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Source: Florida Entomologist, 97(1) : 256-261

Published By: Florida Entomological Society

URL: <https://doi.org/10.1653/024.097.0134>

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

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ABSTRACT

Raoiella indica Hirst (Acari: Tenuipalpidae), a pest of coconut and other species of palms, recently invaded the Western Hemisphere. *Amblyseius largoensis* (Muma) (Acari: Phytoseiidae), is a predatory mite found associated with *R. indica* in different parts of the world. Exclusion and release tactics were used to obtain coconut palms with varying levels of *A. largoensis* in order to quantify their effects on *R. indica* densities. Four treatments consisting of 4 rates of release of *A. largoensis* females (0 = control, 1:10, 1:20 and 1:30 *A. largoensis*: *R. indica*) were tested. The releases of *A. largoensis* resulted in a significant reduction of *R. indica* densities and less damaged leaf area in the treated coconut palms compared to the controls within 3 months. The largest pest density reduction (~92%) was observed at the highest predator release rate (1:10 *A. largoensis*: *R. indica*). The other 2 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, red palm mite, predator releases, damage, biological control.

RESUMEN

Raoiella indica Hirst (Acari: Tenuipalpidae) es una plaga del cocotero y otras especies de palmas que invadió el hemisferio occidental recientemente. *Amblyseius largoensis* (Muma) (Acari: Phytoseiidae), es un ácaro que se encuentra asociado con *R. indica* en varias partes del mundo. Se combinaron técnicas de exclusión y liberación para obtener niveles diferentes de *A. largoensis* en plantas de coco infestadas con *R. indica*. Se evaluaron cuatro tasas de liberación de *A. largoensis* (0 = control, 1:10, 1:20 y 1:30 *A. largoensis*: *R. indica*). La liberación de *A. largoensis* produjo una reducción significativa en las densidades de *R. indica* y del daño foliar ocasionado por este ácaro fitófago. La mayor reducción en densidades de *R. indica* (~92%) se observó en la tasa de liberación más alta (1:10 *A. largoensis*: *R. indica*). Las tasas de liberación de 1:20 y 1:30 (*A. largoensis*: *R. indica*) causaron una reducción significativa pero equivalente (55y 43%, respectivamente) en las densidades de *R. indica*. Los resultados de este estudio indican que *A. largoensis* es un factor importante que causa mortalidad de *R. indica* y debe considerarse como agente de control biológico clave en programas de manejo integrado de *R. indica*.

Palabras Clave: *Cocos nucifera*, especie invasora, ácaro rojo de las palmeras, liberación de depredadores, control biológico, daño.

Raoiella indica Hirst (Acari: Tenuipalpidae) is a polyphagous tenuipalpid mite that recently invaded the Western Hemisphere. It has a wide host-plant range mostly within the Arecaceae (palms), but it also attacks some plants within the Cannaceae, Pandanaceae, Musaceae, Heliconiaceae, Zingiberaceae, and Strelitziaceae (Carrillo et al. 2012a; Godim et al. 2013). Its major host is coconut, *Cocos nucifera* L., on which it reaches high infestation

densities (ca. 4,000 individuals/leaflet [Peña et al. 2009]). Infestations found on other host plants are smaller but still problematic because the plants are important ornamental or native plants (Carrillo et al. 2012a; Godim et al. 2013).

The establishment of *R. indica* in the Caribbean has caused serious economic harm to coconut production with yield reductions estimated to be greater than 50% at some locations

(CARDI 2010). Efforts have been made to identify natural enemies with potential in biological control programs targeting *R. indica*. Surveys for natural enemies conducted in the Philippines (Gallego et al. 2003), Tanzania and Benin (Zannou et al. 2010), India (Taylor et al. 2012), Mauritius (Hoy 2012), Reunion (Domingos et al. 2013), and also in islands of the Caribbean and Florida (Peña et al. 2009), Colombia (Carrillo et al. 2011), Venezuela (Vásquez & De Moraes 2012), Brazil (Domingos et al. 2013) and Mexico (Otero G. personal communication), indicate that *R. indica* has a different natural enemy complex at each site with only one predator species, *Amblyseius largoensis* (Muma) (Acari: Phytoseiidae), present in all the geographical areas (Carrillo et al. 2012b).

