Florida revealed that populations of *A. largoensis* increased after establishment *R. indica* (Peña et al. 2009). Further studies revealed that *A. largoensis* showed high survival rates, short developmental times and high reproductive rates when fed solely on *R. indica* compared with other single food sources (Carrillo et al. 2010, chapter 3). However, it is still unclear whether predation by *A. largoensis* results in reduction of *R. indica* populations. Data addressing that issue may be obtained through studies of functional response (response of individual predators to changes in prey density) and numerical response

(change in density of the predator population in response to changes in prey density) (Solomon 1949).

The functional response of a predator as a function of prey density generally follows one of three mathematical models (Holling 1959a, 1959b, 1961). With a Type I functional response, the number of prey killed increases linearly at constant rate as a function of prey density. With a Type II response the number of prey killed increases up to a maximum (predator saturation) but the proportion of prey killed declines with prey density. With a type III response, predation results in a sigmoidal curve. At low prey densities prey killed is positively density-dependent as a result of an increase in the searching activity of the predator with increasing prey densities. However, at high prey densities predator saturation also occurs. Predators with Type III response are regarded as capable of regulating prey populations (Holling 1965). Predators with type II response have proven to be efficient especially at low prey densities (Krebs 1978; Koehler 1999). The functional response of a predator is not fixed, but can vary depending on many factors, including the developmental stage of its prey (Santos 1975), the plant species upon which the interaction occurs (Skirvin and Fenlon 2001), and the spatial distribution of the prey (Ryoo 1996).

This study was designed to assess the potential of *A. largoensis* to serve as a biocontrol agent for *R. indica*. The specific objectives of the investigation were to: (a) determine the preferences of *A. largoensis* for the different life stages of *R. indica*, and (b) to estimate the functional and numerical responses of this predatory mite to varying densities of its most preferred stage of *R. indica*.

Materials and Methods

Rearing and General Experimental Procedures

Amblyseius largoensis and *R. indica* were obtained from laboratory colonies maintained for approximately two years at the Tropical Research and Education Center (TREC), University of Florida, Homestead. Both mites were reared at $26.5 \pm 1^\circ\text{C}$, RH $70 \pm 5\%$ and 12:12 L:D photoperiod. Sources of colonies and rearing procedures were previously described in Carrillo et al. (2010, chapter 3). All *A. largoensis* used in experiments were mated females in their peak oviposition period (3-6 days posteclosion), reared on *R. indica* (all stages), and starved for 24 hr prior to each experiment. Only replicates in which the female remained within the test arena and produced at least one egg within 48 hr were considered for analysis.

Experiments were conducted at the same environmental conditions used for rearing. The experimental arenas consisted of rectangles (4×2.5 cm) cut from mature coconut (*Cocos nucifera* L.) leaves without any damage or presence of any arthropod. Leaf rectangles were placed with the abaxial surface facing up on cotton squares ($6 \times 6 \times 2$ cm) saturated with water in a plastic tray (hexagonal polystyrene weighing dishes 12.7/ 8.9 cm, Fisherbrand cat. No. 02-202-103). Paper strips (Kimwipe[®], Kimberly –Clark Corporation, Roswell, GA) were placed along the edges of the leaf squares to minimize escape of mites. Arenas were prepared over a period of 7 days. Eggs were obtained by placing 100 *R. indica* ovipositing females in each arena and allowing oviposition for 5 days. After this period females were removed and the desired number of eggs adjusted by removing excess of eggs with a fine brush. Arenas with only *R. indica* larvae, nymphs or adults were obtained by transferring individuals from the stock colony into the arenas. Arenas with combinations of different stages were obtained by first allowing oviposition (on those treatments including eggs), then transferring *R. indica* larvae, nymphs

and/or adults, to adjust the desired ratios between the stages. A small plastic square (0.5 × 0.5 cm) was placed on top of each arena as a shelter for *A. largoensis*.

Raoiella indica* Stage Preference by *A. largoensis

Prey preferences of *A. largoensis* among *R. indica* developmental stages were determined by choice and no-choice tests. Single *A. largoensis* females were transferred into arenas with specific densities of *R. indica*-stages and the numbers of each stage killed during a period of 24 h was recorded by counting the number of shriveled corpses and by subtracting the number of eggs remaining from the number of eggs provided.

In no-choice tests, 30 *R. indica* specimens (eggs, larvae, protonymphs or adult females) per day were offered individually to *A. largoensis* females during two days. Females were transferred to new arenas with the corresponding *R. indica* densities for the second day of evaluation. The no-choice experiment was replicated 15 times and analyzed through 1-way ANOVAs and means separated through Tukey's test (SAS 2003).

Under choice conditions, the following *R. indica*-stage combinations were offered in separate arenas: 30 Eggs:30 Larvae, 30 Eggs:30 Nymphs, 30 Eggs:30 Adults, 30 Larvae:30 Nymphs, 30 Larvae:30 Adults, and 30 Nymphs:30 Adults. Choice experiments were replicated 15 times and the evaluation period for each test was 24 h. Preference was quantified using the index β proposed by Manly et al. (1972):

$$\beta = \left[\frac{\ln \left(\frac{N}{N_c} \right)}{\ln \left(\frac{N}{N_c} \right) + 1} \right]^{-1}$$

where N and N_c are the numbers of each prey-stage provided and N_e and N_{ec} are the numbers of each prey-stage killed. The index assigns preference values from 0 to 1, where 0.5 represents no

preference. Mean β -values were considered significant when 95% confidence intervals based on the t-distribution did not overlap with $\beta=0.5$.

Functional and Numerical Responses of *A. largoensis* to Varying Densities of *R. indica* Eggs

Eight *R. indica* egg densities (5, 10, 20, 30, 40, 60, 80, and 100 eggs/arena) were offered to *A. largoensis* females on the arenas described above. A single *A. largoensis* female was transferred into an arena with a fixed *R. indica* egg-density and the numbers of eggs consumed during a 24-h period was recorded by subtracting the number eggs remaining from the number of eggs provided. Eggs were replenished every day for 4 consecutive days and oviposition by *A. largoensis* was recorded daily. The experiment was replicated 15 times. Data from the first day were excluded from analysis to minimize potential effects from the prior diet of mixed stages. This allowed predators time to adapt physiologically to the new homogeneous diet and its relative level (Castagnoli and Simoni 1999).

The type of functional response of *A. largoensis* preying on *R. indica* eggs was determined using a polynomial logistic regression (Systat Software Inc. 2006) as described by Juliano (2001). This procedure uses the signs of the coefficients in the equation $Ne/N = a + bN + cN^2 + dN^3 + e$, where Ne is the proportion of killed prey and N the prey offered, as indicators of the shape of the functional response. In Type I responses the intercept (a) and the linear coefficient (b) are >0 ; in type II responses the linear coefficient (b) is <0 and the proportion of prey killed declines with an increase in the number of prey offered. In type III responses the linear coefficient (b) is >0 and the quadratic coefficient (d) is <0 (Juliano 2001). After determining the type of the functional response, three models were used to estimate the functional response parameters:

Holling (1959)
$$\frac{N_a}{N} = -\alpha T_h N + \alpha$$

Fan and Petitt (1994)
$$\frac{N}{N_a} = T_h N + \frac{1}{\alpha}$$

Rogers (1972)
$$N_a \alpha = N \{ [1 - \epsilon ([- \alpha T + \alpha T_h] , h N) \}$$

where N_a is the number of prey consumed by each predator during time T (in our experiment $T=1$ day), N is the initial prey density, α is the attack rate coefficient (proportion of prey captured by each predator per unit of searching time) and T_h the handling time (time that the predator spends identifying, pursuing, killing, consuming and digesting a prey).

The functional response models were fitted using the non-linear regression procedure (NLIN; SAS Institute 2003). The numerical response (variation in rate of oviposition by *A. largoensis* as a function of prey density) was calculated through regression analysis using a hyperbolic model (Systat Software Inc. 2006). The hyperbolic model is described by the equation:

$$y = \frac{ax}{b+x}$$

where (y) is the daily oviposition by *A. largoensis* at the various prey densities (x), (a) is the maximum daily oviposition (i.e. plateau), and (b) is the prey density needed to elicit half the maximum response.

Results

Raoiella indica Stage Preference by *A. largoensis*

The no-choice experiments showed a significantly higher consumption of eggs than of other developmental stages of *R. indica* in the two days of evaluation ($F = 111.2$; $df = 3, 57$; $P < 0.001$, Figure 4-1). In addition, consumption of larvae was significantly higher than consumption of nymphs or adults.

The choice experiment indicated that *A. largoensis* showed a significant preference for *R. indica* eggs (95% confidence intervals did not include $\beta=0.5$) when compared to larvae, nymphs or adults. Preference for larvae over adults was also significant (Figure 4-2). No preference was observed when larvae and nymphs or nymphs and adults were offered simultaneously (Figure 4-2). Overall, the results of the choice and no-choice experiments indicate that *A. largoensis* prefers *R. indica* eggs followed by larvae, over other developmental stages.

Functional and Numerical Responses of *A. largoensis* to Varying Densities of *R. indica* Eggs

The daily consumption rate of *R. indica* eggs gradually increased with an increase in prey density (Figure 4-3). Initially, the response curve increased almost linearly with increasing prey density, and then began to level off as consumption rate decreased. Nearly 100% of the prey was consumed at the lowest densities: (5, 10, 20 and 30 eggs/arena/day). Egg consumption by *A. largoensis* females tended to stabilize when more than 40 *R. indica* eggs/day were offered, reaching a plateau at approximately 45 eggs/day.

The binomial logistic regression analysis used to determine the shape of the functional response resulted in the following coefficient estimates: intercept (a) = 0.22 ± 0.005 (SE), $t=40.2$, $P < 0.001$; linear (b) = -0.02 ± 0.001 , $t=-22.41$, $P < 0.001$; quadratic (c) = 0.002 ± 0.005 , $t=15.75$, $P < 0.001$ and cubic (d) = $-9.6 \times 10^{-7} \pm 7.64 \times 10^{-8}$, $t=-12.6$, $P < 0.001$. The linear coefficient (b) was <0 indicating that *A. largoensis* exhibited a Type II functional response. The functional response data of *A. largoensis* preying on *R. indica* eggs successfully fitted to the three tested models (Table 4-1, Figure 4-3). The values of the attack rate coefficient α and the handling time T_h produced by each model are shown in Table 4-1.

The numbers of eggs laid per day per predator (y) and the prey density (x) were highly correlated ($R^2 = 0.98$, $P < 0.001$, Figure 4-4). Daily oviposition of *A. largoensis* females

increased with an increase in the predation rate and tended to stabilize when prey consumption was greater than 30 *R. indica* eggs/day. The maximum daily oviposition (α) of *A. largoensis* was 2.36 ± 0.11 eggs (mean \pm SEM) and the prey density needed to elicit half the maximum response (b) was 15.2 ± 0.11 *R. indica* eggs (mean \pm SEM).

Discussion

The results of our study showed that *A. largoensis* females consumed more eggs than other stages when they were offered separately, and preferred eggs of *R. indica* over other developmental stages when offered the choice. *Amblyseius largoensis* usually required a single attack attempt to successfully penetrate the chorion and suck the egg contents. In contrast, the predator often needed several attack attempts to penetrate the mite cuticle and showed a longer handling time while preying on nymphs or adults. While feeding on nymphs and adults, the predator would often stop feeding, turn to cleaning activities, then return to continue feeding on the same prey item, thus prolonging the handling time. The greater consumption of eggs compared to the other stages could also be explained by differences in the body weight of each stage. Because the body weight of the prey differs among the life stages, the maximum number of each prey stage which the predator can eat varies as well. More important is the nutritional benefit that each stage provides for *A. largoensis*, which could be indirectly estimated by comparing the fecundity of females fed separately on the different stages. That kind of experiment was not carried out in this study. However, the oviposition rate of females fed on an egg diet (2.36 ± 0.11 eggs/day) was higher than those observed in a previous study when a diet of mixed stages was provided (1.63 ± 0.27 , Carrillo et al. 2010, chapter 3), suggesting that a diet of eggs alone may result in a greater or at least similar fecundity than with a diet of mixed prey. Similar results were found with *Neoseiulus fallacis* (Garman); a diet of *Tetranychus urticae* Koch (Acari: Tetranychidae) eggs alone resulted in significantly higher fecundity than a diet of

mixed prey stages (Blackwood et al. 2001). However our results should be analyzed with caution as we did not evaluate the effect of long-term feeding on the different stages which could provide a more accurate estimate of the nutritional benefits of each life stage (Castagnoli and Simoni 1999).

Most studies on prey-stage preferences of phytoseiid mites have been conducted using tetranychid mites as hosts. In studies using *T. urticae* eggs and larvae as prey, Blackwood et al. (2001) suggested that type III generalist predators had no prey-stage preference or preferred larvae over eggs (Blackwood et al. 2001). In our study *A. largoensis*, which was classified as a generalist type III predator by McMurtry and Croft (1997), showed a marked preference for *R. indica* eggs. These results suggest that prey-stage preference of *A. largoensis* varies depending on the prey species and is probably marked by interactions between the searching behavior of the predator, hunger level, prey protection mechanism and nutritional value of each prey individual (Sandness and McMurtry 1970; Blackwood et al. 2001). For instance, the ability of *A. largoensis* to find and capture prey was affected by the webbing of *Oligonychus punicae* (Hirst.) (Sandness and McMurtry 1972); in contrast, *R. indica* females congregate and gradually form large groups of eggs that are exposed without any protection. Thus, *R. indica* eggs could be considered easily accessible prey for *A. largoensis*. The duration of the egg stage is approximately 9 days, which is the longest immature stage of *R. indica* (Nageshachandra and Channabasavanna 1984), suggesting a greater availability of eggs compared to other immature stages. From a management point of view, a preference for *R. indica* eggs could be viewed as a desirable attribute of *A. largoensis*, because it kills the prey before it causes damage to the host plants. However, it is likely that other mortality factors will be required to suppress *R. indica*. Other natural enemies

attacking mobile and reproductive stages of *R. indica* could be very useful for managing this pest.

Predation and oviposition of *A. largoensis* increased as a function of an increase in the population density of *R. indica*. Females consumed almost 100% of prey at relatively low prey densities (<40 eggs/day) suggesting a high searching ability of *A. largoensis*. However, the proportion of prey killed decreased at high populations densities (>40 eggs/day) probably because of satiation or interference on their predation capacity related to prey density (Mori and Chant 1966; Sandness and McMurtry 1970). *Amblyseius largoensis* showed a functional type II response feeding on *R. indica* eggs which suggests that this predator could be more efficient at low to moderate prey densities, a common pattern observed among many predatory mites of the group Gamasina (Koehler 1999) and other predators (Krebs 1978). In accordance with our results, Sandness and McMurtry (1970) studied the functional response of *A. largoensis* fed on *O. punicae* and found a type II response curve. Interestingly, the functional response curves of *A. largoensis* feeding on *O. punicae* and *R. indica* did not show a domed shape. The domed curve has been attributed to a confusion or disturbance component at higher prey densities (Holling 1961; Mori and Chant 1966). For some predators, high prey numbers may cause conflicting stimuli, blocking the feeding response, or causing disturbance resulting in repeated erratic movements. *Amblyseius largoensis* did not show signs of confusion or a reduction on the number of prey killed at high prey population densities which, similar to what was observed with *O. punicae* as prey (Sandness and McMurtry 1970), may explain why a domed curve does not occur.

The type II functional response is the most common functional response of phytoseiid species (Sabelis 1985). A Type II response was also observed in the phytoseiids: *Euseius*

concordis (Chant) and *Phytoseius floridanus* Muma (Sandness and McMurtry 1970), *Phytoseiulus persimilis* Athias-Henriot, *Galendromus occidentalis* (Nesbitt) and *Neoseiulus chilensis* (Laing and Osborn 1974); *Amblyseius longispinosus* (Zhang et al. 1999); *P. persimilis*, *G. occidentalis* (Nesbitt), and *Neoseiulus californicus* (McGregor) (Xiao and Fadamiro 2010); *Chilenseius camposi* González y Schuster (Sepulveda and Carrillo 2008); *Euseius (Amblyseius) finlandicus* and *Amblyseius andersoni* (Koveos and Broufas 2000); *Euseius hibisci* (Chant) (Badii et al. 2004). However, all these examples used tetranychids as prey; in contrast, few studies have addressed the functional response of phytoseiid mites preying on tenuipalpids. Reis et al. (2003) studied the functional response of *Euseius alatus* DeLeon and *Iphiseiodes zuluagai* Denmark and Muma on *Brevipalpus phoenicis* (Geijskes) and found a type II and type I functional responses, respectively. The predatory mite *Amblyseius herbicolus* (Chant) showed a type II functional response when feeding on *B. phoenicis* (Reis et al. 2007). *Euseius mesembrinus* (Dean) showed the same type of response when feeding on *Brevipalpus californicus* (Banks) (Badii et al. 2004). Even though type II responses seem to be common among phytoseiid species it is important to note that functional responses may change depending on the prey stage (Santos 1975), plant species (Skirvin and Fenlon 2001) and spatial distribution pattern of the prey (Ryoo 1996), among other factors.

The best parameter estimate was obtained through the Fan and Petitt model (Table 4-1). Our results coincide with those observed by Sepulveda and Carrillo (2008) and indicate that the Fan and Petitt (1994) equation provides better parameter estimates. Based on our results, Holling's and Rogers's models underestimated (i.e. generated lower values) the handling time of *A. largoensis*. On the other hand, Rogers's model overestimated the attack rate coefficient whereas Holling's model underestimated the same parameter.

Results of the functional and numerical responses suggest that an optimum diet for *A. largoensis*, where prey consumption and oviposition by *A. largoensis* were maximized, is approximately 45 *R. indica* eggs/day. The number of eggs laid/day by *A. largoensis* increased as a function of prey killed, with a positive and highly significant correlation. Similar results were observed for *N. fallacis* (Smith and Newsom 1970) and for *P. persimilis*, *G. occidentalis* and *N. chilensis* fed on tetranychids (Laing and Osborn 1974). Our findings suggest that *A. largoensis* has a high numerical response when feeding on *R. indica*, which could explain previous reports showing that populations of *A. largoensis* increased in numbers on coconut leaves after the arrival of *R. indica* in south Florida (Peña et al. 2009). A similar situation was observed in Puerto Rico and Trinidad and Tobago (Peña et al. 2009; Roda, pers. comm.).

A pattern in the coloration of the alimentary tract of *A. largoensis* was observed. The alimentary tract of *A. largoensis* turns bright red soon after it starts feeding on *R. indica*. In our experiment, only females that ate more than 30 eggs per day retained the red coloration throughout the four days of evaluation. In contrast, females fed with 20 eggs/day or less turned red after feeding but lost the coloration by the following evaluation day. This suggests that predators showing red coloration, which are abundant in field samples (Carrillo, pers. observ.), must be repetitively feeding on a relatively large number (>30 eggs/day) of *R. indica*.

This study was designed to gain insight into the potential capability of *A. largoensis* to control *R. indica*. A previous study demonstrated that *A. largoensis* can complete development and reproduce when fed solely on *R. indica*, showing high survival rates, short developmental times and high reproductive rates compared with single other food sources (Carrillo et al. 2010, chapter 3). Another consideration is that *A. largoensis* has a shorter developmental period than *R. indica*. The predator can complete its immature development in less than one week (5.92 ± 0.67

day, Carrillo et al. 2010), a much shorter developmental period than *R. indica* (24.5 day) (Nageshachandra and Channabasavanna 1984). Results of the present study provide a better understanding of the new association between the Florida predator *A. largoensis* and the invasive *R. indica*. The available evidence suggests that *A. largoensis* could play a role in reducing *R. indica* populations; however, results of the functional response study suggest that this predator could be more efficient at regulating low prey population densities. Nevertheless, it is necessary to consider the results with caution because all experiments have been conducted under simplified conditions using excised leaves. In a whole plant system the predators can disperse and interact with other prey and predator communities, which could significantly affect the efficiency of this predator. Studies on the prey preference of *A. largoensis* and possible intraguild predation may contribute to understanding the potential of this predator as a biological control agent of *R. indica*. In addition, field studies with known proportions of *A. largoensis* and *R. indica* are necessary to determine the efficiency of this predator under more realistic conditions.

Table 4-1. Parameters of the functional response of *A. largoensis* feeding on *R. indica* eggs estimated by three functional response models. Attack rate coefficient α (in units of the proportion of prey captured by each predator per unit of searching time) and handling time T_h (in units of the proportion of 24hr exposure period).

Model	α	T_h	r^2	<i>P</i> value
Rogers	1.84	0.0039	0.95	<0.001
Holling	1.02	0.00617	0.97	<0.001
Fan & Petitt	1.55	0.017	0.98	<0.001

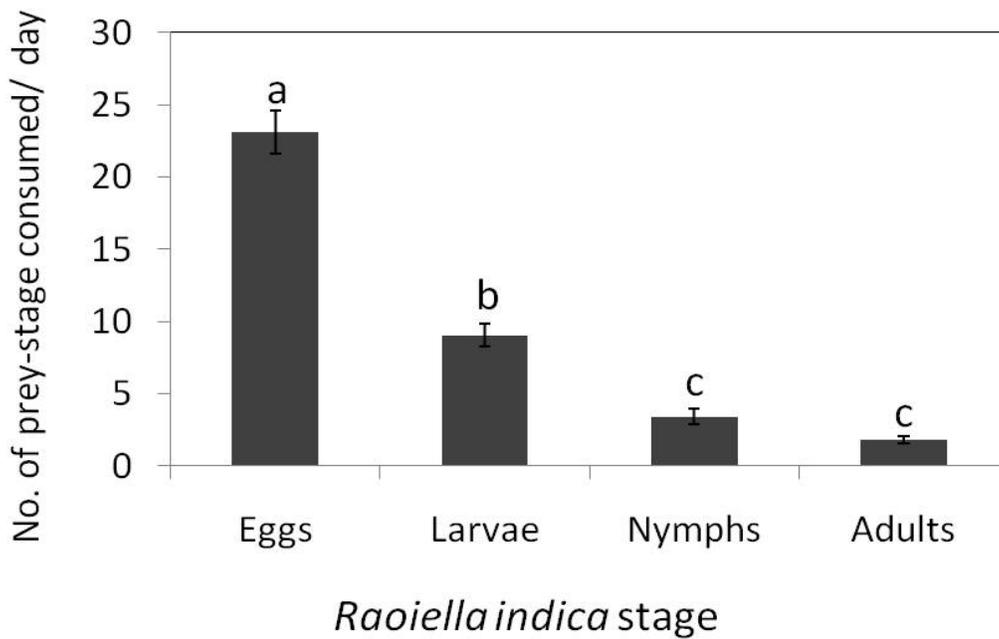


Figure 4-1. Daily consumption and oviposition by *A. largoensis* when offered *R. indica* stages in a no-choice condition. Error bars represent standard error of the mean.

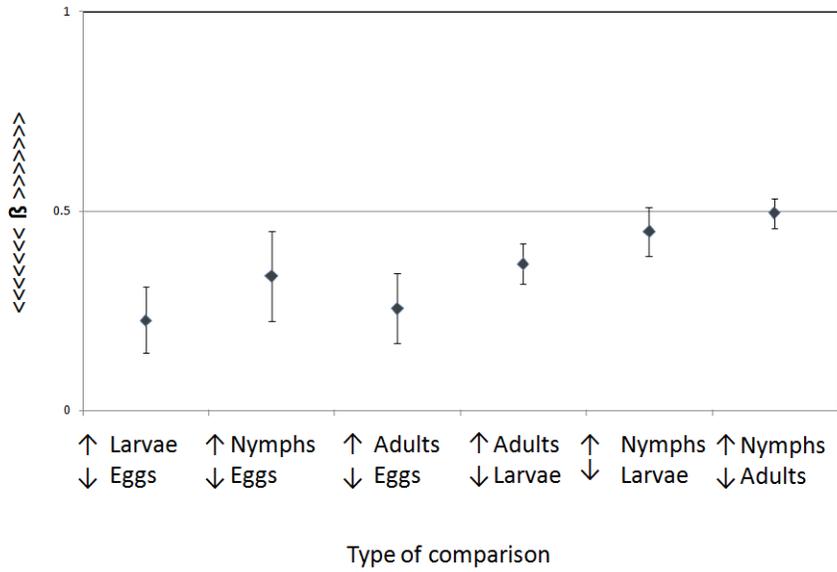


Figure 4-2. Preference of *A. largoensis* females for *R. indica* stages. Paired comparisons among *R. indica* stages. The preference index β assigns preference values from 0 to 1, where 0.5 represents no preference. The position of β in the bottom ($0 < \beta < 0.5$) or top ($0.5 < \beta < 1$) of the graph shows preference for a particular stage as indicated by the arrows in the X axis (i.e. in the first comparison between eggs and larvae there is a preference for eggs). Mean β -values were considered significant when 95% confidence intervals (error bars) based on the t-distribution did not overlap with $\beta=0.5$.

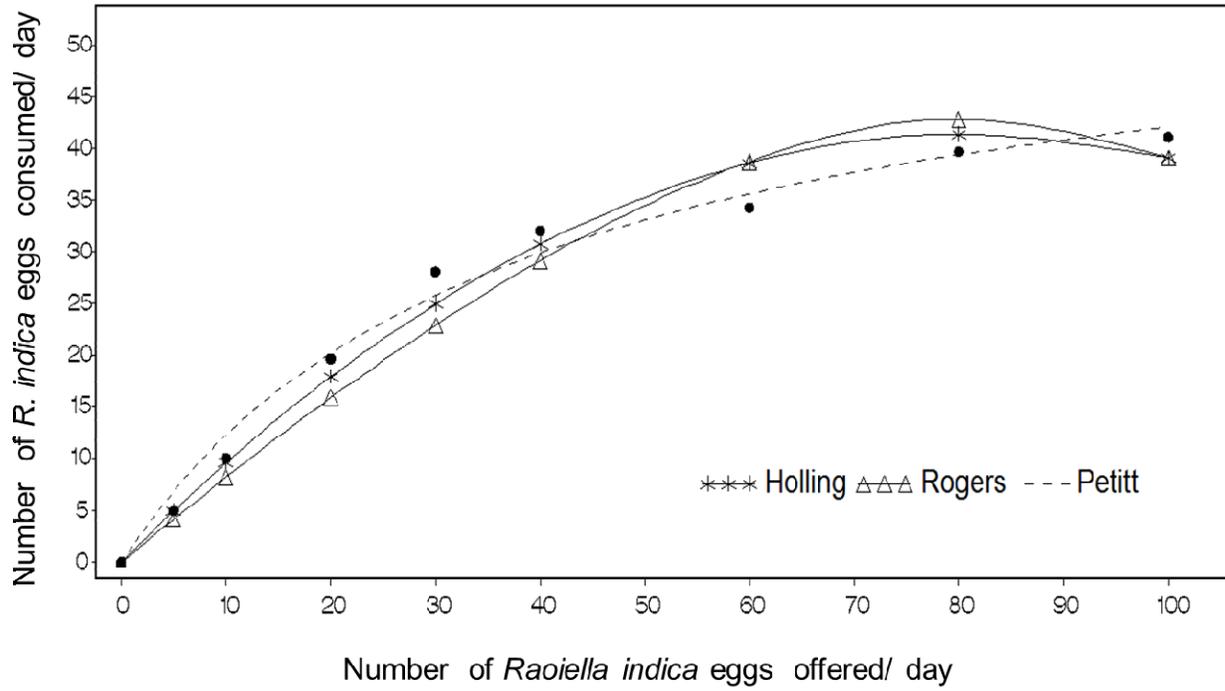


Figure 4-3. Functional response of *A. largoensis* to increasing densities of *R. indica* eggs estimated through three curve fitting models. Dots represent observed data.

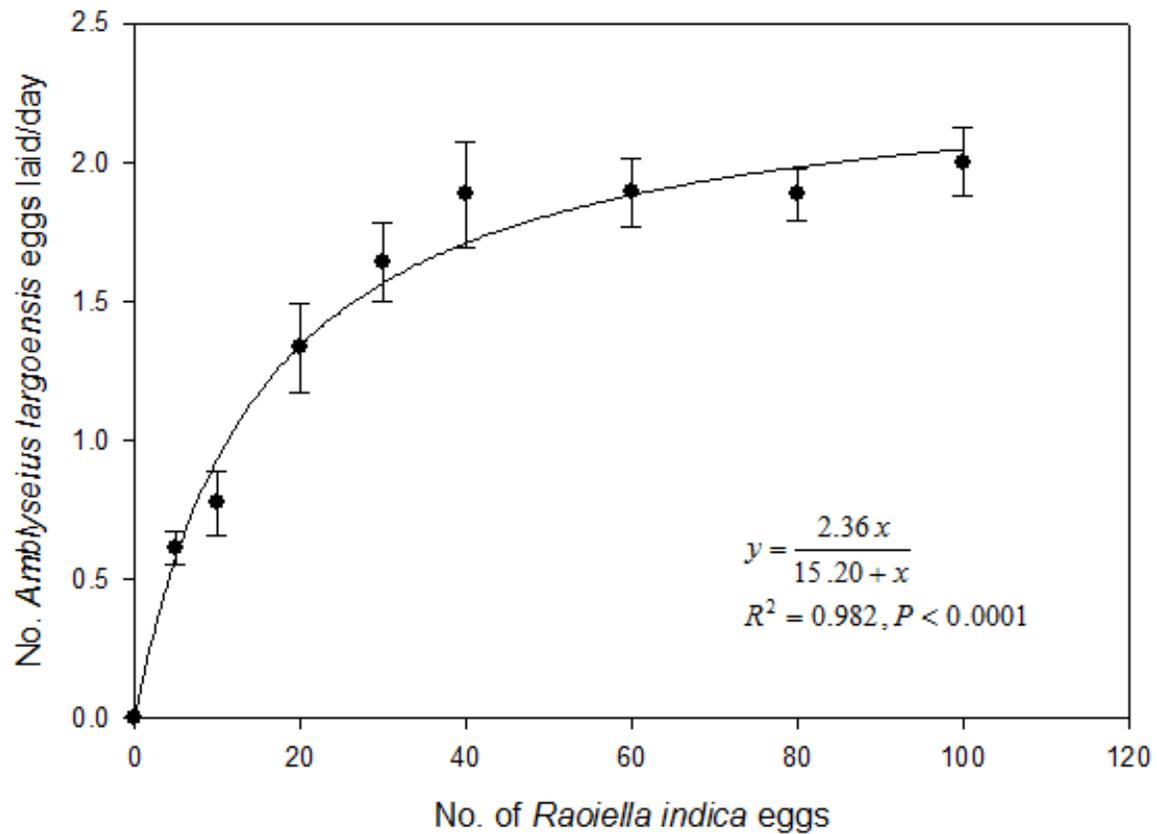


Figure 4-4. Daily oviposition of *A. largoensis* females as a function of the number of *R. indica* eggs consumed/day. Relation calculated through regression analysis using a hyperbolic model. Dots represent observed data. Error bars represent the standard error of the mean.

CHAPTER 5
VARIABILITY IN RESPONSE OF FOUR POPULATIONS OF *AMBLYSEIUS LARGOENSIS*
(ACARI: PHYTOSEIIDAE) TO *RAOIELLA INDICA* (ACARI: TENUIPALPIDAE) AND
TETRANYCHUS GLOVERI (ACARI: TETRANYCHIDAE) EGGS AND LARVA⁴

Summary

Raoiella indica (Acari: Tenuipalpidae) is a phytophagous mite that recently invaded the Neotropical region. A predatory mite *Amblyseius largoensis* (Acari: Phytoseiidae) has been found associated with *R. indica* in Florida. This study evaluated *A. largoensis* by determining its likelihood of consuming eggs and larvae of *R. indica* and *Tetranychus gloveri* (Acari: Tetranychidae) under no-choice and choice conditions. To detect variations in the response of *A. largoensis* to *R. indica*, four populations of predators were examined: (1) predators reared exclusively on *R. indica* in the laboratory for two years, (2) predators reared on *T. gloveri* in the laboratory for 2 months but reared on *R. indica* for two years previously, (3) predators collected from a field infested with *R. indica*, and (4) predators collected from a field that had never been infested with *R. indica*. Results of this study suggest that *A. largoensis* is likely to accept and consume high numbers of *R. indica* eggs regardless of their previous feeding experience. In contrast, all populations consumed relatively fewer *R. indica* larvae than the other prey tested. Predators previously exposed to *R. indica* were more likely to consume *R. indica* larvae. By contrast, predators not previously exposed to *R. indica* showed the lowest likelihood of choosing to feed on this prey item. Plasticity in the response of *A. largoensis* to *R. indica* larvae could be associated with genetic selection, learning, or a combination of both. The possible implications of the observed differences in terms of biological control of *R. indica* are discussed. Key words: mites, invasive species, *Cocos nucifera*, *Raoiella indica*, *Amblyseius largoensis*, prey preference, naïve predators, prey switch, learning, genetic selection.

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Introduction

Raoiella indica Hirst (Acari: Tenuipalpidae) is a phytophagous pest native to tropical and subtropical areas of Asia that recently invaded the Neotropical region (Etienne and Flechtmann 2006; Vásquez et al. 2008; Marsaro Jr. et al. 2009). This mite is a multivoltine and gregarious species that can reach high population densities and cause significant damage to various plant species, especially palms (Arecaceae). Establishment of this invasive species in the Neotropical region has given rise to concerns about its potential effect on several economically and ecologically important plants (Carrillo et al. 2011a, Appendix).

Efforts were undertaken to identify and evaluate potential biological control agents of *R. indica* in the Neotropical region (Peña et al. 2009; Carrillo et al. 2010, chapter 3). The predatory mite *Amblyseius largoensis* Muma (Acari: Phytoseiidae) has been found associated with *R. indica* in several areas of recent invasion including Florida, Puerto Rico, Trinidad and Tobago, Colombia, Cuba, and Mexico. Moreover, this predator has been found associated with *R. indica* in India (Taylor et al. 2011), Philippines (Gallego et al. 2003), Mauritius and Dominica (Hoy, pers. comm.), and Benin and Tanzania (Zannou et al. 2010). It would be important to know whether all the predators identified as *A. largoensis* are equally efficient natural enemies of *R. indica*. The geographically diverse distribution of this phytoseiid suggests that strains, biotypes, or cryptic species could exist (Noronha and Moraes 2004; Bowman 2010), and that differences in predatory behaviors among the various populations of *A. largoensis* could affect their propensity to feed on *R. indica*, as has been found for other phytoseiid species (Tixier et al. 2010).

Information on actual predation by *A. largoensis* upon *R. indica* is limited to a single population of predators that was collected from a coconut plantation located in one of the sites where *R. indica* was first detected in Florida in 2007. This population was used to initiate a

laboratory colony which was used to study survival and reproduction (Carrillo et al. 2010, chapter 3), prey-stage preference, and functional and numerical responses of *A. largoensis* on *R. indica* (Carrillo and Peña 2011, chapter 4). These studies suggested that *R. indica* could be considered an appropriate prey for this population of *A. largoensis* in terms of its reproductive success and consumption rates. However, it is unknown whether other Florida populations of *A. largoensis* will respond differently to *R. indica*.

This study was designed to determine the likelihood of Florida populations of *A. largoensis* using the invasive *R. indica* as prey. Knowledge of the mechanisms that underlie prey acceptance and choice in predatory mites has recently expanded. Studies on the predatory mites *Hypoaspis aculeifer* Canestrini (Acari: Laelapidae) and *Phytoseiulus persimilis* Athias-Henriot (Acari: Phytoseiidae) demonstrated that foraging traits and prey preferences are genetically determined and that genetic polymorphism may occur within local populations (Lesna and Sabelis 1999; Jai et al. 2002). In addition, there is increasing evidence suggesting that learning plays an important role in prey recognition and acceptance in phytoseiid mites (Rahmani et al. 2009; Schausberger et al. 2010). The likelihood of choosing *R. indica* as a prey could vary among local populations of *A. largoensis* as a result of genetically determined prey preferences, predator learning abilities, or a combination of both.

This study evaluated four populations of *A. largoensis* from Florida by determining their likelihood of consuming *R. indica* when offered eggs and larvae of *R. indica* and *Tetranychus gloveri* Banks (Acari: Tetranychidae) under no-choice and choice conditions. To detect possible genetic or behavioral plasticity in the response of *A. largoensis* to *R. indica*, the four populations of predators had different feeding histories (experience feeding on *R. indica*). Prey were chosen

based on a previous study in which *R. indica* and *T. gloveri* were the most suitable prey for the above mentioned laboratory colony of *A. largoensis* (Carrillo et al. 2010, chapter 3).

Materials and Methods

General Experimental Procedures

Experiments were conducted at $26.5 \pm 1^\circ\text{C}$, $70 \pm 5\%$ RH under a 12:12 L:D photoperiod. The experimental arenas consisted of rectangles (4×2.5 cm) cut from mature unblemished coconut (*Cocos nucifera* L.) leaves. Leaf rectangles were placed with the abaxial surface facing up on cotton squares ($6 \times 6 \times 2$ cm) saturated with water in a plastic tray (hexagonal polystyrene weighing dishes 12.7/ 8.9 cm, Fisherbrand® cat. No. 02-202-103). Paper strips (Kimwipe®, Kimberly–Clark Corporation, Roswell, GA) were placed along the edges of the leaf squares to minimize mite escape. A small plastic square (0.5×0.5 cm) was placed on top of each arena as a shelter for *A. largoensis*.

Prey Mites

The two prey species, *R. indica* and *T. gloveri*, were obtained from stock colonies reared on potted coconut palms kept in separate greenhouses following the procedure previously described (Carrillo et al. 2010). Eggs of both prey were obtained by placing 100 ovipositing females in each arena and allowing them to deposit eggs for 5 d. After this period the females were removed and the desired number of eggs adjusted by removing excess eggs with a fine brush. In the case of *T. gloveri*, extensive webbing resulted from the crowded conditions of females on the ovipositing arenas. Such extensive webbing is not observed under ‘natural’ field conditions, so a layer of webbing was carefully removed to expose *T. gloveri* eggs and allow more common conditions for *A. largoensis* to access its prey. Larvae of both prey species were transferred from the stock colonies into the arenas according to the desired prey ratios. Arenas

with two prey species had an additional moistened paper strip on the mid vein of the leaf to allow separate placement of prey, but the strip was removed before releasing the predators.

Predatory Mites

Four populations of *A. largoensis* from Florida with disparate feeding histories (previous diet) were used. Two populations were laboratory colonies that had been fed contrasting diets (following the procedures previously described, Carrillo et al. 2010, chapter 3). The first colony had been fed exclusively with *R. indica* for more than two years and will be referred to as the **R-colony**. This colony was initiated in November 2008 with 150 predators obtained from a coconut palm plantation (Malayan dwarf variety, pesticide-free, 26°02.93 N, 80°09.82 W) that had been infested with *R. indica* since 2007. Individuals collected from the same site and confirmed as *A. largoensis* were added to the colony every six months. The second laboratory colony was initiated with 50 females obtained from the R-colony, which were subjected to a diet shift from *R. indica* to *T. gloveri* two months before testing, which would have allowed the predators to develop approximately 6 generations on the new diet. This colony will be referred to as the **T-colony**. The other two populations were collected from coconut palm plantations and maintained in the laboratory for one week before testing in arenas constructed with the same leaves and prey that were present at the time they were collected. The first field population was obtained from the same coconut plantation where two years before *A. largoensis* was obtained to initiate the R-colony and will be referred to as the **experienced** field population because of its long-term previous exposure to *R. indica*, although we cannot exclude the possibility that this population fed on pollens and *T. gloveri*, as well. The second field population was collected from a pesticide-free coconut plantation (Malayan dwarf variety) that had never been infested with *R. indica* (25°35.49 N, 80°04.03 W, Tropical Research and Education Center, University of Florida, Homestead) and will be referred to as the **naïve** field population for its lack of previous exposure

to *R. indica*. Most likely, it had fed on pollen, *T. gloveri*, *Oligonychus* aff. *modestus* and *Oligonychus* sp. (Acari: Tetranychidae); *Nipaecoccus nipae* (Maskell) (Hemiptera: Pseudococcidae); *Aonidiella orientalis* (Newstead) (Hemiptera: Diaspididae); and *Aleurocanthus woglumi* Ashby (Hemiptera: Aleyrodidae) (Peña et al. 2009; Carrillo et al. 2010, chapter 3).

No-choice Tests

No-choice tests were included to ensure that each population of predators could feed on *R. indica* and *T. gloveri* and to determine whether feeding experience and laboratory rearing could affect prey consumption. Predators from the four populations were offered separately 50 eggs or 50 larvae of either *R. indica* or *T. gloveri*. Ten replicates per treatment (prey item) were used. In addition, absolute controls consisting of arenas with prey but without predators were included to ensure that mortality was caused by predation.

Choice Tests

Prey preference of the four populations was addressed by choice experiments offering 50 individuals of each prey item in the following combinations: *R. indica* eggs and *T. gloveri* eggs, *R. indica* eggs and *T. gloveri* larvae, *R. indica* larvae and *T. gloveri* eggs and *R. indica* larvae and *T. gloveri* larvae. Prey type(s) were randomly assigned to either side of the arena to discard any effect of prey location on the choice by *A. largoensis*. Fifteen replicates per treatment (prey combination) were used, including absolute controls to ensure that prey mortality was caused by predation.

Predators used in all experiments were *A. largoensis* mated females in their oviposition peak (3-5 days after adult emergence) that were starved for 8 hr before being transferred to the arenas. The numbers of each *R. indica* or *T. gloveri* stage consumed during a period of 24 h were recorded by counting the number of shriveled corpses (larvae) and by subtracting the number of

eggs remaining from the number of eggs provided. Only replicates in which the female remained within the test arena and produced at least one egg within 48 hr were considered for analyses.

Data Analysis

Data were separately analyzed for each experiment using SAS 9.2 (SAS Institute Inc.). Predation by the four populations of *A. largoensis* in the no-choice experiments was analyzed by Kruskal-Wallis tests due to variance heterogeneity and non-normality of data. Predation data under choice conditions were normally distributed (Kolmogorov- Smirnov $P > 0.05$) and had homogeneous variances (Levene test $P > 0.05$) and were analyzed by two-tailed t-tests for each *A. largoensis* population and prey combination. The likelihood of choosing one prey item over another (preference) was quantified using the index β proposed by Manly et al. (1972):

$$\beta = \left[\frac{\ln \left(\frac{N'}{N_c'} \right)}{\ln \left(\frac{N}{N_c} \right) + 1} + 1 \right]^{-1}$$

where N and N' are the numbers of each prey-stage provided and N_c and N_c' are the numbers of each prey consumed. The index assigns preference values from 0 to 1, where 0.5 represents no preference. Mean β -values were considered significant when 95% confidence intervals based on the t -distribution did not overlap with $\beta=0.5$. Preference (β) values were normally distributed (Kolmogorov- Smirnov $P > 0.05$) and had homogeneous variances (Levene test $P > 0.05$) in the experiments involving choice between *R. indica* larvae and *T. gloveri* eggs or larvae. Effects of experience and laboratory rearing on prey preference (β) by *A. largoensis* were analyzed through ANOVAs and means separated by Tukey's test. Data of the experiments involving choice tests between *R. indica* eggs and *T. gloveri* eggs or larvae were not normally distributed, so Kruskal-Wallis tests were used in this case.

Results

Prey survival in the absolute controls was always greater than 95%, which ensures that mortality in the choice and no-choice experiments was caused by predation by *A. largoensis* females.