The predatory mite *A. largoensis* 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 fed other prey and pollen (Carrillo et al. 2010). 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 & Peña 2012). Further studies examined the response of 4 populations of *A. largoensis* with previous exposure to *R. indica* or *Tetranychus gloveri* Banks (Acari: Tetranychidae) (Carrillo et al. 2012c). The 4 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, suggesting that *Amblyseius largoensis* may be responding to the invasion by *R. indica* either by learning or evolving genetically to be better predators of this pest (Carrillo et al. 2012c). The combined previous studies indicate that *A. largoensis* is actively responding to the invasion by *R. indica* and provide a framework to hypothesize that this predator has the 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 4 predator-prey ratios.

MATERIALS AND METHODS

The experimental approach combined exclusion and release techniques to obtain different levels of *A. largoensis* on coconut palms infested with *R. indica*. The experiment was conducted

inside a climate-controlled 43.2 m² glasshouse (26.5 ± 4 °C, RH 70 ± 20%) located at the Tropical Research and Education Center in Homestead, Florida (N 25° 35.49' W 80° 04.03'). Thirty-two 1-year-old potted, pest-free and unsprayed Malayan dwarf coconut palms (about 1.5 m tall) 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 cm wide) coated with Tanglefoot® was tied around the base of the frond to exclude any crawling arthropods. Potted palms were arranged on benches and clear plastic sheets (1.5 × 3 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 unwanted arthropods (i.e., *Aonidiella orientalis* Newstead [Hemiptera: Diaspididae], *Tetranychus gloveri* Banks [Acari: Tetranychidae], *A. largoensis*) were removed by hand every 3 days during 2 consecutive weeks before being inoculated with *R. indica*. *Raoiella indica* specimens used for infestation were obtained from a greenhouse colony of the red palm mite (reared as per Carrillo et al. 2010). Two *R. indica*-infested pinnae (containing ca. 30 mites) 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 using a hand lens (20 ×).

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, N 26° 02.93' W 80° 09.82') that had been infested with *R. indica* since 2007, so the predators were experienced on this prey. Predators were confirmed as *A. largoensis* and maintained on rearing arenas containing *R. indica* under laboratory conditions for 1 week before testing. Predators were then transferred from the rearing arenas to the experimental coconut fronds using a camel hair brush. After this, all fronds were carefully inspected every 2 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 effects of release rates on density of *R. indica* eggs and motiles per pinna were 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, and before predators were released, *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 1). Random allocation of treatments ensured similar infestation levels between treatments before predators were released ($F = 0.15$; $df = 3, 8$; $P = 0.92$). The number of predators released ranged from 0 (control treatment) to 4.1 ± 1.1 *A. largoensis* females per pinna according to the release rates and the infestation level (Table 1). Three months after predators were released, *R. indica* mean infestation levels ranged from (124.7 ± 94.1 to 999.6 ± 94.6) *R. indica* motile stages per pinna (Table 1).

The release of *A. largoensis* resulted in a significant reduction in *R. indica* densities relative to the densities found on predator-free palms ($df = 3, 28$; $F = 7.76$; $P < 0.001$). *Raoiella indica* reached densities of 70.7 ± 7.2 individuals/cm² (mean \pm SEM) in predator-free palms, and 5.3 ± 7.1 , 31.7 ± 5.7 and 40.4 ± 5.9 (individuals/cm²) when *A. largoensis* was released at the 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, by the end of 3 months (Fig. 1).

Raoiella indica egg densities on predator-free palms (19.3 ± 2.2 individuals/cm², mean \pm SEM) were similar to those found on palms where predators were released at 1:30 *A. largoensis*: *R. indica* (13.9 ± 1.8 individuals/cm²); however, these were significantly greater than the egg densities found on palms where predators were released at 1:10 and 1:20 (3.3 ± 2.2 and 11.6 ± 1.7). Predator releases resulted in an 82, 39 and 27 percent reductions in *R. indica* egg densities in the 3 *A. largoensis*: *R. indica* release rates, respectively ($df = 3, 28$; $F = 5.13$; $P < 0.01$) (Fig 1A). More motile stages of *R. indica* were present than eggs on the treatments with the 2 lowest *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 (Fig 1C), suggesting that the predators fed preferentially on eggs. 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 3 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 ($df = 3, 28$; $F = 6.87$; $P < 0.001$) (Fig 1B).