No-choice Tests

The four populations of *A. largoensis* consumed equivalent numbers of *R. indica* eggs, with consumption ranging from 44 to 47 eggs per female/day regardless of their previous feeding experience, [χ^2 (3, N=40)= 3.90, $P=0.27$] (Table 5-1). By contrast, the four populations consumed relatively fewer *R. indica* larvae (ranging from 9 to 23 larvae per female/day) compared with the other offered prey items and differed in their predation rates ($P < 0.001$, Table 5-1). The naïve field population, not previously exposed to *R. indica*, consumed significantly fewer *R. indica* larvae than the other three populations [χ^2 (3, N=40)= 19.0, $P<0.0001$]. With respect to use of *T. gloveri* as prey, the four populations preyed on 39 to 44 *T. gloveri* eggs per female/day, showing no statistical differences among the predator populations [χ^2 (3, N=40)= 2.96, $P=0.4$]. Consumption of *T. gloveri* larvae ranged from 36 to 46 larvae per female/day. The *R*-colony, fed on *R. indica* for two years without exposure to *T. gloveri* prey, consumed significantly fewer *T. gloveri* larvae than the other three populations (χ^2 (3, N=40)= 7.5, $P=0.05$).

Choice Tests

All four populations of *A. largoensis* consumed significantly more *R. indica* eggs than *T. gloveri* eggs or larvae (Figure 5-1A). Consequently, preference indices (β) were significant (not overlapping with $\beta = 0.5$) and favoring *R. indica* eggs without significant differences among the four populations tested [χ^2 (3, N=52)= 0.68, $P=0.871$] and χ^2 (3, N=58)= 6.54 $P=0.08$ for the *R*.

indica eggs vs. *T. gloveri* eggs and *R. indica* eggs vs. *T. gloveri* larvae, respectively] (Figure 5-2).

Despite the high rate of consumption of *R. indica* eggs, the likelihood that the four *A. largoensis* populations would consume *R. indica* larvae was generally lower and differed among them (Figure 5-1C & D). The two field populations consumed significantly more *T. gloveri* eggs than *R. indica* larvae, whereas both laboratory colonies consumed similar amounts of these prey items (Figure 5-1C). Preference analysis showed that the *R*-colony, fed on *R. indica* for two years, was more likely to choose to prey on *R. indica* larvae than the other three populations tested, which showed significant preferences for *T. gloveri* eggs ($F=5.29$; $df= 3, 52$; $P<0.003$, Figure 5-2). A significant effect of laboratory rearing on the likelihood of choosing *R. indica* larvae over *T. gloveri* eggs was observed ($F=9.08$; $df= 1, 52$; $P=0.004$). Effects of experience on prey selection approached significant levels ($F=3.99$; $df= 1, 52$; $P=0.05$).

Finally, in the *R. indica* and *T. gloveri* larval-choice test, three populations of *A. largoensis* showed no significant differences in their consumption rates or preference among these prey items (Figures 5-1D and 5-2). However, the naïve field population, not previously exposed to *R. indica*, showed a significantly lower rate of consumption of *R. indica* larvae ($F=5.29$; $df= 3, 40$; $P=0.004$) (Figures 5-1D and 5-2).

Discussion

Our experiments showed that populations of *A. largoensis* consistently fed on *R. indica* eggs but larvae of *R. indica* were less often consumed. *Raoiella indica* eggs were consumed in high numbers under no-choice conditions, and chosen over *T. gloveri* eggs and larvae by the four populations of *A. largoensis* in the choice tests, regardless of their previous feeding experience. Naïve predators, not previously exposed to *R. indica*, responded to *R. indica* eggs similarly as the laboratory colony fed exclusively on *R. indica* for two years, suggesting that the four populations

of *A. largoensis* rapidly associated *R. indica* eggs with a reward and started to consume and prefer this prey item. Thus, the cues used by *A. largoensis* to recognize and associate *R. indica* eggs with a reward must be well defined and easily assimilated by the predator. Previous studies showed that oviposition rates of *A. largoensis* females (i.e., the *R*-colony) fed on a diet of *R. indica* eggs (2.36 ± 0.1153 eggs/ day, Carrillo and Peña 2011) were higher than oviposition rates when the predator was provided a diet of mixed *R. indica* stages (1.63 ± 0.27 eggs/ day, Carrillo et al. 2010), suggesting that a diet of *R. indica* eggs is more rewarding in terms of the predator's reproductive success than a mixed diet. A similar condition was observed with *Metaseiulus occidentalis* (Nesbitt) (Acari: Phytoseiidae), which showed better reproductive parameters when fed on *Tetranychus pacificus* McGregor (Acari: Tetranychidae) eggs compared to a diet of mixed immature stages (Bruce-Oliver and Hoy 1990). Preference of *A. largoensis* for *R. indica* eggs might be explained by its ability to recognize this prey item and associate it with high quality food.

By contrast, the four *A. largoensis* populations differed in their likelihood of preying upon *R. indica* larvae. The no-choice experiments showed that *A. largoensis* generally consumed fewer individuals of *R. indica* larvae compared with the other offered prey items. The laboratory *R*-colony showed the highest likelihood of preying on *R. indica* larvae in the choice experiments and the lowest likelihood of consuming *T. gloveri* larvae in the no-choice experiments, which could have resulted from genetic selection due to it having access only to *R. indica* for over two years (approximately 50 generations). Moreover, prey consumption analysis showed that laboratory colonies had a higher likelihood of consuming *R. indica* larvae over *T. gloveri* eggs than the two field populations. These results suggest that laboratory colonies could have been genetically selected to prey on *R. indica*, and that this genetic change was not eliminated by two

months of rearing on *T. gloveri* in the *T*-colony. For instance, the three populations that had previous exposure to *R. indica* (*R*-colony, *T*-colony and the experienced field population) consumed significantly more *R. indica* larvae than the naïve field population with no previous exposure to *R. indica*. The naïve field population showed the lowest likelihood of consuming *R. indica* larvae both in choice and no-choice experiments, suggesting that its lack of experience in preying on *R. indica* might have affected its ability to consume this prey.

Our experiments did not test *R. indica* nymphs and adults as prey. However, previous studies indicated that female *A. largoensis* (the *R*-colony) had difficulty handling nymphs and adults and consumed significantly more *R. indica* eggs and larvae than nymphs and adults (Carrillo and Peña 2011, chapter 4). While preying on nymphs and adults, predators would often stop feeding, turn to cleaning activities, then return to continue feeding on the same prey item, thus prolonging the handling time. Consequently, one could assume that the likelihood of consuming *R. indica* nymphs and adults would have been lower than the likelihood of consuming *R. indica* eggs or larvae observed in this study.

Overall, the results of our studies suggest that multiple mechanisms can be involved in the response of *A. largoensis* to *R. indica*. It is possible that these mechanisms do not act independently but are conditionally expressed depending on the conditions that the predator faces (Sabelis and Lesna 2010). For instance, it is likely that populations of *A. largoensis* are subject to relatively long periods of exposure to *R. indica*, which has become by far the dominant arthropod on coconuts in areas of recent invasion. Under these circumstances, genetically determined prey preferences could be selected and the likelihood of *A. largoensis* preying on *R. indica* active stages may increase. Simultaneously, experienced predators could learn to optimize their foraging activities and also increase the likelihood of preying on *R. indica* larvae. However,

when food quality declines and predators are forced to disperse, dispersal might bring *A. largoensis* to a location where another food type is dominant. Generalist predators must retain the ability to switch prey (Sabelis and Lesna 2010). Our results suggest that *A. largoensis* can switch to novel prey after a period of starvation despite possible conditioning of prey preferences due to genetic selection or experience. In conclusion, *A. largoensis* showed plasticity in its use of *R. indica* larvae, which could be related to genetic selection, learning or a combination of both. Moreover, the environmental conditions that predators face can modulate the expression of these.

Another factor that could affect the prey preference of phytoseiid mites is the presence of feeding attractants and deterrents (Hoy and Smilanick 1981; Vet and Dicke 1992). It has been suggested that *R. indica* could produce repellent compounds because of the presence of droplets located at the tips of the dorsal body setae and at the tip of the egg's pedicel. The marked preference for *R. indica* eggs observed in the present study and the high 'profitability' of this prey for *A. largoensis* (Carrillo et al. 2010, chapter 3) do not support the hypothesis of toxic or repellent compounds being present in the droplets of *R. indica* eggs. However, it is possible that the droplets found on other *R. indica* larvae (and other life stages) could have a different composition and affect the ability of *A. largoensis* to feed on them. The possible existence of kairomones or allomones produced by *R. indica*, together with herbivore-induced plant volatiles that may alter the searching behavior of *A. largoensis*, should be investigated to better understand their role on the prey choice of this predator. In addition, mites can have other defense mechanisms that could directly influence their attractiveness to predators. The hardness of the cuticle could serve as a defense mechanism against predators (Alberti and Crooker 1985). Observations made during the experiments showed that *A. largoensis* usually required a single attack to successfully penetrate the chorion and imbibe all of the egg contents. In contrast, *A.*

largoensis females probed several times in order to penetrate the larval cuticle, had a longer handling time, and did not consume the larval prey entirely. Thus, preference of *A. largoensis* for *R. indica* eggs and the lack of preference for larvae could be related to the energy spent and the nutritional reward obtained when feeding on each of them.

Findings of this study suggest that disparate populations of *A. largoensis* may be responding to the invasion by *R. indica* either by learning or evolving genetically to be better predators of this invasive pest. These findings may be important for future biological control programs targeting *R. indica*. Since *R. indica* gained importance as an invasive pest in the Neotropics, surveys for natural enemies in several places of the world (Roda et al. 2008; Peña et al. 2009; Ramos et al. 2010; Zannou et al. 2010; Carrillo et al. 2011; Taylor et al. 2011; Hoy, pers. comm.) have identified *A. largoensis* as the most abundant predator, and often the only phytoseiid species associated with *R. indica*. Further studies are needed to determine the efficacy of these predator populations in controlling *R. indica*, and whether there are additional differences in populations of *A. largoensis* with regard to their use of *R. indica*.

Table 5-1. Predation of *R. indica* and *T. gloveri* eggs and larvae by four local populations of *A. largoensis* with disparate feeding history (previous diet) under no-choice conditions. Mean number prey items consumed in 24 hrs \pm Std. Dev.

<i>Amblyseius largoensis</i> Population	Number of individuals consumed/day			
	<i>R. indica</i> eggs	<i>R. indica</i> larvae	<i>T. gloveri</i> eggs	<i>T. gloveri</i> larvae
R-colony	47.2 \pm 2.7 a	23.4 \pm 6.7 a	39.4 \pm 8.2 a	35.5 \pm 11.1 b
Experienced field	44.4 \pm 4.2 a	24.3 \pm 7.1 a	40.5 \pm 3.9 a	41.7 \pm 6.4 a
T-colony	46.6 \pm 3.5 a	21.7 \pm 5.4 a	42.6 \pm 5.8 a	46.3 \pm 2.3 a
Naïve field	45.5 \pm 3.0 a	9.2 \pm 4.8 b	43.7 \pm 5.0 a	41.3 \pm 9.6 a
χ^2 (3,N=40)	3.9	18.9	2.96	7.5
<i>P</i> value	0.3	<0.001	0.4	0.05

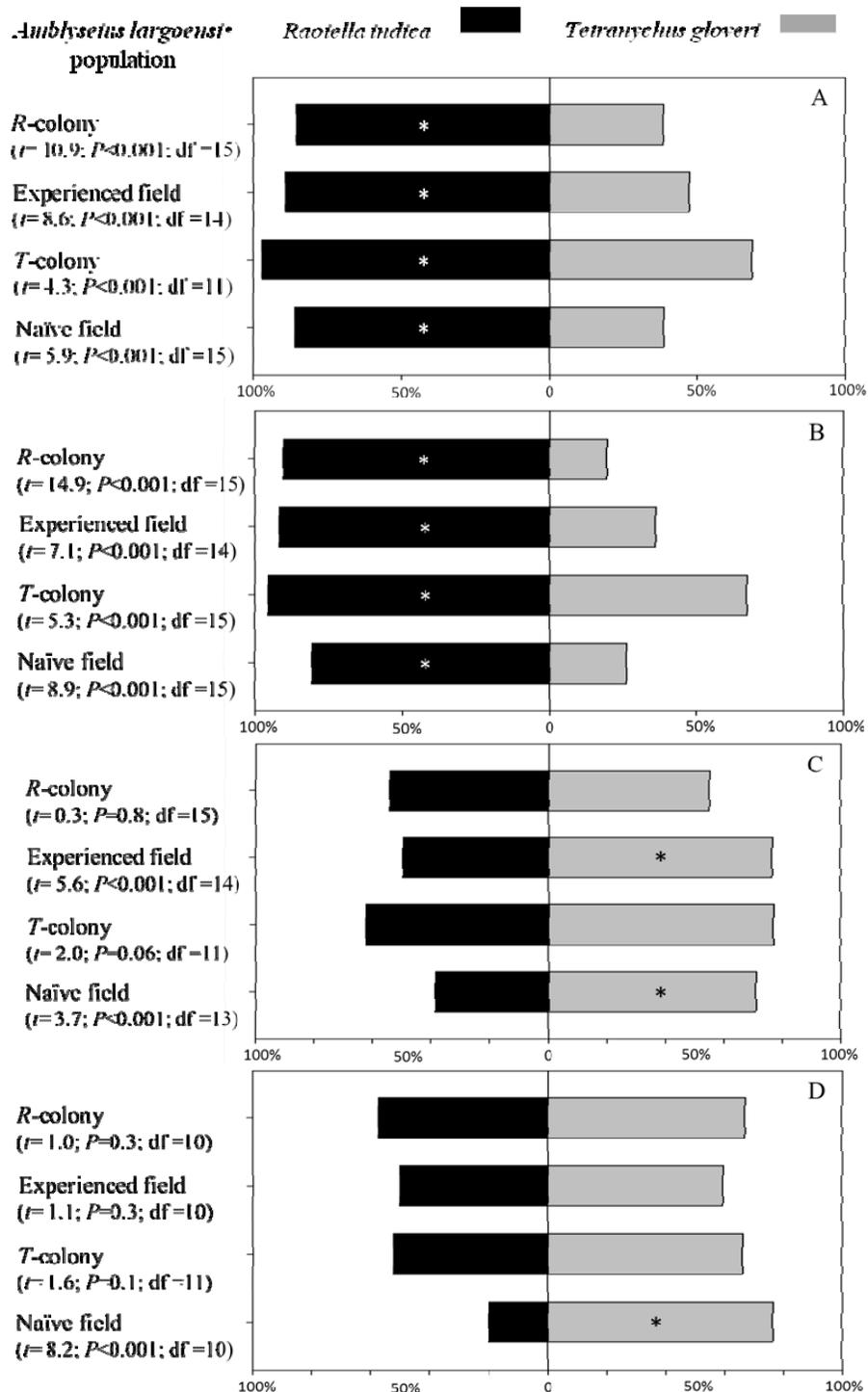


Figure 5-1. Percentage of individuals of *R. indica* (black bars) and *T. gloveri* (grey bars) consumed by four populations of *A. largoensis* with disparate feeding histories (previous diet) under choice conditions. (A) *R. indica* eggs vs. *T. gloveri* eggs (B) *R. indica* eggs vs. *T. gloveri* larvae (C) *R. indica* larvae vs. *T. gloveri* eggs (D) *R. indica* larvae vs. *T. gloveri* larvae.* Represents significant differences in two-tailed *t*-tests ($P<0.05$) for each *A. largoensis* population and prey combination.

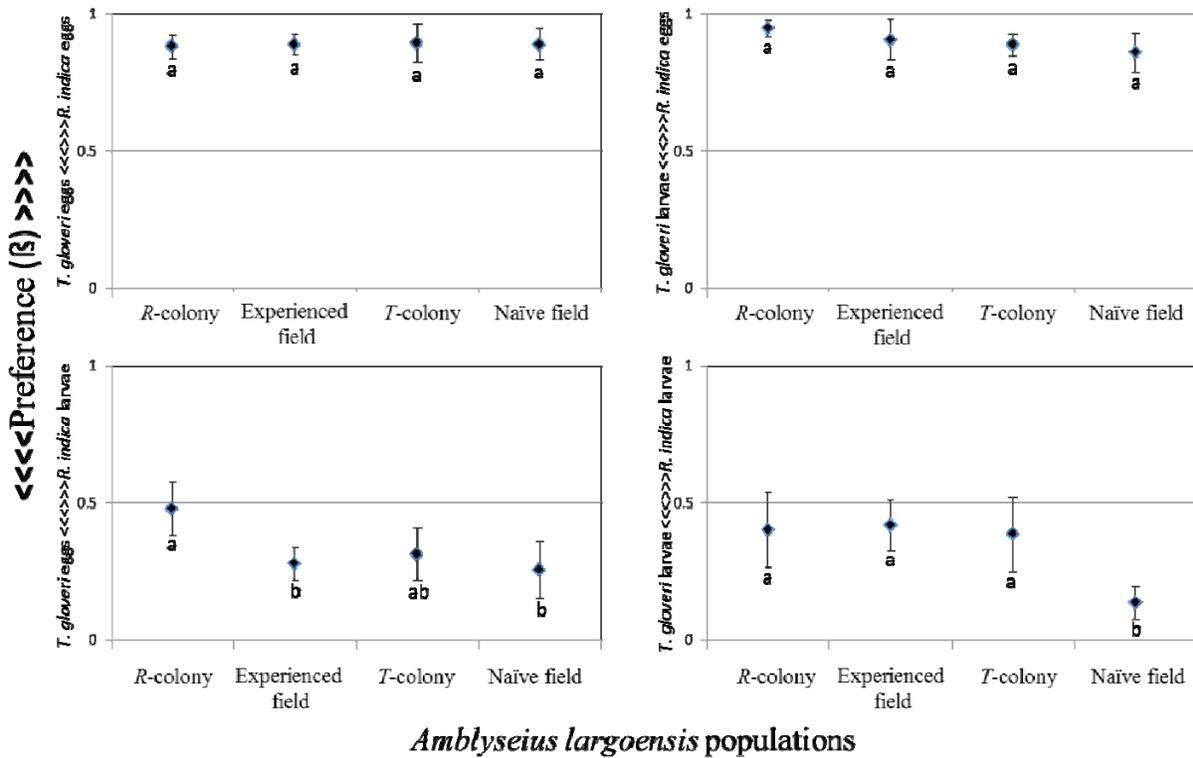


Figure 5-2. Prey preference of four local populations of *A. largoensis* with disparate feeding history (previous diet) when preying on *R. indica* and *T. gloveri* eggs and larvae under choice conditions. The preference index β assigns preference values from 0 to 1, where 0.5 represents no preference. Mean β -values we considered significant when 95% confidence intervals (error bars) based on the t-distribution did not overlap with $\beta=0.5$. Differences in β -values among the four populations of *A. largoensis* represented by letters were analyzed through ANOVAs or Kruskal-Wallis tests depending on normality of data.

CHAPTER 6
EFFECT OF *AMBLYSEIUS LARGOENSIS* (ACARI: PHYTOSEIIDAE) ON *RAOIELLA INDICA* (ACARI: TENUIPALPIDAE) USING PREDATOR EXCLUSION AND PREDATOR RELEASE TECHNIQUES

Summary

Exclusion and release tactics were used to obtain coconut palms with disparate levels of *A. largoensis* in order to quantify their effects on *R. indica* densities. Four treatments consisting of a range of *A. largoensis* release rates (0= control, 1:10, 1:20 and 1:30 *A. largoensis*: *R. indica*) were tested. The release of *A. largoensis* resulted in a significant reduction of *R. indica* densities and less leaf area damaged in the coconut palms compared to the controls. The largest pest density reduction (~92%) was observed at the highest predator release rate (1:10 *A. largoensis*: *R. indica*). The other two release rates (1:20 and 1:30 *A. largoensis*: *R. indica*) caused significant and equivalent reductions in pest densities (55 and 43%, respectively). Results of this study support the hypothesis that *A. largoensis* is an important mortality factor of *R. indica* and should be considered as a key biological control agent in IPM programs targeting *R. indica*. Key words: mites, *Cocos nucifera*, invasive species, *Raoiella indica*, *Amblyseius largoensis*, exclusion, predator release, pest densities, damage.

Introduction

The arrival and establishment of *Raoiella indica* has had a serious economic effect on coconut production and the nursery palm industry in the Caribbean, Florida and other sites in the Neotropics (Roda et al. 2008; Carrillo et al. 2011a, Appendix). Efforts have been made to identify natural enemies with potential in biological control and IPM programs targeting *R. indica*. The predatory mite *Amblyseius largoensis* (Muma) (Acari: Phytoseiidae) responded numerically to the arrival of *R. indica* in Florida (Peña et al. 2009), which motivated detailed studies on the efficiency of this predator. An initial study determined that this predator was able

to feed, develop and reproduce on a diet consisting solely of *R. indica* showing ‘better’ reproductive parameters than when feeding on other prey and pollen (Carrillo et al. 2010, chapter 3). Follow-up studies determined that *A. largoensis* responded both functionally and numerically to increasing densities of *R. indica*, and showed a marked preference for eggs over larvae, nymphs and adults of the phytophagous mite (Carrillo and Peña 2011, chapter 4). Further studies examined the response of four populations of *A. largoensis* with disparate previous exposure to *R. indica* and *Tetranychus gloveri* (Acari: Tetranychidae). The four populations of *A. largoensis*, including predators never exposed to *R. indica*, were likely to accept and consume high numbers of *R. indica* eggs. However, predators previously exposed to *R. indica* were more likely to feed on *R. indica* larvae than were naïve predators. The combined previous studies (Chapters 2-5) indicate that *A. largoensis* is actively responding to the invasion by *R. indica* and provide a framework to hypothesize that this predator has potential to be used in biological control and IPM programs targeting *R. indica*. The objective of this study was to provide a quantitative evaluation of the ability of *A. largoensis* to reduce *R. indica* densities at four predator-prey ratios.

Materials and Methods

The experimental approach combined exclusion and release techniques to obtain differential levels of *A. largoensis* on palms infested with *R. indica*. The experiment was conducted inside a climate-controlled 43.2 m² glasshouse house ($26.5 \pm 4^\circ\text{C}$, RH $70 \pm 20\%$) located at the Tropical Research and Education Center in Homestead, FL ($25^\circ35.49\text{ N}$, $80^\circ04.03\text{ W}$). Thirty-two 1-year-old potted, pest-free and unsprayed Malayan dwarf coconut palms (about 1.5 m in height) were used in this experiment. A middle frond in each palm was selected as the experimental unit and remaining fronds were manually removed. A yellow “tagging” tape (~5 cms wide) coated with Tanglefoot[®] was tied around the base of the frond to exclude any crawling arthropods. Palms were arranged on benches and clear plastic sheets ($1.5 \times 3\text{ m}$) were hung from

the roof of the glasshouse to isolate each individual palm. Fronds were then inspected with a hand lens (20 ×) and any arthropod [*Aonidiella orientalis* Newstead (Hemiptera: Diaspididae), *Tetranychus gloveri* Banks (Acari: Tetranychidae), *A. largoensis*] was removed by hand every three days during 2 consecutive weeks before being inoculated with *R. indica*. *Raoiella indica* specimens used for infestation were obtained from a red palm mite colony stock (Carrillo et al. 2010). Two *R. indica*-infested pinnae were then attached to the abaxial surface of each palm frond with a hair clip. Infested palms were left undisturbed for 30 days. After this period, *R. indica* establishment was evaluated by counting the number of motile stages and eggs per pinna. Treatments were allocated to each palm depending on the degree of infestation. Four *A. largoensis* release rates (0= control, 1:10, 1:20 and 1:30 *A. largoensis*: *R. indica*) were tested with 8 replicates per treatment. Predators were obtained from a coconut palm plantation (Malayan dwarf variety, pesticide-free, 26°02.93 N, 80°09.82 W) infested with *R. indica* since 2007. Predators were confirmed as *A. largoensis* and maintained on rearing arenas under laboratory conditions for one week before testing. Predators were then transferred from the rearing arenas to the experimental coconut fronds using a camel hair brush. After treatment, all fronds were carefully inspected every two weeks with a 20× hand lens and any undesirable predators or phytophagous arthropods removed. Three months later, fronds were excised, taken to the laboratory, and the total number of *R. indica* and *A. largoensis* eggs and motiles per pinna were inspected under a dissecting microscope (50×). The surface area of each of the pinnae was estimated by measuring its width and height and calculating the area using $\frac{1}{2}$ base × height in order to account for the triangular shape of the pinnae. Leaf damage was quantified by measuring the width and height of necrotic areas and calculated in the same manner.

Statistical Analysis: *Raoiella indica* and *A. largoensis* density values were normally distributed (Kolmogorov-Smirnov $P > 0.05$) and had homogeneous variances (Levene test $P > 0.05$). The effect of releasing rates on density of *R. indica* eggs and motiles per pinna was analyzed through covariance analysis and Tukey separation tests (Proc ANCOVA SAS Inc.). The number of predators released per plant was used as the covariate and the analysis was weighted with the number of pinnae per palm. Data on the leaf area damaged (showing necrosis) were not normally distributed, so Kruskal-Wallis tests were used in this case.

Results

One month after inoculation, *R. indica* mean infestation levels ranged from 41.3 ± 14.6 to 46.6 ± 16.5 (Mean \pm SEM) *R. indica* motile stages per pinna (Table 6-1). Random allocation of treatments ensured similar infestation levels between treatments before predators were released ($df= 3, 8; F= 0.15; P= 0.92$). The number of predators released ranged from 0 (control treatment) to 4.1 ± 1.1 *A. largoensis* females per pinnae according to the release rates and the infestation level (Table 6-1). Three months after predators were released, *R. indica* mean infestation levels ranged from $(124.7 \pm 94.1$ to $999.6 \pm 94.6)$ *R. indica* motile stages per pinna (Table 6-1, Figure 6-1).

Although release of *A. largoensis* females did not result in decline of *R. indica* initial infestation levels, the release of *A. largoensis* resulted in a significant reduction in *R. indica* densities relative to the densities found on predator-free palms ($df= 4, 28; F= 7.76; P < 0.001$). *Raoiella indica* reached 70.7 ± 7.2 individuals/ cm^2 (mean \pm SEM) in predator-free palms, and 5.3 ± 7.1 , 31.7 ± 5.7 and 40.4 ± 5.9 (individuals/ cm^2) when *A. largoensis* was released at three rates of 1:10, 1:20 and 1:30 *A. largoensis*: *R. indica*, resulting in a 92, 54 and 42 percent reduction of *R. indica* densities, respectively. The highest *R. indica* density reduction was observed at the highest predator release rate (Figure 6-2).

More motile stages of *R. indica* were present than eggs on the two treatments with lower *A. largoensis* release rates and on the predator-free palms. In contrast, *R. indica* populations consisted of more *R. indica* eggs than motile stages in the treatment with the highest *A. largoensis* release rate (Figure 6-2C). Motile stage densities in predator-free palms (51.5 ± 5.9 *R. indica* motiles / cm², mean \pm SEM) were significantly higher than those found in the release treatments (2.0 ± 5.8 , 20.1 ± 4.7 and 26.5 ± 4.9 *R. indica* motiles/cm², mean \pm SEM), resulting in 96, 61 and 48 percent reduction of *R. indica* motile stages in the 1:10, 1:20 and 1:30 *A. largoensis*: *R. indica* release rates, respectively (df= 4, 28; $F= 6.87$; $P < 0.001$) (Figure 6-2B). *Raoiella indica* egg densities on predator-free palms (19.3 ± 2.2 individuals/ cm², mean \pm SEM) were significantly higher than those found in palms where predators were released (3.3 ± 2.2 , 11.6 ± 1.7 and 13.9 ± 1.8); predator releases resulted in an 82, 39 and 27 percent reduction in *R. indica* egg densities in the three *A. largoensis*/*R. indica* release rates, respectively (df= 4, 28; $F= 5.13$; $P < 0.01$) (Figure 6-2A).

Amblyseius largoensis established on all palms where it was released and was not detected in the control treatments. However, the numbers of *A. largoensis* recovered in the final destructive sampling (0.25 ± 0.8 , 0.36 ± 1.4 , 0.4 ± 1.3 and 0 ± 0 predators per/pinnae, in the 1:10, 1:20 and 1:30 *A. largoensis*: *R. indica* release rates, respectively) were lower than the numbers released (Table 6-1), and did not vary along the release treatments (df= 4, 28; $F= 10.04$; $P < 0.001$). Besides *A. largoensis*, no other predators were detected.

The leaf area per pinna and the number of pinnae per palm were equivalent among the palms assigned to each *A. largoensis* release rate ($F= 0.69$ $P=0.56$ and $F= 0.11.4$ $P=0.95$, respectively). In contrast, the percentage of leaf area showing necrosis was significantly higher

in palms where *A. largoensis* was excluded compared to palms where the predator was released [χ^2 (3, N=32)= 99.83, $P<0.0001$].

Discussion

Convincing evidence of the efficacy of a natural enemy requires not only quantitative evaluations of pest densities with and without the presence of the natural enemy, but also needs to differentiate the mortality caused by the natural enemy from other biotic and abiotic factors (Luck et al 1988; Van Driesche and Bellows 1996). In our experiment, the use of exclusion tactics (sticky barriers, hand removal and cages) was used to quantify *R. indica* densities in palms with different ratios of *A. largoensis*, including predator-free palms. No other predators were detected and palms were kept under controlled environmental conditions, which suggest that the observed differences in *R. indica* densities could only be attributed to *A. largoensis*.

Previous studies indicated that *A. largoensis* had a marked preference for *R. indica* eggs and difficulties feeding on motile stages (Carrillo and Peña 2011, chapter 4). In this experiment, pest density reductions were observed both in the egg and motile stages of *R. indica*. However, the proportion of eggs and motile stages in the treatment with the highest release rate, where the highest pest reduction was observed, differed from all other treatments. This could have been caused by a higher predation of eggs in this treatment that resulted in fewer individuals developing into motile stages. By contrast, the treatments with the two lowest release rates and the predator-free treatment resulted in an accumulation of motile stages and substantially higher prey densities. It is possible that at low predator densities more *R. indica* individuals completed the egg stage and became larvae, a stage when they are less likely preyed upon by *A. largoensis*. These results suggest that *A. largoensis* can be effective in controlling *R. indica* only when it consumes a large proportion of *R. indica* eggs and reduces the accumulation of motile stages. Moreover, the ability of *A. largoensis* to suppress *R. indica* might depend not only on the

predator –prey ratio but also on the population density of *R. indica*. It is possible that as for other generalist predators (James 1990; McMurtry 1992), *A. largoensis* could have its major effect at low population densities of *R. indica*.

Predation on *R. indica* by *A. largoensis* resulted in a reduction of the coconut leaf area showing damage. The proportion of damaged leaf tissue differed between treated and untreated palms, but not among the three release rates tested. Predator-free palms showed necrosis four months after being inoculated with *R. indica*. Therefore, the experiment was concluded at that time in order to prevent advanced necrosis and subsequent reduction of host plant quality, which would eventually affect *R. indica* survivorship. In conclusion, our results suggest that *A. largoensis* can reduce *R. indica* densities and the damage inflicted to coconut fronds under greenhouse conditions.

Although *A. largoensis* caused a significant reduction in *R. indica* densities relative to the predator-free palms, predators were unable to eliminate *R. indica* during the period evaluated. It is possible that a higher number of predators is required to suppress *R. indica* or that a longer time could have resulted in elimination of *R. indica*. The highest pest reduction (~92% of total density) in our experiment was observed at the highest release rate (1:10 *A. largoensis*: *R. indica*), which suggests that inundative releases at higher release rates than the ones used in this experiment could result in larger pest density reductions and better control of *R. indica*.

The use of exclusion tactics was useful to quantify *R. indica* densities in palms with different levels of *A. largoensis*. An alternative to exclusion by physical means used in our experiment is the use of acaricides to reduce mite numbers (Braun et al. 1987; McMurtry et al. 1992; Cuthbertson et al. 2003). Some chemicals are highly toxic to predators but less toxic to phytophagous mites (Roush and Hoy 1978) so they can be used for predator exclusion purposes.

However, the use of pesticides has often led to problems of interpreting results because of possible pesticide-induced physiological effects on the plants (Jones et al. 1983), pesticide-induced sex ratio bias and stimulation of the reproductive potential of the prey population (hormoligosis) (Bartlett 1968; Dittrich et al. 1974; Hoy et al. 1979; Maggi and Leigh 1983; Cuthbertson et al. 2003). Despite these problems, acaricidal disruption is a quick and easy way to evaluate the effect of natural enemies at a large scale (Luck et al. 1988). Thus, the feasibility of using this technique to test the effects of different of *A. largoensis* release rates on *R. indica* populations under natural conditions should be investigated.

Our experiments showed that *A. largoensis* is an important mortality factor of *R. indica*, and its effects can reduce damage on coconut leaves caused by this pest. However, additional mortality factors may be needed to control *R. indica* populations, especially mortality factors affecting motile stages of *R. indica*.

Table 6-1. Initial mean *R. indica* infestations and *A. largoensis* release rates on coconut palms and mean *R. indica* infestations and number of *A. largoensis* recovered three months after predator release ($26.5 \pm 4^\circ\text{C}$, RH $70 \pm 20\%$) in a climate-controlled glasshouse at TREC, Homestead, FL.

Treatment release rates <i>A. largoensis</i> : <i>R. indica</i>	Infestation level before predator release		Infestation level and predators recovered	
	<i>R. indica</i> motiles/pinna (mean \pm SEM)	<i>A. largoensis</i> released/pinna (mean \pm SEM)	<i>R. indica</i> motiles/pinna (mean \pm SEM)	<i>A. largoensis</i> recovered/pinna (mean \pm SEM)
1 : 10	41.3 \pm 10.9	4.1 \pm 1.1	124.7 \pm 94.1	0.3 \pm 0.1
1 : 20	46.6 \pm 11.6	2.3 \pm 0.6	376.7 \pm 75.4	0.3 \pm 0.1
1 : 30	41.5 \pm 7.50	1.3 \pm 0.2	546.7 \pm 78.1	0.4 \pm 0.1
0 : X(Control)	43.3 \pm 10.9	0.0 \pm 0.0	999.5 \pm 94.5	0.0

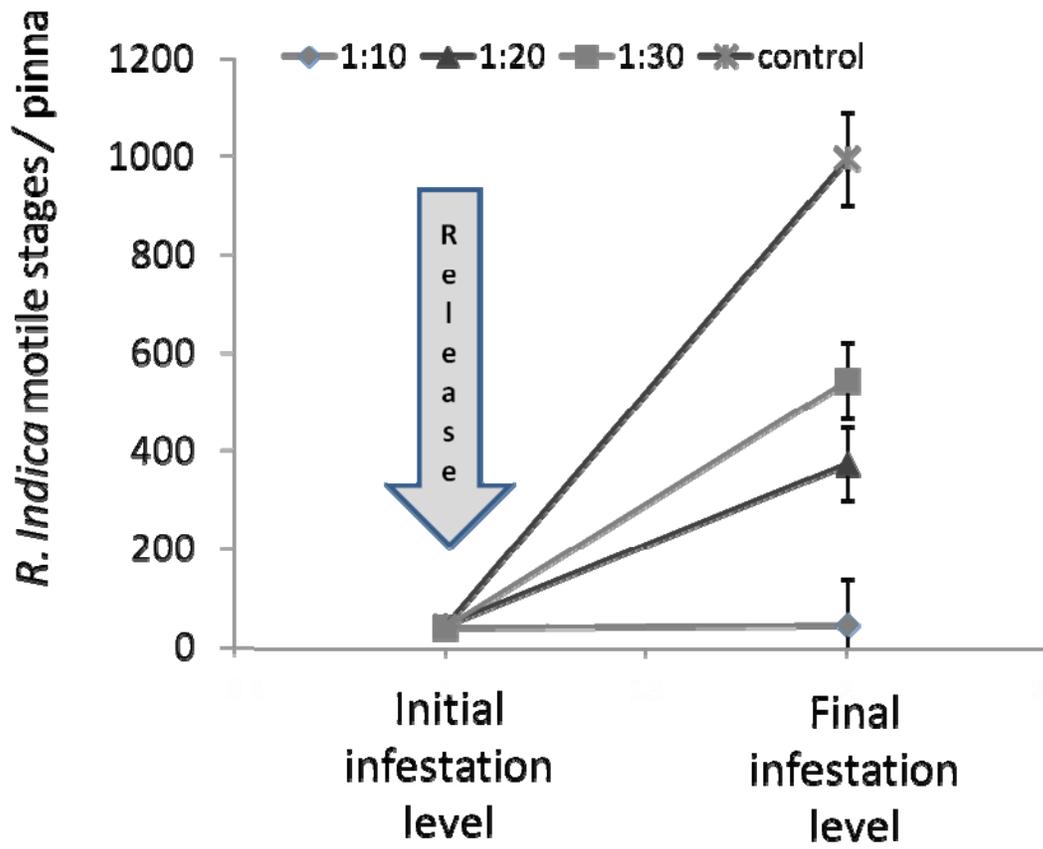
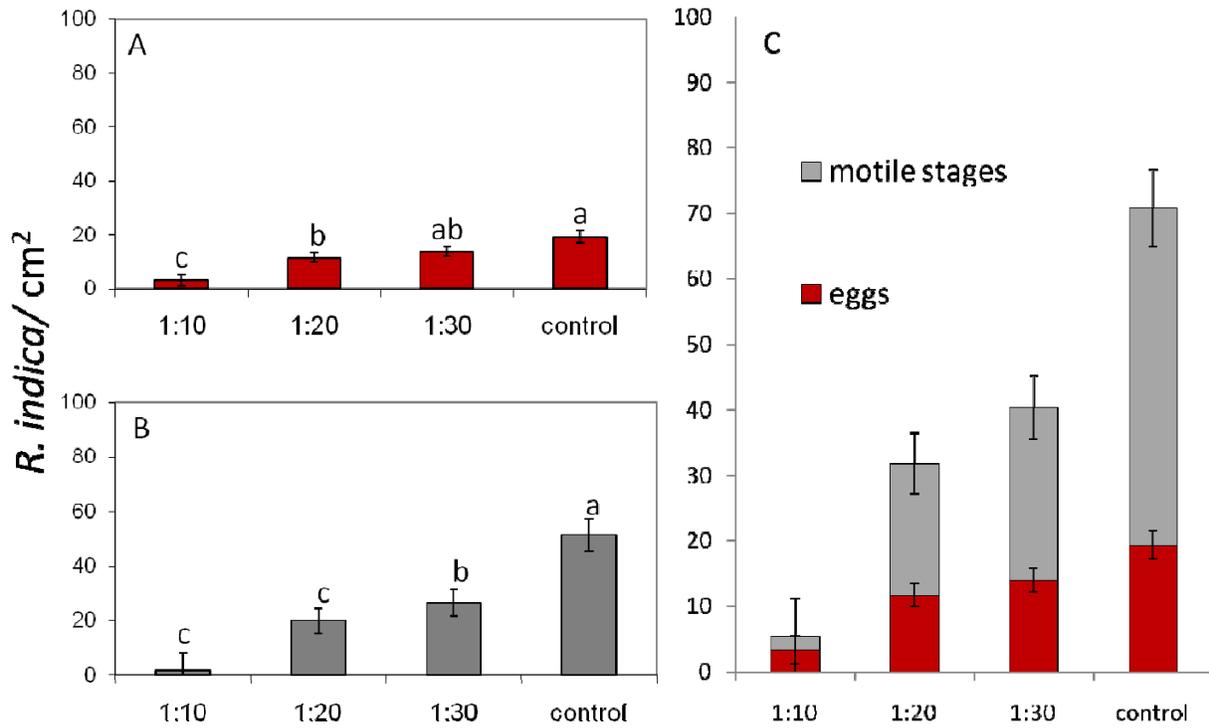


Figure 6-1. Effect of four release rates of *A. largoensis* (0= control, 1:10, 1:20, 1:30 *A. largoensis*: *R. indica*) on *R. indica* motile stage densities (mean \pm SEM represented by the error bars).



A. largoensis release rates

Figure 6-2. Effect of four release rates of *A. largoensis* (0= control, 1:10, 1:20, 1:30 *A. largoensis*/*R. indica*) on *R. indica* densities. A. *R. indica* egg density B. *R. indica* motile stages density, C. *R. indica* total density showing the proportion of eggs and motile stages. Error bars represent standard errors.

CHAPTER 7 CONCLUSIONS

The objective of this dissertation was to determine the role of key mortality factors affecting *R. indica* within the natural enemy complex that inhabits coconut palms in Florida. Previous reports (Peña et al. 2009) and the information presented here indicate that 9 predator species have been reported feeding on *R. indica* in Florida. However, with the exception of *A. largoensis*, all other predators are found only occasionally, in low numbers and depend on other prey species (Chapter 2). The studies presented here suggest that *A. largoensis* is the most important biotic factor causing mortality of *R. indica* in Florida.

The first study dealt with the development and reproduction of *A. largoensis* feeding on *R. indica*, pollen, and on different potential prey. In contrast to the results of tests with other predators, the study showed that *R. indica* is a good prey for *A. largoensis*. Predators fed on a *R. indica* diet had faster development and greater intrinsic rate increase than those fed on other prey or pollen, suggesting that the observed relationship between these two species could be important in terms of biological control of *R. indica*.

The second study of prey stage-preferences and functional and numerical responses of *A. largoensis* to *R. indica* density, was useful to gain insight on the predator-prey interactions between these two species. The study showed that *A. largoensis* has a marked preference for *R. indica* eggs over other developmental stages, which has implications for the effectiveness of the predator. By consuming primarily eggs, the predator kills its prey before it can cause injury to the host and before it reproduces (Huffaker et al. 1970). In contrast, it was observed that the predator had difficulties preying on the active stages of *R. indica*, indicating that other mortality factors are necessary to hold in check the reproductive stages. The second part of the study also showed that *A. largoensis* is capable of responding both functionally and numerically to changes

in the population density of *R. indica*. These results signaled that *A. largoensis* could prey on *R. indica* in a density dependent manner by increasing prey consumption and their own reproduction in response to increasing prey density, an important attribute for potentially effective natural enemies (Rosen and Huffaker 1983).

The third study explored differences in the use of *R. indica* by four populations of *A. largoensis* that differed in their previous exposure to *R. indica*. This study revealed that all predators, including those with no previous exposure to *R. indica*, had a high likelihood of consuming and preferring *R. indica* eggs over eggs and larvae of *T. gloveri*. However, it also showed that populations of *A. largoensis* varied in their consumption of *R. indica* motiles. Predators with previous exposure to the invasive species were more likely to consume *R. indica* larvae than naïve predators. Overall, the findings of this study suggest that disparate populations of *A. largoensis* may be responding to the invasion by *R. indica* either by learning or evolving genetically to be better predators of this invasive pest.

Finally, the fourth study using predator exclusion and release techniques proved that *A. largoensis* can cause significant reduction in pest densities and can be considered an important mortality factor of *R. indica* in Florida, and also contributes to a reduction of the damage to coconut caused by this pest. However, additional biotic mortality factors may be needed to suppress the high populations of this pest observed after its recent invasion of Florida.

One method to achieve greater pest suppression could be through augmenting the effect of biotic factors that are already acting over *R. indica* in Florida. For instance, besides *A. largoensis*, the other natural enemy showing some potential as predator of *R. indica* is the lacewing species *Ceraochrysa claveri* Navás (Neuroptera:Chrysopidae). Although this predator depends on other prey to complete development, it has been repeatedly found associated with *R.*

indica. It has been observed that its first larval instars are voracious predators of this pest. Methods of increasing the effects of *A. largoensis* and *C. claveri* on *R. indica* merit investigation. Techniques such as augmentative release, use of predator attractants and provision of alternative food sources for increasing predator populations in *R. indica* infested areas should be explored. In addition, research towards identifying chemical control practices that can preserve these natural enemies for their use in local IPM plans targeting *R. indica* should be conducted.