Amblyseius largoensis established on all palms where it was released and was not detected in the control treatments, indicating that the isolation methods used were successful over the 4 months of the experiment. 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/pinna, in the 1:10, 1:20 and 1:30 *A. largoensis*: *R. indica* release rates, respectively) were lower than the numbers released (Table 1), and did not vary along the release treatments ($df = 3, 28$; $F = 10.04$; $P < 0.001$). 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 ($F =$

TABLE 1. LEVELS OF INFESTATION OF *RAOIELLA INDICA* (MEAN \pm SEM) 1 MONTH AFTER BEING ESTABLISHED ON PREDATOR-FREE COCONUT PALMS AND JUST BEFORE *AMBLYSEIUS LARGOENSIS* PREDATORS WERE INTRODUCED AT VARIOUS PREDATOR TO PREY RATIOS; AND THE POPULATION LEVELS OF PREY AND PREDATORS THREE MONTHS LATER. THE EXPERIMENT WAS CONDUCTED AT HOMESTEAD, FLORIDA IN A CLIMATE-CONTROLLED GLASSHOUSE (26.5 ± 4 °C, RH 70 \pm 20%).

Release rates <i>A. largoensis</i> : <i>R. indica</i>	Infestation levels after one month and just before predator releases		Infestation levels and predators recovered 3 months after predator releases	
	<i>R. indica</i> motiles/pinna	<i>A. largoensis</i> released/pinna	<i>R. indica</i> motiles/pinna	<i>A. largoensis</i> recovered/pinna
1 : 10	41.3 ± 10.9	4.1 ± 1.1	124.7 ± 94.1	0.3 ± 0.1
1 : 20	46.6 ± 11.6	2.3 ± 0.6	376.7 ± 75.4	0.3 ± 0.1
1 : 30	41.5 ± 7.50	1.3 ± 0.2	546.7 ± 78.1	0.4 ± 0.1
0 : (Control)	43.3 ± 10.9	0.0 ± 0.0	999.5 ± 94.5	0.0