An alternative to biological control with the natural enemy fauna present in Florida is the introduction of natural enemies in a classical biological control approach. Considering that the natural enemy complex of *R. indica* in Florida is composed of only generalist predators it will be desirable to find natural enemies more narrowly specific to *R. indica*. However, until now there are no reports or evidence of the existence of more effective predators in any place recently surveyed for natural enemies of *R. indica*. Other geographical areas should also be searched for natural enemies of this pest. The high diversity of *Raoiella* species in the Middle East region (Chapter 1) suggests the species could have originated in this region and, theoretically, its most effective natural enemies could be found there. Nevertheless, results from one of the studies of this dissertation suggest that a long time of association between *A. largoensis* and *R. indica* could result in more aggressive strains of this predator. The possible existence of more aggressive strains, biotypes or cryptic species of *A. largoensis* from other parts of the world that are reproductively isolated from the *A. largoensis* already present in Florida should be investigated. Moreover, the recent report of four species of acaropathogenic fungi attacking *R. indica* in Puerto Rico (Rodrigues and Colon, unpublished) is promising and deserves special attention. The tropical conditions and high *R. indica* populations found in areas of recent invasion could favor

epizootics caused by acaropathogenic fungi which could cause a decline in *R. indica* densities and possibly enhance the regulatory capacity of *A. largoensis* and other predators.

In closing, an important consideration for this research is that all studies were conducted excluding abiotic factors that could cause mortality on *R. indica*. In natural conditions populations of *R. indica* will be affected not only by natural enemies, but also by factors such as temperature and precipitation (Moutia 1958; Sakar and Somchoudhury 1989). For instance, *R. indica* infested areas in three sites in Florida (Palm Beach, Broward and Miami-Dade counties) were sampled from January 2008 to March 2010. At all three sites the populations were highest in the first four months after the initial infestation and a steady negative trend on population density was observed thereafter. The decline in *R. indica* densities could be related to the subtropical conditions of the state as well as the build-up of predators, primarily *A. largoensis* (Duncan et al. 2010; Peña, unpublished). As seen in other invasive species, the rates of population growth can vary markedly during the early stages of invasion (Crooks and Soule 1999). It is possible that the high rates of population growth that *R. indica* has displayed in its early stages of invasion will change over time when other mortality factors present in the environment affect this invasive species. It will be important to quantify the effects of abiotic factors on *R. indica*. Moreover, the complementarity or antagonisms between abiotic and biotic factors affecting *R. indica* should be investigated to better understand the population dynamics of this invasive pest in Florida.

APPENDIX
HOST PLANT RANGE OF *RAOIELLA INDICA* HIRST (ACARI: TENUIPALPIDAE) IN
AREAS OF INVASION OF THE NEW WORLD⁵

Summary

Raoiella indica has spread rapidly through the Neotropical region where the mite damages economically and ecologically important plants. Three studies were conducted to determine the host plant range of *R. indica*, using the presence of colonies containing all life stages as an indicator of reproductive suitability. Periodic surveys at the Fairchild Tropical Botanic Garden (Miami Dade County, FL, USA) and the Royal Botanical Gardens (Port of Spain, Trinidad and Tobago) identified 27 new reproductive host plants. The reproductive suitability of 2 dicotyledonous species and 3 native Florida palm species was examined. An updated list of reproductive host plants of *R. indica* is presented. All reported reproductive hosts (91 plant species) of *R. indica* are monocots from the orders Arecales (Arecaceae), Zingiberales (Heliconiaceae, Musaceae, Strelitziaceae, Zingiberaceae) and Pandanales (Pandanaceae). Most are palms of the family Arecaceae that originated in areas of the Eastern Hemisphere; about one fourth of the reported hosts are native to the New World and could be considered new host associations of *R. indica*. Six years after the initial detection in the Caribbean, *R. indica* has expanded its host plant range. Here we report 27 new reproductive host of *R. indica* that represent 30 % of increase on previous host plant records. As this mite continues spreading in the Neotropical region a great diversity of plants could potentially be affected. Key words: *Raoiella indica*, reproductive hosts, monocotyledons, Arecaceae, palms.

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Introduction

Raoiella indica Hirst (Acari: Tenuipalpidae), also called the red palm mite, is a phytophagous mite that recently invaded the Western Hemisphere. This mite is a polyphagous species that can reach very high populations and cause significant damage to various plant species. It is also the first mite species observed feeding through the stomata of its host plants (Ochoa et al. 2011). Through this specialized feeding habit *R. indica* probably interferes with the photosynthesis and respiration processes of their host. However, the damage caused by this species to most of its host plants has not yet been characterized. In coconut, *R. indica* feeding causes an initial bronzing of the leaves which later turns into necrotic tissues.

During 2004, *R. indica* was detected in Martinique and St. Lucia, and rapidly expanded its geographical range throughout the Caribbean (Kane et al. 2005; Etienne and Flechtmann 2006; Rodrigues et al. 2007). In December 2007, *R. indica* was detected in the West Palm Beach area of south Florida (FDACS 2007) and spread to six counties of the state thereafter. *Raoiella indica* has also reached Venezuela (Vásquez et al. 2008), Brazil (Navia et al. 2011), Colombia (Carrillo et al. 2011b) and Mexico (NAPPO 2009). Concerns have arisen about the consequences of the establishment of this exotic species in the Neotropical region where economically and ecologically important plants could potentially be affected.

Most studies and reports of this mite have focused on one of its more suitable and economically important host, *Cocos nucifera* (Arecales: Arecaceae) (Nageshachandra and Channabasavanna 1984 and 1983b; Sarkar and Somchoudry 1989; Peña et al. 2009). Few studies have addressed the potential effects of *R. indica* on other host plants in the New World. In 2006, Welbourn made available a list of reported host plants of *R. indica* in the Caribbean region comprising 59 plant species. All the reported plants were monocotyledons that belong to the orders Arecales [family Arecaceae (42 species)], Zingiberales [families Musaceae (6),

Heliconiaceae (5), Strelitziaceae (2) and Zingiberaceae (3)] or Pandanales [family Pandanaceae (1)] (Table A-4). In 2009, Cocco and Hoy published an expanded list of 72 reported host plants. Interestingly, these reports included 7 dicotyledon plant species from the families Aceraceae (1), Celastraceae (1), Myrtaceae (2), Lamiaceae (1) Oleaceae (1) and Fabaceae (1) raising the question whether this mite is a highly polyphagous species or a stenophagous species that feeds and reproduces on relatively few plant families within the orders Arecales, Zingiberales and Pandanales. As this exotic species keeps expanding its geographical range in the New World, a comprehensive study on the host plant range of *R. indica* is needed in order to devise feasible management options for the pest.

We present results of three studies that contribute to the knowledge of the host plant range of *R. indica* in the New World. We evaluated the potential host range of red palm mite by surveying two botanical gardens that had extensive tropical plant and palm collections located in areas of recent *R. indica* invasion (Miami Dade, FL, USA and Port of Spain, Trinidad and Tobago). The reproductive suitability of 2 dicotyledonous species and 3 native Florida palm species was examined through infesting the plants with known numbers of *R. indica* in a plant nursery. Further field surveys evaluated the viability of the Florida native palms as reproductive hosts. Based on the studies, we present an updated host plant list and discuss the known distribution and phylogenetic placement of the reported reproductive hosts of *R. indica*.

Material and Methods

Fairchild Tropical Botanic Garden Survey, Florida, USA

This botanical garden, located in the Coral Gables area of Miami Dade County, has a large collection of taxonomically arranged and well-documented tropical plants with emphasis on palms. All plants in the Palmetum area of the garden reachable with an extension pole (5 m) were inspected three times (25 February 2009, 24 November 2009 and 25 February 2010). Plants

were inspected by looking at the underside of five fronds per plant using a hand lens (20×). Plants with at least one *R. indica* individual were recorded and samples of 5 pinnae/ plant were taken to the laboratory to count the total number of individuals (eggs, immatures and adults) per pinna under a dissecting microscope (50×). Leaf area was measured using a leaf area meter (LI-3000C Portable area meter; LI-COR Biosciences) and mite densities estimated by dividing the number of mites by the area per pinna. Plants with established *R. indica* colonies, having all developmental stages (egg, larva, protonymph, deutonymph and adult), were considered reproductive hosts. Plants with only *R. indica* adults were considered non-reproductive hosts. In addition, all natural enemies (predators or pathogens) found associated with *R. indica* were also recorded and counted. The study was conducted approximately one year after the first report of *R. indica* in the area.

Royal Botanical Gardens Survey, Port of Spain, Trinidad and Tobago

Twenty five species of palms were selected from this 61.8 acre botanical garden located at 10.675284,-61.513696. Three trees were selected for each species when possible. The trees received no regular fertilizer or pesticide application, but mowing was performed periodically around them. The size of the palm depended on the species and ranged from 2-8 meters tall. One frond from the upper 1/3rd of the canopy (leaf 3 from the central spike), the middle 1/3rd canopy and the lower 1/3rd canopy were removed during the dry season (March 26-April 14, 2008) and again in the rainy season (May 29-June 11, 2008). The fronds were individually placed into plastic bags and transported to the laboratory in coolers. All stages of red palm mites (adults, nymphs, larvae and eggs) and phytoseiid motiles (adults, nymphs and larvae) were counted using a mite brushing machine (Leedom Engineering, Twain Harte, CA). As in the Fairchild Tropical Botanic Garden study, plants with *R. indica* colonies of all stages (egg, larva, protonymph,

deutonymph and adult) were considered reproductive hosts while plants with only *R. indica* adults were considered non-reproductive hosts. The surface area of each of frond was estimated taking the width and height for the section and calculating the area using $\frac{1}{2}$ base \times height in order to account for the triangular shape of the sample. In addition, phytoseiid mites were collected from each palm species from which they were encountered for identification. The study was conducted approximately 2 years after the first report of red palm mite in the area.

Evaluation of 3 Florida Native Palms as Reproductive Host of *R. indica*

The potential of three species of native palms, cabbage palm (*Sabal palmetto*), saw palmetto (*Serenoa repens*), and Florida thatch (*Thrinax radiata*), as reproductive hosts of *R. indica* was evaluated under nursery (University of Florida, West Palm Beach Extension Office, Palm Beach County, FL) and field conditions. Coconut was included in the study as positive control plant.

In the nursery experiment, potted (3 gal. vol.) palms of each species were infested by placing 20 field collected *R. indica* adult females per leaf on 5 randomly selected leaves (total of 100 adults per palm). The five infested leaves were marked in each palm and *R. indica* infestation or lack of, examined every month thereafter during 5 months (August 2008 to December 2008) until the coconut palms started to show severe bronzing and leaf necrosis. All *R. indica* eggs, nymphs and adults were counted in 15 randomly selected locations of the frond using a hand lens (10 \times) with a viewing field of 6.45 cm². The experiment had 4 treatments (palm species) and 4 replications. All palms were free of pesticides, fertilized (Osmocote® 15-9-12/ N-P-K at a rate of 2 Tbsp per 3-gal pot) and watered daily using automated overhead irrigation. The 3 native palms had been grown for approximately 2-years and the coconut palms for 1-year prior to infestation.

In addition to the nursery experiment, eight locations in South Florida known to have *R. indica* infestations (4 in Miami-Dade County, 2 in Broward County and 2 in Palm Beach County) were surveyed every 2 months from December 2008 to August 2009. Each site was approximately 2 km² and contained at least one cabbage palmetto, 1 saw palmetto and 1 Florida thatch palm. All study sites were located within 300 to 800 meters of coconut palms with moderate to high infestation of *R. indica*, which were considered a source of *R. indica* infestation for the native palms. At each site, one plant of each palm species was used to count *R. indica* each sample date. Three fronds were chosen randomly and *R. indica* counts done as described for the nursery evaluation. Coconut palms were evaluated by taking 15 pinnae randomly selected from a single frond located in the lower canopy.

Evaluation of Two Dicotyledon Plant Species, *Phaseolus vulgaris* and *Ocimum basilicum*, as Hosts of *R. indica*

Potted *O. basilicum* (approximately 4 months old, obtained from a local nursery), *C. nucifera* plants (approx 1 year old) and *P. vulgaris* (grown from seed for 4 weeks) were infested with *R. indica* in a similar way to the nursery experiment previously described. Fifty field-collected adult *R. indica* females were placed on the bean and basil leaves and on 1 selected coconut frond. The plants were then examined weekly for *R. indica* or lack of for 5 weeks. All *R. indica* life stages were counted using 10× hand lens. After 6 weeks, all plants were taken to the laboratory and destructively sampled. The total number of *R. indica* (eggs, immatures and adults) per leaf or pinna was counted using a dissecting microscope (50×). The leaf area for each plant was estimated by taking the width and height for the section and calculating the area using $\frac{1}{2}$ base × height in order to account for the triangular shape of the sample. This experiment had ten replicates.

Results

Fairchild Tropical Botanic Garden Survey, Florida, USA

In the first survey (Feb 25 2009) *R. indica* was found on three palms at variable population densities (Table A-1). *Cocos nucifera* and *Phoenix canariensis* had established colonies of *R. indica* whereas *Roystonea lenis* had only isolated individuals (adults) that were not forming colonies. In the second survey (Nov 24 2009), *R. indica* was found on 24 palm species at variable population densities; eighteen had established colonies and six had isolated individuals (adults) not forming colonies (Table A-1). As in the first survey, *C. nucifera* and *P. canariensis* had *R. indica* colonies. In contrast, no mites were found on *R. lenis* during the second survey. In the third survey (Feb 25 2010), *R. indica* was found on 26 palm species at variable population densities; 24 had established colonies and two had isolated individuals not forming colonies (Table A-1). Among the 24 plants with multigenerational colonies 12 also had colonies in the previous survey. In contrast, six palm species that had established colonies during the second survey completely lost *R. indica* or had only isolated individuals on them during the third survey (Table A-1). Phytoseiid mites, identified as *Amblyseius largoensis* (Acari: Phytoseiidae), were found associated with *R. indica* on 12 and 13 of the plants during the second and third survey respectively (Table A-1).

Overall, *R. indica* was found only on 36 palms among the high diversity of palm species in Fairchild Tropical Botanic Garden (a complete list of the plants is available at <http://www.fairchildgarden.org/livingcollections/listoflivingplantsinfairchildtropicalbotanicgarden/>). Thirty one of these palms were considered reproductive hosts because they had established colonies with all developmental stages. Among the reproductive hosts, nine palm species were previously reported as hosts of *R. indica* and 22 palm species are reported here for the first time (Table A-4).

Royal Botanical Garden Survey, Port of Spain, Trinidad

The number of *R. indica* varied greatly between the sample periods and on the 25 palm species (Table A-2). *Raoiella indica* numbers were highest in the dry season and decreased 80-100% with the onset of the rainy season on palms sampled at both time periods. Mite populations were highest on coconut (*C. nucifera*), pygmy date palm (*Phoenix roebelenii*) and lady palm (*Rhapis excelsa*) during the dry season (Table A-2). *Raoiella indica* were relatively rare on 68% of the species sampled where 7 species of palm had no mites, 5 species had very low populations (< 0.05 *R. indica*/cm²) and 5 other species had low numbers (< 1 *R. indica*/cm²). *Raoiella indica* were found throughout the palm canopy and were not found in greater numbers in any portion of the canopy.

All phytoseiid mites collected were identified as *Amblyseius largoensis* (Welbourn, pers. comm.). Seventy eight percent of the palms with *R. indica* also had phytoseiid mites present. Only three species of palms had *R. indica* and no detectable populations of phytoseiid mites (Table A-2). Only one palm species, *Elaeis guineensis*, had phytoseiid mites and no detectable population of red palm mites. The highest number of phytoseiids was found on the palms with the highest number of *R. indica*. Similar to *R. indica*, the numbers of phytoseiids were highest in the dry season.

In summary, *R. indica* was found on 18 palm species out of the 25 palms that were sampled (Table A-2). Fourteen of these palms were considered reproductive hosts for having established colonies with all developmental stages. Among the reproductive hosts eight palm species were previously reported as hosts of *R. indica* and six palms are reported here for the first time (Table A-4).

Evaluation of 3 Florida Native Palms as Reproductive Host of *R. indica*

In the nursery experiment, *R. indica* colonies developed on the coconut palms but did not develop on the native palms (Figure A-1). *Raoiella indica* adults were recorded on Florida thatch during the first September sampling, however no colonies were found on the succeeding sample dates. *Raoiella indica* populations on coconut gradually increased with the highest density recorded in November (15.5 ± 2.1 *R. indica*/ cm²), then decreased in December (Figure A-1).

In the field evaluation, *R. indica* colonies were found on coconuts at all sites and on all sampling dates with peak population palms occurring on June 2009. *Raoiella indica* were not found on the native palms during the first 2 sampling dates (Figure A-2). In the February 2009 survey, *R. indica* adults were recorded on Florida thatch at Site 2 in Miami Dade and at Site 6 in Broward County (Figure A-2). In April 2009, *R. indica* colonies were found on Florida thatch at all sites except site 4 but the densities (0.24 ± 0.1 *R. indica*/ cm²) were low compared to the ones found on coconuts (6.9 ± 1.8 *R. indica*/ cm²). *Raoiella indica* were never found on cabbage and saw palmettos.

The nursery and field studies suggest that cabbage palms and saw palmetto are not reproductive hosts of *R. indica*. Detection of established *R. indica* colonies on Florida thatch from the field evaluation confirmed this palm as a reproductive host.

Evaluation of Two Dicotyledon Plant Species, *P. vulgaris* and *O. basilicum*, as Hosts of *R. indica*

Adult *R. indica* adults were found on all plant species one and two weeks after infestation. However, only the coconut palms had established colonies with adults and all immature stages. By the third week, *R. indica* was found only on coconut. On the final sample date, *R. indica* adults and established colonies only occurred on coconut (Table A-3). These controlled

infestations studies suggest that *P. vulgaris* and *O. basilicum* are not reproductive hosts for *R. indica*.

Updated List of Reported Host Plants of *R. indica*

The surveys of the botanical gardens in Florida and Trinidad identified 27 new reproductive host plants of *R. indica* (Table A-4). The new reports compromise 13 genera of the family Arecaceae, seven of which had not been previously reported as *R. indica* hosts [*Arenga* (6 spp.), *Heterospathe* (4 spp.), *Allagoptera* (1 sp.), *Brahea* (1 sp.), *Gaussia* (1 sp.), *Guihaia* (1 sp.), *Latania* (1 sp.) and *Neoveitchia* (1 sp.)] and six from which other species were previously reported [*Livistona* (7 spp.), *Acanthophoenix* (1 sp.), *Caryota* (1 sp.), *Licuala* (1 sp.), *Phoenix* (1 sp.) and *Pritchardia* (1 sp.)].

All dicotyledonous plants were excluded from the reproductive host plant list presented here. Four dicotyledonous plants (*Eugenia* sp., *Eucalyptus* sp., *Olea* sp. and *Cassine transvaalensis*) were included in a previous list citing a reference by Kane and Ochoa (2006). However, the authors referred to these plants as hosts of *Raoiella* spp., not necessarily of *R. indica*. The references on *Phaseolus* sp. and *O. basilicum* as hosts of *R. indica* do not substantiate its reproductive host status (Chaudhri et al. 1974; Gupta 1984). In contrast, our controlled infestation experiments showed that *P. vulgaris* and *O. basilicum* were not suitable hosts for *R. indica*. The other mention of a dicotyledonous plant (*Acer* sp.) as host of *R. indica* was probably induced by a spelling error (Ochoa, pers. observ.). The original document (Mitrofanov and Strunkova 1979) cited a study on *Areca* sp. but it was misspelled once as *Acer* sp. leading to erroneous citations and translations thereafter. In conclusion, it is unclear whether *R. indica* can reproduce on any dicotyledonous plant. In contrast, all the available evidence suggests that *R. indica* feeds and reproduces only on monocot hosts.

The host plant list of *R. indica* now comprises 91 plants species. All reported reproductive hosts of *R. indica* are monocots in the orders Arecales (Arecaceae), Zingiberales (Heliconiaceae, Musaceae, Strelitziaceae, Zingiberaceae) and Pandanales (Pandanaaceae) (Table A-4). Most ($\approx 80\%$) are palms of the family Arecaceae. Within the Arecaceae (as classified by Baker et al. 2009) the subfamily Arecoideae is represented by 33 species in four tribes (Areceae 19 spp., Cocoseae 10 spp., Chamaedoreae 2 spp. and Roystoneae 2 spp.), Coryphoideae are represented by 38 species in six tribes (Trachycarpeae 18 spp., Caryoteae 8 spp., Phoeniceae 5 spp., Thrinaceae (= Cryosophileae) 4 spp., Borasseae 2 spp. and Coryphea 1 sp.) and Ceroxyloideae are represented by two species of the tribe Cyclospatheae (Table A-4).

Discussion

Knowledge of the reproductive host range of *R. indica* is important to identify plants that could transport and increase the infestations of this exotic species in areas of invasion of the New World. The best way to determine whether a plant is a reproductive host of *R. indica* is to infest the potential host with known numbers and ages of mites and compare the population changes to infested known reproductive hosts. In the field, parameters are needed to verify that plants growing in natural conditions can be considered reproductive hosts of *R. indica*. Observations in areas with high *R. indica* infestations suggest that mites can be blown by the wind and land on various plants that are not necessarily reproductive hosts of *R. indica*. In this study, the status as a reproductive host was limited to plants where multigenerational *R. indica* colonies were found.

Our studies showed a high variability of *R. indica* population densities on the disparate reproductive host plants. While most plants had relatively low *R. indica* densities, other reproductive hosts had mite densities similar to those found to cause damage on coconut. Plants with relatively high populations of *R. indica* included 11 species found in the Florida survey (*A. tremula*, *A. engleri*, *A. australasica*, *C. urens*, *G. princeps*, *G. grossefibrosa*, *H. elata*, *H.*

intermedia, *L. mariae*, *L. muelleri*, *L. rigida*, *N. storckii*, and *P. canariensis*), five in the Trinidad survey (*A. merrillii*, *P. aucalis*, *P. pacifica*, *P. macarthurii*, *W. robusta*) and two found in both places (*P. roebelenii* and *R. excelsa*). These species are capable of sustaining large *R. indica* populations and could serve as a source for infestations at other locations. Interestingly, three of these species (*A. tremula*, *A. engleri* and *H. intermedia*) had high populations on one sampling date but few or no mites on the succeeding sampling dates, despite the palms being under the same environmental conditions as other species that retained large numbers of *R. indica*. A similar situation was observed in other plants (*Heliconia* sp., *L. australis* and *L. carinensis* in Florida and *A. pinnata* in Trinidad) suggesting that not all reproductive hosts will necessarily maintain high populations of *R. indica*. In general, results of these studies suggest that there are differences in the fitness of *R. indica* on the different host plants. The fitness of a phytophagous arthropod on its host plants can be influenced by direct interactions between the arthropod and plant, including differences in adaptation to plant constitutive and induced defenses (e.g., chemical detoxification ability, Agrawal 2000), and the physical characteristics of the plant surface. For instance, the feeding habits of *R. indica* suggest that the host plants must have special stomata that could allow mites to insert their stylet and feed inside them (Ochoa et al. 2011). Also, the performance of a phytophagous arthropod on a host plant can result from interactions with competitors or enemies at other trophic levels, interactions with mutualists, or abiotic differences (e.g., temperature, sunlight) in the microhabitats in which the plant species grow (Thompson 1988). For example, a large drop in red palm mite populations during the rainy season was observed in Trinidad suggesting that *R. indica* is may be easily dislodged off of certain species of palm. The ability of *R. indica* to remain attached to the palm may be a possible reason for the differences in populations on the different palm species.

Most *R. indica* host plants ($\approx 70\%$, Table A-4) originated in the Eastern Hemisphere, primarily in tropical areas of Asia, Australia and/or Africa (Uhl and Dransfield 1987), where *R. indica* is widely distributed. Nevertheless, about one fourth of the reported hosts are native to the New World and could be considered new host associations of *R. indica*. This suggests that *R. indica* has a high ability to adapt to new environments and feed on new plant species. However, not all New World palms are vulnerable to *R. indica* infestations. Our study showed that Florida native palms such as that cabbage palm and saw palmetto were not suitable hosts for *R. indica*. In another study Cocco and Hoy (2009) also found that *R. indica* females did not survive on cabbage and saw palmettos leaf disks. In the same study, *R. indica* completed a generation on the Florida needle palm (*Rhapidophyllum hystrix*) but with a longer development time, higher mortality, and lower fecundity than when reared on coconut leaf disks. In the botanical garden surveys *R. indica* populations were low on the majority of the ‘new’ host associations recorded. Most plant species with high *R. indica* densities were native to the Eastern Hemisphere; *G. princeps* and *W. robusta* were exceptions where mite populations were comparable to or higher than *R. indica* numbers found on coconut. These observations suggest that the evolution of host range patterns of *R. indica* in the New World is a complex process that needs to be investigated in detail to determine the potential threats that this exotic species could represent for native palm species and important crops in the Neotropical region. The evolution of host associations has been studied in other phytophagous mite species. Gould (1979) demonstrated that host range evolution in *Tetranychus urticae* (Acari: Tetranychidae) can be a rapid process influenced by the ecological proximity of the plant species and cross-adaptation to sets of plants.

Most *R. indica* hosts are palms of the family Arecaceae with two subfamilies, Arecoideae and Coryphoideae, accounting for 75% of the reported host plants. Within the Arecoideae, plants

in four tribes (Areceae, Chamaedoreae, Cocoseae and Roystoneae) have been reported as *R. indica* reproductive hosts (Table A-4). All reported hosts of the tribe Areceae are native to the Eastern Hemisphere. In contrast, *R. indica* host species in the tribes Chamaedoreae, and Roystoneae are native to the Neotropics (Uhl and Dransfield 1987). Within the Cocoseae, six reported hosts are native to the Neotropics and four to the 'Old World'. This tribe contains several economically important plants, including coconut, a major host of *R. indica* with pantropical distribution (Gunn 2004). Another important plant *E. guineensis* (African oil palm) was reported as reproductive host of *R. indica* (Welbourn 2009), but in both the Fairchild and Trinidad botanical garden surveys *R. indica* populations were not detected on that host. The Cocoseae contain other species of value in local Neotropical economies that could be vulnerable to *R. indica*. For example, plants in the genera *Bactris* and *Attalea* are widely used as food and fiber source. Further studies are required to determine the susceptibility of these plants to damage caused by the invasive mite. Another important subfamily of palms, in terms of the host range of *R. indica*, is the Coryphoideae. Plants in five tribes within the Coryphoideae (Borasseae, Caryoteae, Corypheae, Trachycarpeae, Phoeniceae and Thrinaceae) have been reported as *R. indica* reproductive hosts (Table A-4). All reported hosts of the Borasseae, Caryoteae, Corypheae and Phoeniceae are native to the Eastern Hemisphere (Uhl and Dransfield 1987). Reported hosts of the tribe Trachycarpeae included primarily species from the Old World but also four species from the Neotropics (*W. filifera*, *W. robusta*, *A. wrightii* and *B. armata*) (Table A-4). In contrast, all *R. indica* reproductive hosts in the tribe Thrinaceae (=Cryosophileae) are native to the New World (Uhl and Dransfield 1987).

Besides palms, high populations of *R. indica* have been observed on other economically important hosts, including bananas (Musaceae), Heliconias (Heliconiaceae) and bird of paradise

(Strelitziaceae). However, the pest status of *R. indica* on these plants is unknown. Further studies are needed to determine the potential effect *R. indica* may have on these economically and ecologically important plants, which are widely distributed in the Neotropics.

In closing, almost six years after the initial detection of in the Caribbean, *R. indica* has expanded its host plant range to various plants. Here we report 27 reproductive hosts of *R. indica* that represent a 30 % increase on previous host plant records. As this mite continues spreading throughout the Neotropical region a great diversity of plants could potentially be affected. More detailed studies are necessary to determine the potential effects of *R. indica* in its areas of invasion.

Table 1-A. Mean densities \pm SEM of *R. indica* and phytoseiid mites found on various palms in the Fairchild Tropical Botanic Garden, Miami, FL.

Date Plant	24-Feb-09		24-Nov-09		25-Feb-10	
	RPM/ cm ²	Phyto. / cm ²	RPM/ cm ²	Phyto. / cm ²	RPM/ cm ²	Phyto. / cm ²
<i>Cocos nucifera</i>	0.59 \pm 0.25*	0	2.49 \pm 0.59*	0.03 \pm 0.01	1.62 \pm 0.03*	0.03 \pm 0.01
<i>Phoenix canariensis</i>	1.38 \pm 0.43*	0	3.58 \pm 1.1*	0	3.20 \pm 1.50*	0
<i>Roystonea lenis</i>	0.01 \pm 0.01	0				
<i>Arenga undulatifolia</i>	-	-	0.28 \pm 0.14*	0.01 \pm 0.00	1.88 \pm 1.00*	0.01 \pm 0.00
<i>Arenga tremula</i>	-	-	0.65 \pm 0.38*	0.04 \pm 0.01	0.02 \pm 0.01	0.01 \pm 0.01
<i>Arenga engleri</i>	-	-	0.67 \pm 0.34*	0.03 \pm 0.02	0	0
<i>Arenga australasica</i>	-	-	0.71 \pm 0.31*	0.03 \pm 0.01	0.20 \pm 0.07*	0.01 \pm 0.01
<i>Attalea butyracea</i>	-	-	0.01 \pm 0.01	0	0	0
<i>Heliconia sp.</i>	-	-	0.09 \pm 0.04*	0.02 \pm 0.00	0.01 \pm 0.01	0
<i>Heterospathe elata</i> var. <i>palauensis</i>	-	-	0.50 \pm 0.22*	0.05 \pm 0.03	2.09 \pm 0.63*	0.04 \pm 0.01
<i>Heterospathe intermedia</i>	-	-	0.94 \pm 0.58*	0	0	0
<i>Licuala spinosa</i>	-	-	0.01 \pm 0.01	0	0	0
<i>Livistona australis</i>	-	-	0.38 \pm 0.17*	0.01 \pm 0.01	0	0
<i>Livistona carinensis</i>	-	-	0.32 \pm 0.18*	0.01 \pm 0.01	0	0
<i>Livistona fulva</i>	-	-	0.23 \pm 0.07*	0.01 \pm 0.00	0.32 \pm 0.12*	0.01 \pm 0.00
<i>Livistona mariae</i>	-	-	1.50 \pm 0.83*	0	3.07 \pm 1.10*	0.03 \pm 0.01
<i>Livistona muelleri</i>	-	-	3.31 \pm 1.58*	0.01 \pm 0.01	0.77 \pm 0.07*	0.03 \pm 0.02
<i>Livistona rigida</i>	-	-	0.44 \pm 0.17*	0	0.61 \pm 0.27*	0.01 \pm 0.01
<i>Neoveitchia storckii</i>	-	-	0.37 \pm 0.16*	0.03 \pm 0.01	0.18 \pm 0.05*	0.01 \pm 0.00
<i>Musa acuminata</i>						
Cavendish	-	-	0.39 \pm 0.10*	0	0.49 \pm 0.17*	0
<i>Phoenix roebelenii</i>	-	-	2.31 \pm 2.12*	0	1.93 \pm 0.28*	0
<i>Allagoptera leucocalyx</i>	-	-	0.01 \pm 0.02	0	0	0
<i>Musa liukuensis</i>	-	-	0.01 \pm 0.01	0	0	0
<i>Pritchardia affinis</i>	-	-	0.01 \pm 0.01	0	0	0
<i>Acanthophoenix rubra</i>	-	-	-	-	0.21 \pm 0.05*	0
<i>Caryota urens</i>	-	-	-	-	0.90 \pm 0.17*	0.03 \pm 0.01
<i>Gaussia princeps</i>	-	-	-	-	3.85 \pm 0.43*	0
<i>Brahea armata</i>	-	-	-	-	0.10 \pm 0.08*	0
<i>Phoenix sp.</i>	-	-	-	-	0.19 \pm 0.04*	0
<i>Arenga microcarpa</i>	-	-	-	-	0.38 \pm 0.09*	0
<i>Phoenix reclinata</i>	-	-	-	-	0.50 \pm 0.17*	0.01 \pm 0.01
<i>Heterospathe elmeri</i>	-	-	-	-	0.23 \pm 0.10*	0
<i>Heterospathe negrosensis</i>	-	-	-	-	0.03 \pm 0.01*	0
<i>Guihaia grossefibrosa</i>	-	-	-	-	2.14 \pm 0.89*	0
<i>Rhapis excelsa</i>	-	-	-	-	1.06 \pm 0.33*	0
<i>Allagoptera arenaria</i>	-	-	-	-	0.42 \pm 0.14*	0.01 \pm 0.01

* reproductive host, plants with established *R. indica* colonies having all developmental stages (egg, larva, protonymph, deutonymph and adult).

Table A-2. Mean number of red palm mites \pm SE per cm^2 and phytoseiid mites \pm SE per cm^2 found in palms located at the Botanical Gardens, Trinidad. Samples were collected in the dry season (March 2008) and rainy season (June 2008).

Date	March 2008 (dry season)		June 2008 (rainy season)	
Plant	RPM/ cm^2	Phytoseiids/ cm^2	RPM/ cm^2	Phytoseiids/ cm^2
<i>Cocos nucifera</i>	5.76 \pm 1.75*	0.04 \pm 0.02	0.58* \pm 0.29	0.01 \pm 3.8x ⁻³
<i>Arenga pinnata</i>	0.32 \pm 0.24*	3.3x ⁻³ \pm 3.3x ⁻³	0	0
<i>Pritchardia pacifica</i>	1.05 \pm 0.52*	2.0x ⁻³ \pm 6.8x ⁻⁴	0.22* \pm 0.11	1.9x ⁻³ \pm 1.2x ⁻³
<i>Syagrus romanzoffiana</i>	0.04 \pm 0.03*	5x ⁻⁴ \pm 5.0x ⁻⁴	***	***
<i>Licuala spinosa</i>	0.05 \pm 0.02*	5.7x ⁻⁴ \pm 3.7x ⁻⁴	***	***
<i>Phoenix acaulis</i>	1.38 \pm 0.34*	2.9x ⁻³ \pm 1.2x ⁻³	***	***
<i>Phoenix roebelenii</i>	3.89 \pm 2*	2.2x ⁻³ \pm 2.2x ⁻³	***	***
<i>Ptychosperma macarthurii</i>	1.47 \pm 0.68*	4.6x ⁻³ \pm 3.1x ⁻³	***	***
<i>Washingtonia robusta</i>	0.86 \pm 0.21*	6.3x ⁻⁴ \pm 4.9x ⁻⁴	***	***
<i>Adonidia merrillii</i>	1.65 \pm 0.61*	7.3x ⁻³ \pm 2.0x ⁻³	***	***
<i>Latania</i> sp. **	0.15*	6.0x ⁻³	***	***
<i>Rhapis excelsa</i> **	4.33*	0.02	***	***
<i>Caryota urens</i> **	0.21*	0	***	***
<i>Roystonea oleracea</i>	0.003 \pm 0.002	2.5x ⁻⁴ \pm 2.5x ⁻⁴	***	***
<i>Sabal glaucescens</i>	0.001 \pm 8.4x ⁻⁴	0	***	***
<i>Bactris</i> spp. **	0.01	0	***	***
<i>Elaeis guineensis</i>	0	4x ⁻⁴	0	5.2x ⁻³
<i>Chrysalidocarpus lutescens</i>	0	0	***	***
<i>Hyophorbe lagenicaulis</i>	0	0	***	***
<i>Scheelea urbaniana</i>	0	0	0	0
<i>Livistona rotundifolia</i>	***	***	0.07 \pm 0.04*	1.3x ⁻³ \pm 7.7x ⁻⁴
<i>Sabal umbraculifera</i>	***	***	0.001 \pm 7.4x ⁻	1.5x ⁻³ \pm 7.4x ⁻⁴
<i>Euterpe oleracea</i>	***	***	0	0
<i>Livistona chinensis</i>	***	***	0	0
<i>Thrinax floridana</i> (<i>T. radiata</i>)	***	***	0	0

* reproductive host, plants with established *R. indica* colonies having all developmental stages (egg, larva, protonymph, deutonymph and adult).

** only one palm available.

*** No samples taken

Table A-3. Average counts of *R. indica* and phytoseiid mites on basil, bean and coconut six weeks after being infested with 50 *R. indica* females (Means \pm SEM).

Plant	Average mite count/ cm^2	
	<i>R. indica</i>	Predatory Mite
Basil	0.00 \pm 0.00	3.50x ⁻⁴ \pm 5.30x ⁻⁵
Bean	0.00 \pm 0.00	4.20x ⁻⁴ \pm 7.50x ⁻⁵
Coconut	0.02 \pm 0.003	0.002 \pm 0.00

Table A-4. Reported host plant species of *Raoiella indica*. Subfamily and tribe classification (Baker et al. 2009)

Order	Family	Subfamily	Tribe	Scientific name	Report	
Arecales	Areceaceae	Arecoideae	Areceae	<i>Archontophoenix alexandrae</i> (F. Muell.) H. Wendl. & Drude	Welbourn (2009)	
				<i>Acanthophoenix rubra</i> (Bory) H. Wendl.	Fairchild, new report	
				<i>Areca catechu</i> L.	Nagesha-Chandra & Channabasavanna (1984)	
				<i>Areca</i> sp.	Pritchard & Baker (1958), Fairchild	
				<i>Neoveitchia storckii</i> (Wendl)	Fairchild, new report	
				<i>Dypsis decaryi</i> (Jum.) Beentje & J. Dransf.	Welbourn (2009)	
				<i>Dypsis lutescens</i> (H. Wendl.) Beentje & J. Dransf. (= <i>Chrysalidocarpus</i>)	Kane et al. (2005)	
				<i>Adonidia merrillii</i> (Becc.) Becc. (= <i>Veitchia</i>)	Fletchmann & Etienne (2004), Trinidad	
				<i>Ptychosperma elegans</i> (R.Br.) Blume	Welbourn (2009)	
				<i>Ptychosperma macarthurii</i> (H. Wendl. ex HJ Veitch) H. Wendl. ex Hook. f.	Etienne & Fletchmann (2006), Trinidad	
				<i>Ptychosperma</i> sp.	Cocco & Hoy (2009)	
				<i>Veitchia arecina</i> Becc.	Cocco & Hoy (2009)	
				<i>Veitchia</i> sp.	Welbourn (2009)	
				<i>Wodyetia bifurcata</i> A.K. Irvine	Welbourn (2009)	
				<i>Dictyosperma album</i> (Bory) H. Wendl. & Drude ex Scheff.	Moutia (1958)	
				<i>Heterospathe elmeri</i> Becc. in Leafl.	Fairchild, new report	
				<i>Heterospathe negrosensis</i> Becc.	Fairchild, new report	
				<i>Heterospathe elata</i> Hough & Hubb	Fairchild, new report	
				<i>Heterospathe intermedia</i> (Becc.) Fernando	Fairchild, new report	
				Chamaedoreae	<i>Chamaedorea</i> sp. *	Welbourn (2009)
					<i>Gaussia princeps</i> (Scott) *	Fairchild, new report
				Cocoseae	<i>Butia capitata</i> (Mart.) Becc.	Welbourn (2009)
					<i>Cocos nucifera</i> L.	Hirst (1924), Welbourn (2009) Fairchild, Trinidad
<i>Syagrus schizophylla</i> (Mart.) Glass. *	Welbourn (2009)					

Table A-4 Continuation

Order	Family	Subfamily	Tribe	Scientific name	Report
				<i>Syagrus romanzoffiana</i> (Cham.) Glass. *	Kane et al. (2005), Trinidad
				<i>Allagoptera arenaria</i> (Gomes) O. Kintze*	Fairchild, new report
				<i>Beccariophoenix madagascariensis</i> Jum. & H. Perrier	Welbourn (2009)
				<i>Aiphanes caryotifolia</i> (Kunth) H. A. Wendl.*	Welbourn (2009)
				<i>Aiphanes</i> sp. *	Kane et al. (2005)
				<i>Bactris plumeriana</i> Mart. *	Welbourn (2009)
				<i>Elaeis guineensis</i> Jacq.	Welbourn (2009), NR-2
			Roystoneeae	<i>Roystonea borinquena</i> Cook *	Welbourn (2009)
				<i>Roystonea regia</i> (Kunth) Cook*	Welbourn (2009)
		Ceroxyloideae	Cyclospatheae	<i>Pseudophoenix sargentii</i> H. Wendl. *	Welbourn (2009)
				<i>Pseudophoenix vinifera</i> (Mart.) Becc. *	Welbourn (2009)
		Coryphoideae	Borasseae	<i>Bismarckia nobilis</i> Hildebr. & Wendl.	Welbourn (2009)
				<i>Latania</i> sp.	Trinidad, new report
			Caryoteae	<i>Arenga australasica</i> (H. L. Wendl. & Drude) S. T. Blake	Fairchild, new report
				<i>Arenga engleri</i> Becc.	Fairchild, new report
				<i>Arenga pinnata</i> (Wurmb) Merrill	Trinidad, new report
				<i>Arenga tremula</i> (Blanco) Becc.	Fairchild, new report
				<i>Arenga undulatifolia</i> Becc.	Fairchild, new report
				<i>Arenga microcarpa</i> Becc.	Fairchild, new report
				<i>Caryota mitis</i> Lour	Etienne & Fletchmann (2006), Fairchild, Trinidad
				<i>Caryota urens</i> L.	Florida & Trinidad, new report
			Corypheae	<i>Corypha umbraculifera</i> L.	Welbourn (2009)
			Livistoneae (Trachycarpeae)	<i>Licuala grandis</i> Wendl.	Etienne & Fletchmann (2006)
				<i>Licuala spinosa</i> Thunberg	Trinidad, new report, NR-1
				<i>Livistona australis</i> (R. Br.) Mart.	Fairchild, new report
				<i>Livistona carinensis</i> (Chiov.) Dransf.& Uhl	Fairchild, new report

Table A-4 Continuation

Order	Family	Subfamily	Tribe	Scientific name	Report
				<i>Livistona fulva</i> A.N. Rodd	Fairchild, new report
				<i>Livistona mariae</i> F.Muell	Fairchild, new report
				<i>Livistona muelleri</i> F.Muell.	Fairchild, new report
				<i>Livistona rigida</i> Becc.	Fairchild, new report
				<i>Livistona rotundifolia</i> (Lam.) Mart.	Trinidad, new report
				<i>Livistona chinensis</i> (Jacq.) R. Br. ex Mart.	Welbourn (2009), NR-2
				<i>Pritchardia pacifica</i> Seem. & H. Wendl.	Etienne & Fletchmann (2006), Trinidad
				<i>Pritchardia vuylstekeana</i> H. Wendl.	Cocco & Hoy (2009)
				<i>Washingtonia filifera</i> (Linden ex André) H. Wendl. *	Welbourn (2009)
				<i>Washingtonia robusta</i> H. Wendl. *	Etienne & Fletchmann (2006), Trinidad
				<i>Acoelorrhaphe wrightii</i> (Griseb. & H. Wendl.) H. Wendl. ex Becc. *	Welbourn (2009)*
				<i>Brahea armata</i> S. Watson *	Fairchild, new report
				<i>Guihaia grossefibrosa</i> J.Dransf., S.K.Lee & F.N.Wei	Fairchild, new report
				<i>Rhapis excelsa</i> (Thunb.) A. Henry ex Rehder	Welbourn (2009), Fairchild, Trinidad
			Phoeniceae	<i>Phoenix acaulis</i> Roxb.	Trinidad, new report
				<i>Phoenix canariensis</i> hort. ex Chabaud	Etienne & Fletchmann (2006), Fairchild
				<i>Phoenix dactylifera</i> L.	Sayed (1942)
				<i>Phoenix reclinata</i> Jacq.	Welbourn (2009), Fairchild
				<i>Phoenix roebelenii</i> O'Brien	Welbourn (2009), Fairchild, Trinidad
			Thrinaceae (Cryosophileae)	<i>Coccothrinax argentata</i> (Jacq.) L. H. Bailey *	Cocco & Hoy (2009)
				<i>Coccothrinax miraguama</i> (Kunth) Becc. *	Welbourn (2009)
				<i>Schippia concolor</i> Burret *	Welbourn (2009)
				<i>Thrinax radiata</i> Loddiges ex J.A. & J.H. Schultes (= <i>T. floridana</i>) *	Welbourn (2009)

Table A-4 Continuation

Order	Family	Subfamily	Tribe	Scientific name	Report	
Zingiberales	Heliconiaceae			<i>Heliconia bihai</i> L. *	Welbourn (2009)	
				<i>Heliconia caribaea</i> Lam. *	Welbourn (2009)	
				<i>Heliconia psittacorum</i> Sassy	Welbourn (2009)	
				<i>Heliconia rostrata</i> Ruiz & Pavon *	Etienne & Fletchmann (2006)	
				<i>Heliconia</i> sp. *	Peña et al. (2006), Fairchild	
		Strelitziaceae			<i>Ravenala madagascariensis</i> Sonn.	Welbourn (2009)
					<i>Strelitzia reginae</i> Ait.	Etienne & Fletchmann (2006)
		Musaceae			<i>Musa acuminata</i> Colla	Kane et al. (2005), Fairchild
					<i>Musa balbisiana</i> Colla	Kane et al. (2005)
					<i>Musa corniculata</i> Rumph.	Welbourn (2009)
					<i>Musa</i> sp.	Etienne & Fletchmann (2006)
					<i>Musa uranoscopus</i> Lour. (= <i>M. coccinea</i>)	Kane et al. (2005)
					<i>Musa paradisiaca</i> L.	Kane et al. (2005)
		Zingiberaceae			<i>Alpinia purpurata</i> (Vieillard) K. Schumann	Welbourn (2009)
					<i>Alpinia zerumbet</i> (Pers.) Burt. & R. M. Sm.	Welbourn (2009)
					<i>Etilingera elatior</i> (Jack) R. M. Sm.	Etienne & Fletchmann (2006)
					<i>Zingiber</i> sp.	Peña et al. (2006)
Pandanales	Pandaneaceae			<i>Pandanus</i> sp.	Kane & Ochoa (2006)	
				<i>Pandanus utilis</i> Bory	Welbourn (2009)	

- Plant names followed by * are native to the Neotropics according to Uhl and Dransfield 1987.
- NR= Non reproductive hosts.