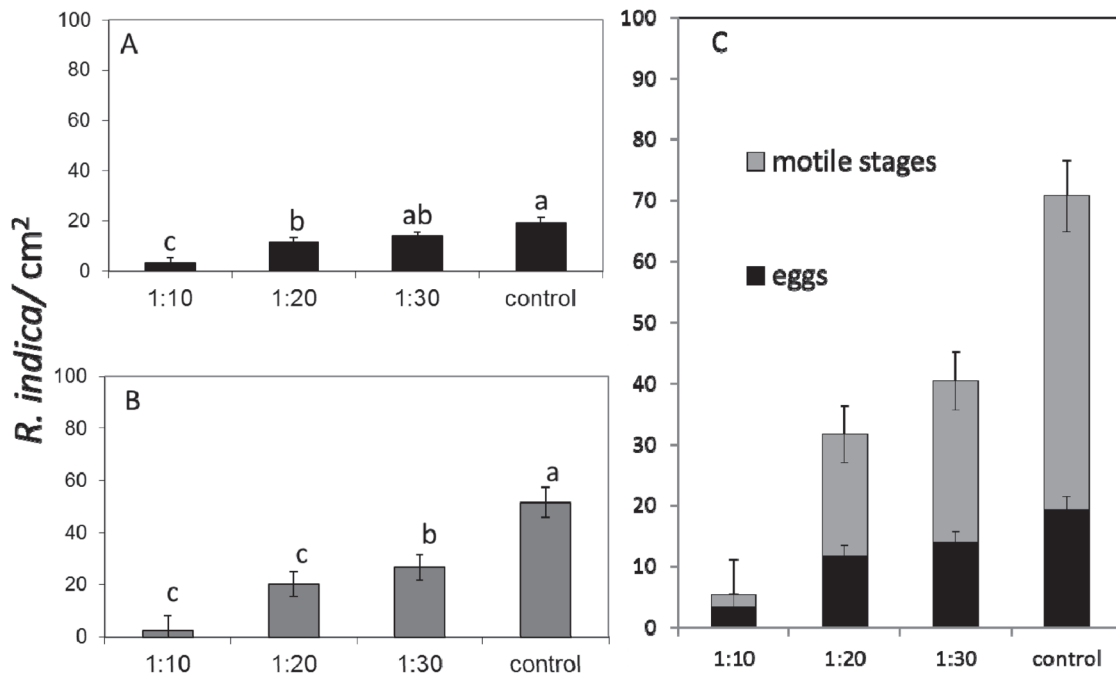


Fig 1. Effect of 4 release rates of *A. largoensis* (0= control, 1:10, 1:20, 1:30 *A. largoensis*/*R. indica*) on *R. indica* densities. A. *R. indica* egg density B. *R. indica* motile stages density, C. *R. indica* total density showing the proportion of eggs and motile stages. Error bars represent standard errors.

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 ($\chi^2 [3, N = 32] = 99.83, P < 0.0001$).

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 clear separation of the mortality caused by the natural enemy from that of other biotic and abiotic factors (Luck 1988; Van Driesche & Bellows 1996). In our experiment, exclusion tactics (sticky barriers, hand removal and cages) were used to quantify *R. indica* densities in palms with different ratios of *A. largoensis*, including predator-free palms. No other predators were detected and since palms were kept under controlled environmental conditions, the observed differences in *R. indica* densities could be attributed to *A. largoensis* alone.

Previous studies indicated that *A. largoensis* has a marked preference for *R. indica* eggs and has difficulty feeding on the motile stages (Carrillo & Peña 2012). 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 greatest pest reduction was observed, differed significantly from all other treatments. This result 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 2 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 to be 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 consequently 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 (i.e., as in 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 significantly between treated and un-

treated palms, but not among the 3 release rates tested. Predator-free palms showed necrosis 4 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.

Exclusion tactics were useful to quantify *R. indica* densities in palms with different levels of *A. largoensis*. However, a relatively low number of predators were recorded after the fronds were destructively sampled, suggesting that the physical barrier could have affected the establishment and reproduction of the predators. An alternative to exclusion by the physical means used in our experiment is the use of acaricides to reduce mite numbers (Braun et al. 1987; McMurtry 1992; Cuthbertson et al. 2003). Some chemicals are highly toxic to predators but less toxic to phytophagous mites (Roush & Hoy 1978), so they can be used for predator exclusion purposes. However, the use of pesticides has often led to problems in 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 (hormologosis) (Bartlett 1968; Dittrich et al. 1974; Hoy et al. 1979; Maggi & Leigh 1983; Cuthbertson et al. 2003). Despite these problems, acaricidal disruption is a quick and easy way to evaluate the effect of natural enemies on a large scale (Luck 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.

Raoiella indica populations in Florida have shown a steady decline that 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). However, *R. indica* populations in the Caribbean and in areas of recent introduction are still causing damage, which suggests that additional mortality factors may be needed to effectively suppress the populations of this invasive pest, especially mortality factors affecting motile stages of *R. indica*. Assis et al. (2013) identified acaricides that are selective for *A. largoensis* such as fenpyroximate and spiroticlofen, which could be used to integrate chemical and biological control strategies against *R. indica*. In addition, provision of alternative food sources could increase predator populations resulting in a better potential control of *R. indica*.

In conclusion, our results suggest that *A. largoensis* is an important mortality factor that can reduce *R. indica* densities and the damage inflicted on coconut fronds under greenhouse conditions. Moreover, the search for additional natural enemies, including fungal pathogens should be intensified to suppress the populations of this invasive pest in tropical areas, and areas recently invaded by this pest.

ACKNOWLEDGMENTS

The authors thank Katia Santos (UF-TREC) for her hard work in setting up and maintaining the experiments, Dr. James Colee (UF-IFAS-Statistics Department) for help with the statistical analysis, and Rita E. Duncan, Ana Vargas and Jose Alegria (UF-TREC) for their help. This research was partially funded by a TSTAR Grant to JEP.

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