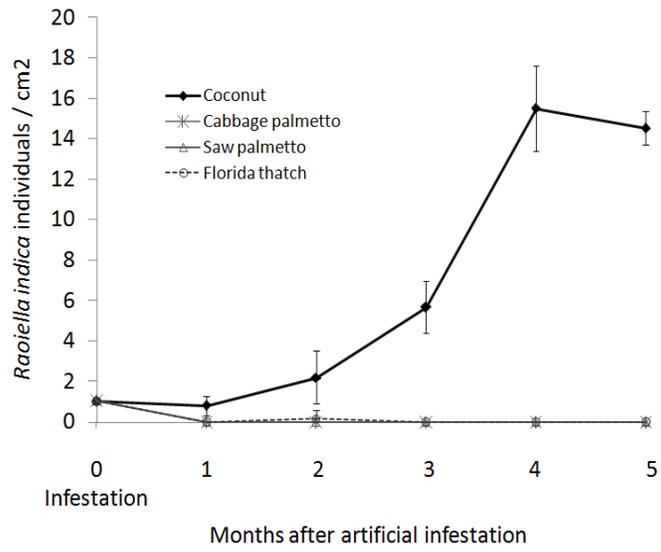


Figure A-1. Monthly average counts of *R. indica* on the 3 native palms and coconut palm after being artificially infested.

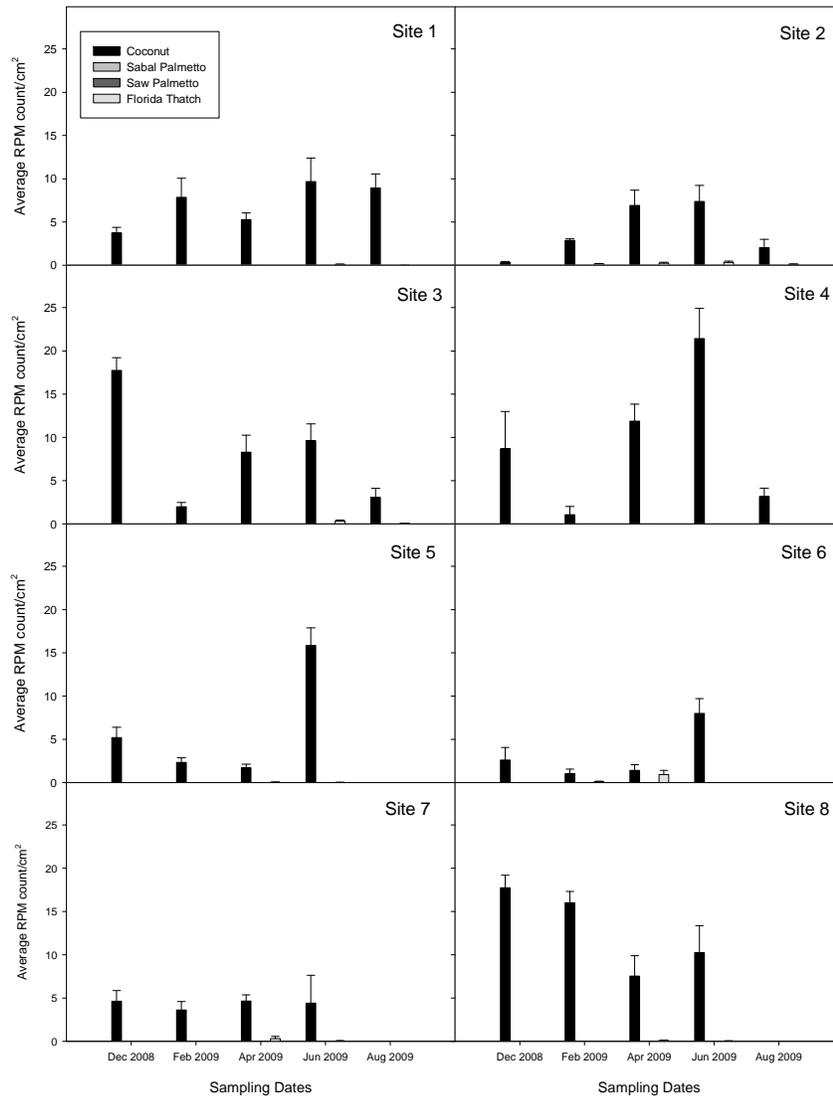


Figure A-2. Average *R. indica* count on 3 native palms and coconut palms in Miami-Dade County (Sites 1-4), Broward County (Sites 5-6), and Palm Beach County (Sites 7-8).

LIST OF REFERENCES

- Alberti G, Crooker, AR (1985) Internal anatomy. In: Helle W, Sabelis MW (eds) Spider mites: their biology, natural enemies, and control, vol A. Elsevier, Amsterdam, Netherlands, pp 29–311
- Agrawal AA (2000) Host-range evolution: adaptation and trade-offs in fitness of mites on alternative hosts. *Ecology* 81(2):500–508
- Andrewartha HG, Birch LC (1954) The distribution and abundance of animals. University of Chicago Press, Chicago, Illinois, pp 782
- Askari M, Arbabi M, Golmohammad ZN (2002) Plant mite fauna of Sistan-Baluchestan and Hormozgan provinces. *J Entomol Soc Iran* 22(1):87–88
- Badii MH, Hernandez-Ortiz E, Flores A, Landeros JN (2004) Prey stage preference and functional response of *Euseius hibisci* to *Tetranychus urticae* (Acari: Phytoseiidae). *Exp Appl Acarol* 34:263–273
- Baker WJ, Savolainen V, Asmussen-Lange CB, Chase MW, Dransfield J, Forest F, Harley MM, Uhl NW, Wilkinson M (2009) Complete generic-level phylogenetic analyses of palms (Arecaceae) with comparisons of supertree and supermatrix approaches. *Syst Biol* 58(2):240–256
- Bartlett, BR (1968) Outbreaks of two-spotted spider mites and cotton aphids following pesticide treatment. I. Pest stimulation vs. natural enemies destruction as the cause of outbreaks. *J Econ Entomol* 61:297–303
- Bellotti AC, Smith L, Lapointe SL (1999) Recent advances in cassava pest management. *Annu Rev Entomol* 44:343–370
- Bibinger DJ, Weber DC, Hull LA (2009) Stethorini in biological control. *Biol Control* 51:268–283
- Blackwood JS, Luh HK, Croft BA (2004) Evaluation of prey-stage preference as an indicator of life-style type in phytoseiid mites. *Exp Appl Acarol* 33:261–280
- Blackwood JS, Schausberger P, Croft BA (2001) Prey-stage preference in generalist and specialist phytoseiid mites (Acari: Phytoseiidae) when offered *Tetranychus urticae* (Acari: Tetranychidae) eggs and larvae. *Environ Entomol* 30:1103–1111
- Bowman HM (2010) Molecular discrimination of phytoseiids associated with the red palm mite *Raoiella indica* (Acari: Tenuipalpidae) from Mauritius and South Florida. MSc Thesis, University of Florida, Gainesville, Florida, pp 136

- Braun AR, Guerrero, JM, Belloti AC, Wilson LT (1987) Relative toxicity of permethrin to *Mononychellus progresivus* Doreste and *Tetranychus urticae* Koch (Acari: Tetranychidae) and their predators *Amblyseius limonicus* Garman & McGregor (Acari: Phytoseiidae) and *Oligota minuta* Cameron (Coleoptera: Staphylinidae): bioassays and field validation. *Environ Entomol* 16:545–550
- Bruce-Oliver SJ, Hoy MA (1990) Effect of prey stage on life-table attributes of a genetically manipulated strain of *Metaseiulus occidentalis* (Acari: Phytoseiidae). *Exp Appl Acarol* 9:201–217
- CARDI (2010) Caribbean Agricultural Research and Development Institute. Natural resource management, invasive species. Available via DIALOG. <http://www.cardi.org/default.asp?id=46>. Accessed July 2010
- Carrillo D, Peña JE, Hoy MA, Frank JH (2010) Development and reproduction of *Amblyseius largoensis* (Acari: Phytoseiidae) feeding on pollen, *Raoiella indica* (Acari: Tenuipalpidae), and other microarthropods inhabiting coconuts in Florida, USA. *Exp Appl Acarol* 52:119–129
- Carrillo D, Amalin D, Hosein F, Roda A, Duncan R, Peña JE (2011a) Host plant range of *Raoiella indica* Hirst (Acari: Tenuipalpidae) in areas of invasion of the New World. Submitted to *Exp Appl Acarol*
- Carrillo D, Navia D, Ferragut F, Peña JE (2011b) First report of *Raoiella indica* HIRST (Acari: Tenuipalpidae) in Colombia. *Fla Entomol* 94(2):370–371
- Carrillo D, Peña JE (2011) Prey-stage preferences and functional numerical responses of *Amblyseius largoensis* (Acari: Phytoseiidae) to *Raoiella indica* (Acari: Tenuipalpidae). Submitted to *Exp Appl Acarol*
- Castagnoli M, Simoni S (1999) Effect of long-term feeding history on functional and numerical response of *Neoseiulus californicus* (Acari: Phytoseiidae). *Exp Appl Acarol* 23:217–234
- Chaudhri WM, Akbar S, Rasol A (1974) Taxonomic studies of the mites belonging to the families Tenuipalpidae, Tetranychidae, Tuckerellidae, Caligonellidae, Stigmaeidae and Phytoseiidae. University of Agriculture, Lyallpur, Pakistan, PL-480 Project on Mites, pp 250
- Chinnamade-Gowda C, Mallik B (2010) Fauna of Phytoseiid mites associated with plants in southern Karnataka, India. XIII International Congress of Acarology, Recife, Brazil, Abstract Book, pp 55–56
- Cocco A, Hoy MA (2009) Feeding, reproduction, and development of the red palm mite (Acari: Tenuipalpidae) on selected palms and banana cultivars in quarantine. *Fla Entomol* 92:276–291
- Crooks JA, Soule ME (1999) Lag times in population explosions of invasive species: causes and implications. In: Sandlund OT, Schei PJ, Viken A (eds) *Invasive species and biodiversity management*. Kluwer Academic Publishers, Dordrecht, The Netherlands, pp 103–125

- Cuthbertson AGS, Bell AC, Murchie AK (2003) Impact of the predatory mite *Anystis baccarum* (Prostigmata: anystidae) on apple rust mite *Aculus schlechtendali* (Prostigmata: Eriophyidae) populations in Northern Ireland Bramley orchards. *Ann Appl Biol* 142(1):107–114
- Daneshvar H (1980) Some predator mites from northern and western Iran. *Entomol Phytopathol Appl* 48(1):87–96
- Daniel M (1981) Bionomics of the predaceous mite *Amblyseius channabasavanni* (Acari: Phytoseiidae) predaceous on the palm mite. In: Channabasavanna GP (ed) Contributions to acarology in India. Anubhava Printers, Bangalore, pp 167–172
- De la Torre PE, Suarez A, Iris A (2010) Presencia del acaro *Raoiella indica* Hirst (Acari: Tenuipalpidae) en Cuba. *Rev Proteccion Veg* 25(1):1–4
- Denmark HA, Muma MH (1989) A revision of the genus *Amblyseius* Berlese, 1914 (Acari: Phytoseiidae). Occasional papers of the Florida state collection of arthropods, USA, 4, pp 149
- Dittrich VP, Streibert P, Bathe PA (1974) An old case reopened: mite stimulation by insecticide residues. *Environ Entomol* 3:534–539
- Dunca RE, Carrillo D, Peña JE (2010) Population dynamics of the red palm mite, *Raoiella indica* (Acari: Tenuipalpidae) in Florida, USA. XIII International Congress of Acarology, Recife, Brazil, Abstract Book. p 74
- Elder RJ, Smith D (1995) Mass rearing of *Aonidiella orientalis* (Newstead) (Hemiptera: Diaspididae) on butternut gramina. *J Austral Entomol Soc* 34:253–254
- Elton CS (1958) The ecology of invasions by animals and plants. Methuen and Co., Ltd, London, UK, pp 183
- Elwan A (2000) A survey of the insect and mite pests associated with date palm trees in Al-Dakhliya region, Sultanate of Oman. *Egypt J Agr Res* 78:653–664
- EPPO (2009) *Raoiella indica* (Acari: Tenuipalpidae). In: European and Mediterranean plant protection organization. Available via DIALOG. http://www.eppo.org/QUARANTINE/Alert_List/insects/raoiella_indica.htm. Accessed Sept 2009
- Etienne J, Flechtmann CHW (2006) First record of *Raoiella indica* (Hirst, 1924) (Acari: Tenuipalpidae) in Guadeloupe and Saint Martin, West Indies. *Int J Acarol* 32:331–332
- Fan Y, Petit F (1994) Parameter estimation of functional response. *Environ Entomol* 23:785–794
- FDACS (2007 and 2011) Red palm mite infestation identified in Palm Gardens. In: Florida Department of Agriculture and Consumer Services. Available via DIALOG. http://www.doacs.state.fl.us/press/2007/12052007_2.html. Accessed Sept 2009

- Flechtmann CHW, Etienne J (2004) The red palm mite, *Raoiella indica* Hirst, a threat to palms in the Americas (Acari: Prostigmata: Tenuipalpidae). *Syst Appl Acarol* 9:109–110
- Flores-Galano G, Montoya A, Rodriguez H (2010) Biología de *Raoiella indica* Hirst (Acari: Tenuipalpidae) sobre *Areca catechu* L. *Rev Proteccion Veg* 25(1):11–16
- Frank JH, Bennett FD, Cromroy HL (1992) Distribution and prey records for *Oligota minuta* (Coleoptera: Staphylinidae) a predator of mites. *Fla Entomol* 75(3):376–380
- Gagné RJ (2004) A catalog of the Cecidomyiidae (Diptera) of the world. *Mem Entomol Soc Wash* 25:1–408
- Gallego CE, Aterrado ED, Batomalaque CG (2003) Biology of the false spider mite, *Rarosiella cocosae* Rimando, infesting coconut palms in Camiguin, northern Mindanao (Philippines). *Philipp Entomol* 17(2):187
- Gallego CE, Batomalaque CG (2004) Biology, fecundity and predatory capacity of *Telsimia ephippiger* Chapin (Coleoptera Coccinellidae) false spider mite predator of the coconut. *Philipp Entomol* 18(2):166
- Galvao AS, Gondim MGC, Moraes GJ, De Oliveira JV (2007) Biology of *Amblyseius largoensis* (Muma) (Acari: Phytoseiidae), a potential predator of *Aceria guerreronis* Keifer (Acari: Eriophyidae) on coconut trees. *Neotrop Entomol* 36:465–470
- Gerson U, Venezian A, Blumberg D (1983) Phytophagous mites on date palms in Israel. *Fruits* 38:133–135
- Gomes FP, Prado CHBA (2007) Ecophysiology of coconut palm under water stress. *Braz J Plant Physiol* 19(4):377–391
- Gonzales AI, Ramos M (2010) Desarrollo y reproducción de *Raoiella indica* Hirst (Acari: Tenuipalpidae) en laboratorio. *Rev Proteccion Veg* 25(1):7–10
- Gould F (1979) Rapid host range evolution in a population of the phytophagous mite *Tetranychus urticae* Koch. *Evolution* 33(3):791–802
- Gunn BF (2004) The phylogeny of the Coccoceae (Arecaceae) with emphasis on *Cocos nucifera*. *Ann Missouri Bot Gard* 91:505–522
- Gupta SK (2001) A conspectus of natural enemies of phytophagous mites and mites as potential biocontrol agents of agricultural pests in India. In: Halliday RB, Walter DE, Proctor HC, Norton RA, Colloff MJ (eds) *Proceedings of the 10th International Congress of Acarology*, Collingwood, Australia. CSIRO Publishing, pp 484–497
- Gupta SK (1998) Present state of knowledge on bioecological studies of plant mites including agricultural pests and predatory mites in India. In: Sinha MP (ed) *Advances in ecobiological research Vol 2*. APH Publishing Corporation, New Delhi, India, pp 117–132

- Gupta YN (1984) On a collection of tetranychoid mites from Tamil Nadu with description of a new species of *Aponychus* (Acari: Tetranychidae). *Bull Zool Surv India* 6: 237–245
- Gutiérrez B, Rodrigues N, Garcia A (2007) Situación actual del cocotero in al municipio Valdez del estado Sucre. In: *Producción y negocio*. Available via DIALOG. http://www.produccionynegocio.com/edicion_20/cocotero.htm. Accessed 29 Sept 2009
- Hastie E, Benegas A, Rodríguez H (2010) Inventario de ácaros depredadores asociados a fitoácaros en plantas de las familias Arecaceae y Musaceae. *Rev Prot Veg* 25:17–25
- Helle W, Bolland HR, Haitmans WRB (1980) Chromosomes and types of parthenogenesis in the false spider mites (Acari: Tenuipalpidae). *Genetica* 54(1):45–50
- Herren HR, Neuenschwander P (1991) Biological control of cassava pests in Africa. *Annu Rev Entomol* 36:257–83
- Hirst S (1924) On some new species of red spider. *Ann Mag Nat Hist* 14:522–527
- Hokkanen H., Pimentel D (1984) New approach for selecting biological control agents. *Can Ent* 116:1109–1121
- Holling C (1959a) The components of predation as revealed by a study of small mammal predation of european pine sawfly. *Can Entomol* 91:293–329
- Holling C (1959b) Some characteristics of simple types of predation and parasitism. *Can Entomol* 91:385–398
- Holling C (1961) Principles of insect predation. *Annu Rev Entomol* 6:163–182
- Holling C (1965) The functional response of predators to prey density and its role in mimicry and population regulation. *Mem Entomol Soc Can* 45:5–60
- Hoy MA, Flaherty D, Peacock W, Culver D (1979) Vineyard and laboratory evaluations of methomyl, dimethoate, and permethrin for grape pest management program in the San Joaquin Valley of California. *J Econ Entomol* 72:250–255
- Hoy MA, Smilanick JM (1981) Non-random prey location by the phytoseiid predator *Metasieulus occidentalis*: differential responses to several spidermite species. *Entomol Exp Appl* 29:241–253
- Hoy MA (2011) *Agricultural acarology: introduction to integrated mite management*. CRC Press, Boca Raton, Florida, p 129
- Huffaker CB, van de Vrie M, McMurtry JA (1970) Ecology of tetranychid mites and their natural enemies: a review. II. Tetranychid populations and their possible control by predators: an evaluation. *Hilgardia* 40:391–458

- Jagdish PS, Nageshachandra BK (1981) Biology of *Typhlodromips tetranychivorous* (Acari: Phytoseiidae) on red palm mite *Raoiella indica* (Acari: Tenuipalpidae). In: Channabasavanna GP (ed) 1st Indian Symposium in Acarology. April 23–25, Bangalore, India, p 173
- Jai F, Margolies DC, Boyer JE, Charlton RE (2002) Genetic variation in foraging traits among inbred lines of a predatory mite. *Heredity* 89:371–379
- James DG (1990) Biological control of *Tetranychus urticae* (Koch) (Acari: Tetranychidae) in New South Wales peach orchards: the role of *Amblyseius victoriensis* (Acari: Phytoseiidae). *Aust J Zool* 37:645–655
- Jones VP (1983) Pest resurgence of, and sampling plans for, the citrus red mite, *Panonychus citri* (McGregor) (Acari: Tetranychidae). PhD dissertation, Univ of California, Riverside, California, pp 104
- Juliano SA (2001) Nonlinear curve fitting: predation and functional response curves. In: Cheiner SM, Gurven J (eds) Design and analysis of ecological experiments, 2nd ed Chapman & Hall, New York, pp 178–196
- Kamburov SS (1971) Feeding, development, and reproduction of *Amblyseius largoensis* on various food substances. *J Econ Entomol* 64:643–648
- Kane EC, Ochoa R (2006) Detection and Identification of *Raoiella indica* Hirst (Acari: Tenuipalpidae). Agency Report. USDA ARS Systematic Entomology Laboratory Beltsville, MD. Available via DIALOG. http://entnemdept.ufl.edu/pestaalert/Raoiella_indica_Guide.pdf. Accessed May 2011
- Kane EC, Ochoa R, Mathurin G, Erbe EF (2005) *Raoiella indica* Hirst (Acari: Tenuipalpidae): An island-hopping mite pest in the Caribbean. Available via DIALOG. http://www.sel.barc.usda.gov/acari/PDF/Raoiella_indica-Kane_et_al.pdf. Accessed July 2010
- Kapur AP (1961) A new species of *Stethorus* Weise (Coleoptera: Coccinellidae), feeding on Arecanut palm mites in Kerala, Southern India. *Entomophaga* 6(1):35–38
- Koehler HH (1999) Predatory mites (Gamasina, Mesostigmata). *Agric Ecosyst Environ* 74:395–410
- Koveos DS, Broufas GD (2000) Functional response of *Euseius finlandicus* and *Amblyseius andersoni* to *Panonychus ulmi* on apple and peach leaves in the laboratory. *Exp Appl Acarol* 24:247–256
- Krantz GW, Walter DE (2009) A manual of acarology, third edition. Texas Tech University press, Lubbock Texas, pp 807
- Krebs CJ (1978) Ecology, the experimental analysis of distribution and abundance, 2nd edition, Harper and Row, New York, USA, pp 694

- Laing JE, Osborn JAL (1974) The effect of prey density on the functional and numerical response of three species of predatory mites. *Entomophaga* 19:267–277
- Lawson-Balagbo LM, Gondim MGC, Moraes GJ, Hanna R, Schausberger P (2008) Exploration of the acarine fauna on coconut palm in Brazil with emphasis on *Aceria guerreronis* (Acari : Eriophyidae) and its natural enemies. *Bull Entomol Res* 98:83–96
- Lesna I, Sabelis MW (1999) Diet dependent female choice for males with ‘good genes’. *Nature* 401:581–584
- Luck RF (1988) Experimental methods for evaluating arthropod natural enemies. *Annu Rev Entomol* 33:367–391
- Maggi VL, Leigh TF (1983) Fecundity response of the twospotted spider mite to cotton treated with methyl parathion or phosphoric acid. *J Econ Entomol* 76:20–25
- Maia AHN, Luiz AJB, Campanhola C (2000) Statistical inference on associated fertility life table parameters using Jackknife technique: computational aspects. *J Econ Entomol* 93:511–518
- Manly BFJ, Miller P, Cook LM (1972) Analysis of a selective predation experiment. *Am Nat* 106:719–736
- Marsaro Jr AL; Navia D, Gondim Jr MG, Silva FR, Moraes GJ (2009) Chegou ao Brasil-o ácaro vermelho das palmeiras *Raoiella indica*. *Cultivar, Hortaliças e Frutas* 57:31
- McMurtry JA (1992) Dynamics and potential impact of generalist phytoseiids in agroecosystems and possibilities for establishment of exotic species. *Exp Appl Acarol* 14:371–382
- McMurtry JA, Croft BA (1997) Life-styles of phytoseiid mites and their role in biological control. *Annu Rev Entomol* 42:291–321
- McMurtry JA, Moraes GJ (1984) Some phytoseiid mites from the South Pacific, with descriptions of new species and a definition of the *Amblyseius largoensis* group. *Int J Acarol* 10(1):27–37
- McMurtry JA, Morse JG, Johnson HG (1992) Studies of the impact of *Euseius* species (Acari: Phytoseiidae) on citrus mites using predator exclusion and predator release experiments. *Exp Appl Acarol* 15:233–248
- Mesa NC, Ochoa R, Welbourn WC, Evans GA, Moraes GJ (2009) A catalog of the Tenuipalpidae (Acari) of the world with a key to genera. *Zootaxa* 2098:185
- Meyerdirk DE, Khasimuddin S, Bashir M (1988) Importation, colonization and establishment of *Anagyrus indicus* (Hymenoptera: Encyrtidae) on *Nipaecoccus viridis* (Homoptera: Pseudococcidae) in Jordan. *Entomophaga* 33:229–237

- Mitrofanov VI, Strunkova ZI (1979) Ofredelitel kleshcheiploskotelok (a key to false spider mites). Donish, pp 148 (in Russian)
- Moraes GJ, Kreiter S, Lofego AC (2000) Plant mites (Acari) of the French Antilles. 3. Phytoseiidae (Gamasida). *Acarologia* 40(3):237–264
- Moraes GJ, McMurtry JA, Denmark HA, Campos CB (2004) A revised catalog of the mite family Phytoseiidae. *Zootaxa* 434:494
- Mori H, Chant D (1966) The influence of prey density, relative humidity and starvation on the predaceous behavior of *Phytoseiulus persimilis* Athias-Henriot (Acarina: Phytoseiidae). *Can J Zool* 44:483–491
- Moutia LA (1958) Contribution to study of some phytophagous Acarina and their predators in Mauritius. *Bull Entomol Res* 49:59–75
- Muma MH (1955) Phytoseiidae (Acarina) associated with citrus in Florida. *Ann Entomol Soc Am* 48(4):262–272
- Muma MH (1975) Mites associated with citrus in Florida. Bulletin No. 640A. Agricultural Experiment Stations, Institute of Food and Agricultural Sciences, University of Florida, p 92
- Nageshachandra BK, ChannaBasavanna GP (1983a) *Stethorus keralicus* (Coleoptera: Coccinellidae) feeding on *Raoiella macfarlanei* infesting roseapple. *Acarol Newsletter* 13:3
- Nageshachandra BK, Channabasavanna GP (1983b) Studies on seasonal fluctuation of the population of *Raoiella indica* Hirst (Acari: Tenuipalpidae) on coconut with reference to weather parameters. *Indian J Acarol* 8:104–111
- Nageshachandra BK, Channabasavanna GP (1984) Development and ecology of *Raoiella indica* Hirst (Acari: Tenuipalpidae) on coconut. In: Griffiths DA, Bowman CE (eds), *Acarology VI*. Ellis Horwood Publishers, Chicester, UK, pp 785–790
- Nangia N, ChannaBasavanna GP (1989) Feeding potential of *Amblyseius tetranychivorus* (Acari: Phytoseiidae), an indigenous predator on selected tetranychid and tenuipalpid mites. *Indian J Acarol* 10:75–81
- NAPPO (2009) North American Plant Protection Organization. Detection of the red palm mite (*Raoiella indica*) in Cancun and Isla Mujeres, Quintana Roo, Mexico. Available via DIALOG: <http://www.pestalert.org/oprDetail.cfm?oprID=406>. Accessed 30 December 2010
- Navia D, Marsaro AL, Silva FR, Gondim MGC, Moraes GJ (2011) First report of the red palm mite, *Raoiella indica* Hirst (Acari: Tenuipalpidae), in Brazil. *Neotrop Entomol* (accepted 29 Nov 2010)
- Navia D, Ochoa R, Welbourn C, Ferragut F (2010) Adventive Eriophyoid mites: a global review of their impact, pathways, prevention and challenges. *Exp Appl Acarol* 51:225–255

- Noronha ACDS, Moraes, GJ (2004) Reproductive compatibility between mite populations previously identified as *Euseius concordis* (Acari: Phytoseiidae). *Exp Appl Acarol* 32:271–279
- Ochoa R, Beard JJ, Bauchan GR, Kane EC, Dowling APG, Erbe EF (2011) Herbivore exploits chink in armor of Host. *Am Entomol* 57(1):26–29
- Peña JE, Rodrigues JCV, Roda A, Carrillo D, Osborne LS (2009) Predator-prey dynamics and strategies for control of the red palm mite (*Raoiella indica*) (Acari: Tenuipalpidae) in areas of invasion in the Neotropics. Proceedings of the 2nd Meeting of IOBC/WPRS, Work group integrated control of plant feeding mites. Florence, Italy 9–12 March 2009, pp 69–79
- Peña JE, Mannion CM, Howard FW, Hoy MA (2006) *Raoiella indica* (Prostigmata: Tenuipalpidae): the red palm mite: a potential invasive pest of palms and bananas and other tropical crops in Florida. In: University of Florida IFAS extension. Available via DIALOG. <http://edis.ifas.ufl.edu/IN681>. Accessed Sept 2009
- Pimentel D, Zuniga R, Morrison D (2005) Update of the environmental and economic costs associated with alien-invasive species in the United States. *Ecol Econ* 52:273–288
- Pritchard AE, Baker EW (1958) The false spider mites (Acarina: Tenuipalpidae). University of California publications in entomology 14(3):175–274
- Puttarudriah M, ChannaBasavanna GP (1956) Some beneficial coccinellids of Mysore. *J Bombay Nat Hist Soc* 54:156–159
- Puttaswamy, Rangaswamy HR (1976) *Stethorus keralicus* Kapur (Coleoptera: Coccinellidae), a predator of the areca palm mite. *Curr Res* 5:27–28
- RADA (2007) The red palm mite in Jamaica. Rural Agricultural Development Authority of Jamaica. Available via DIALOG. <http://www.moa.gov.jm/food/files/THE%20RED%20PALM%20MITE%20IN%20JAMAICA.pdf>. Accessed May 2011
- Rahmani H, Hoffman D, Walzer A, Schausberger P (2009) Adaptive learning in the foraging behaviour of the predatory mite *Phytoseiulus persimilis*. *Behav Ecol* 20:46–950
- Rajagopal V, Kasturi-Bai KV, Voleti SR (1990) Screening of coconut genotypes for drought tolerance. *Oleagineux* 45:215–223
- Ramos M, Gonzales AI, Gonzales M (2010) Management strategy of *Raoiella indica* Hirst in Cuba, based on biology, host plants, seasonal occurrence and use of acaricide. XIII International Congress of Acarology, Recife, Brazil, Abstract Book, pp 218–219
- Reis A, Gondim JM, Moraes GJ, Hanna R, Schausberger P, Lawson-Balagbo L, Barros R (2008) Population dynamics of *Aceria guerreronis* Keifer (Acari: Eriophyidae) and associated predators on coconut fruits in northeastern Brazil. *Neotrop Entomol* 37:467–462

- Reis PR, Sousa EO, Teodoro AV, Neto MP (2003) Effect of prey densities on the functional and numerical response of two species of predaceous mites (Acari: Phytoseiidae). *Neotrop Entomol* 32:461–467
- Reis PR, Teodoro AV, Neto MP, Silva EA (2007) Life history of *Amblyseius herbicolus* (Acari: Phytoseiidae) on coffee plant. *Neotrop Entomol* 36:282–287
- Ricciardi A, Palmer ME, Yan D (2011) Should biological invasions be managed as natural disasters? *BioScience* 61(4):312–317
- Roda A, Dowling A, Welbourn C, Peña JE, Rodrigues JCV, Hoy MA, Ochoa R, Duncan RA, De Chi W (2008) Red palm mite situation in the Caribbean and Florida. *Proc Caribbean Food Crops Soc* 44(1):80–87
- Rodrigues JCV, Kitajima EW, Childers CC, Chagas CM (2003) Citrus leprosis virus vectored by *Brevipalpus phoenicis* (Acari: Tenuipalpidae) in citrus in Brazil. *Exp Appl Acarol* 30(1/3):161–179
- Rodrigues JCV, Ochoa R, Kane EC (2007) First report of *Raoiella indica* Hirst (Acari: Tenuipalpidae) and its damage to coconut palms in Puerto Rico and Culebra Island. *Int J Acarol* 33:3–5
- Rodriguez H, Ramos M (2004) Biology and feeding behavior of *Amblyseius largoensis* (Muma) (Acari: Phytoseiidae) on *Polyphagotarsonemus latus* (Banks) (Acari: Tarsonemidae). *Rev Proteccion Veg* 19:73–79
- Rogers D (1972) Random search and insect population models. *J Animal Ecol* 18:1–35
- Rosen D, Huffaker CB (1983) An overview of desired attributes of effective biological control agents, with particular emphasis on mites. In: Hoy MA, Cunningham GL, Kuntson L (eds) *Biological control of pests by mites*. Div Agr Sci, Univ Calif Publ 3304, pp 2–11
- Roush RT, Hoy MA (1978) Relative toxicity of permethrin to a predator, *Metaseiulus occidentalis*, and its prey, *Tetranychus urticae*. *Environ Entomol* 7(2):287–289
- Ryoo M (1996) Studies on the basic component of the predation of *Phytoseiulus persimilis* (Acari: Phytoseiidae). *Res Pop Ecol* 28:17–26
- Sabelis MW (1985) Predator–prey interaction: predation on spider mites. In: Helle W, Sabelis MW (eds) *Spider mites: their biology, natural enemies and control*. Elsevier, Amsterdam, Netherlands, pp 103–129
- Sabelis MW, Lesna I (2010) Does artificial selection for fixed prey preference affect learning in a predatory mite? Experiments to unravel mechanisms underlying polyphagy in *Hypoaspis aculeifer*. *Acarologia* 50(2):257–268
- Sandness JN, McMurtry JA (1970) Functional response of three species of Phytoseiidae (Acarina) to prey density. *Can Entomol* 102:692–704

- Sandness JN, McMurtry JA (1972) Prey consumption behavior of *Amblyseius largoensis* in relation to hunger. *Can Entomol* 104:461–470
- Santos MA (1975) Functional and numerical responses of the predatory mite, *Amblyseius fallacis*, to prey density. *Environ Entomol* 4:89–992
- Sarkar PK, Somchoudhury AK (1989) Influence of major abiotic factors on the seasonal incidence of *Raoiella indica* and *Tetranychus fijiensis* on coconut. In: Channabasavanna GP, Viraktamath CA (eds) *Progress in Acarology*. Oxford and IBH, New Delhi, India, pp 59–65
- SAS Institute Inc (2003) SAS 9.1 for Windows. Cary, NC, USA
- SAS Institute Inc SAS 9.2 for Windows. Cary, NC, USA
- Sayed MT (1942) Contribution to the knowledge of Acarina in Egypt: 1. The genus *Raoiella* Hirst (Pseudotetranychidae: Tetranychidae). *Bull Soc Ent Egypte* 26:81–91
- Sayed MT (1950) On the taxonomy of Tetranychidae and allied genera. A new family and two new sub-families in Acarina. *Proceedings of the 8th International Congress of Entomology*, Stockholm, Sweden, 1012–1017
- Schausberger P, Croft BA (1999) Activity, feeding, and development among larvae of specialist and generalist phytoseiid mite species (Acari: Phytoseiidae). *Environ Entomol* 28:322–329
- Schausberger P, Walzer A, Hoffman D, Rahmani H (2010) Food imprinting revisited: early learning in foraging predatory mites. *Behaviour* 147:883–897
- Sepulveda F, Carrillo R (2008) Functional response of the predatory mite *Chileseius camposi* (Acarina: Phytoseiidae) on densities of its prey, *Panonychus ulmi* (Acarina: Tetranychidae). *Int J Trop Biol* 56(3):1255–1260
- Sheeja UM, Ramani N (2009) Feeding potential of *Lasioseius* sp. (Acari Mesostigmata), a promising predator of the red palm mite *Raoiella indica* (Acari: Tenuipalpidae). *Karnataka J Agric Sci* 23(3):698–700
- Skirvin DJ, Fenlon JS (2001) Plant species modifies the functional response of *Phytoseiulus persimilis* (Acari:Phytoseiidae) to *Tetranychus urticae* (Acari: Tetranychidae): implications for biological control. *Bull Entomol Res* 91:61–67
- Smith JC, Newsom LD (1970) The biology of *Amblyseius fallacis* (Acarina: Phytoseiidae) at various temperature and photoperiod regimes. *Ann Entomol Soc Am* 63:460–462
- Solomon ME (1949) The natural control of animal populations. *J Anim Ecol* 18:1–35
- Somchoudhry AK, Sarkar PK (1987) Observations on natural enemies found in association with coconut mite, *Raoiella indica* Hirst. *Bulletin of Entomology* 28:104–107

- Systat Software Inc (2006) SigmaPlot 10 user's manual. Systat Software Inc, Point Richmond, CA.
- Taylor B, Rahman PM, Murphy ST, Sudheendrakumar VV (2011) Population dynamics of the red palm mite (*Raoiella indica*) and phytoseiid predators on two host palm species in southwest India. Submitted to Exp Appl Acarol
- TDA (2008) Texas Department of Agriculture. Red Palm Mite, *Raoiella indica* Hirst. Available via DIALOG.
http://www.agr.state.tx.us/agr/main_render/0,1968,1848_27454_0_0,00.html?channelId=27454. Accessed May 2011
- Tixier M-S, Ferrero M, Okassa M, Guichou S, Kreiter S (2010) On the specific identity of specimens of *Phytoseiulus longipes* Evans (Mesostigmata: Phytoseiidae) showing different feeding behaviours: morphological and molecular analyses. Bull Entomol Res
doi:10.1017/S0007485309990617
- Thompson JN (1988) Evolutionary ecology of the relationship between oviposition preference and performance of offspring in phytophagous insects. Entomol Exp Appl 47: 3–14
- Uhl NW, Dransfield J (1987) Genera Palmarum: a classification of palms based on the work of HE Moore Jr Lawrence Kansas: International Palm Society and L. H Bailey Hortorium.
- Van Driesche RG, Bellows TS Jr. (1996) Biological control. Kluwer Academic Publishers, Massachusetts, USA, pp 539
- Van Rijn PCJ, Tanigoshi LK (1999) Pollen as food for the predatory mites *Iphiseius degenerans* and *Neoseiulus cucumeris* (Acari: Phytoseiidae): dietary range and life history. Exp Appl Acarol 23:785–802
- Vásquez C, Quirós GM, Aponte O, Sandoval DMF (2008) First report of *Raoiella indica* Hirst (Acari: Tenuipalpidae) in South America. Neotrop Entomol 37:739–740
- Vet LEM, Dicke M (1992) Ecology of infochemical use by natural enemies in a tritrophic context. Annu Rev Entomol 37:141–72
- Ward DB, Austin DF, Coile NC (2003) Endangered and threatened plants of Florida, ranked in order of rarity. Castanea 68:160–174
- Watson DM, Du TY, Li M, Xiong JJ, Liu DG, Huang MD, Rae DJ, Beattie GAC (2000a) Life history and feeding biology of the predatory thrips, *Aleurodothrips fasciapennis* (Thysanoptera: Phlaeothripidae). Bull Entomol Res 88:351–357
- Watson DM, Du TY, Li M, Xiong JJ, Liu DG, Huang MD, Rae DJ, Beattie GAC (2000b) The effect of two prey species, *Chrysomphalus aonidum* and *Corcyra cephalonica*, on the quantity of the predatory thrips, *Aleurodothrips fasciapennis* reared in the laboratory. BioControl 45:45–61

- Welbourn C (2009) Pest alert: red palm mite, *Raoiella indica* Hirst (Acari: Tenuipalpidae). In: Florida Department of Agriculture and Consumer Services. Available via DIALOG. <http://www.doacs.state.fl.us/pi/enpp/ento/r.indica.html>. Accessed May 2011
- Xiao Y, Fadamiro HY (2010) Functional responses and prey-stage preferences of three species of predacious mites (Acari: Phytoseiidae) on citrus red mite, *Panonychus citri* (Acari: Tetranychidae). Biol Control 53:345–352
- YadavBabu RK, Manjunatha M (2007) Seasonal incidence of mite population in arecanut. Karnataka J Agric Sci 20(2):401–402
- Yaninek JS, Herren HR (1988) Introduction and spread of the cassava green mite, *Mononychellus tanajoa* (Bondar) (Acari: Tetranychidae), an exotic pest in Africa, and the search for appropriate control methods: a review. Bull Entomol Res 78:1–13
- Yue BS, Tsai JH (1996) Development, survivorship, and reproduction of *Amblyseius largoensis* (Acari: Phytoseiidae) on selected plant pollens and temperatures. Environ Entomol 25:488–494
- Zaher MA, Wafa AK, Yousef AA (1969) Biological studies on *Raoiella indica* Hirst and *Phyllozetanochus aegyptiacus* Sayed infesting date palm trees in U.A.R. (Acarina: Tenuipalpidae). Z Angew Entomol 63:406–411
- Zannou ID, Negloh K, Hanna R, Houadakpode S, Sabelis MW (2010) Mite diversity in coconut habitat in West and East Africa. XIII International Congress of Acarology, Recife, Brazil, Abstract Book, p 295
- Zhang Y, Zhang Z, Lin J, Liu Q (1999) Biology of *Typhlodromus bombusae* (Acari: Phytoseiidae) a predator of *Schizotetranychus nanjingensis* (Acari: Tetranychidae) injurious to bamboo in Fujian, China. Syst Appl Acarol 4:57–62

BIOGRAPHICAL SKETCH

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