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Management strategy of *Raoiella indica* Hirst (Acari: Tenuipalpidae) in Cuba*

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Abstract

The Red Palm Mite (RPM), *Raoiella indica* Hirst, has been reported to attack several plant species in the Americas, mainly palm and banana species. High population levels of this mite have been found damaging coconut and banana in Cuba. The objectives of this work were to estimate the potential of *R. indica* as a pest in Cuba and to evaluate the effectiveness of Dicofol and *Bacillus thuringiensis* for its control. The following activities were conducted to meet the first objective: a) evaluation of its host range; b) comparison of its biotic potential on coconut and banana leaves; and c) determination of its population dynamics on banana (*Musa acuminata* Colla Cavendish subgroup). To meet the second objective, a study was conducted to determine the effect of the chemical acaricide Dicofol and line 13 of *Bacillus thuringiensis* (LBt-13) in the control of RPM. Twenty one plant species were found as host, of which 11 are Arecaceae, three Musaceae, two Heliconiaceae, two Zingiberaceae, one Strelitziaceae and two Cycadaceae. *Mycrocycas calocoma* (Miq.) A.D.C and *Cycas* sp. are reported as new hosts for this mite. The immature phase was completed in about the same time on coconut and banana leaves, respectively 31.4 ± 3.3 and 33.4 ± 4.8 days, 26.3 ± 1.3°C, 75 ± 4% relative humidity and 14:10 daily photophase. Female oviposition period, longevity and total oviposition were higher on coconut than on banana. RPM population levels tended to increase in months with low rainfall and to decrease in months with high rainfall. The predator *Amblyseius largoensis* (Muma) (Phytoseiidae) was the only predator found associated with RPM. In general, there was a correspondence between prey and predator population trends. Dicofol caused total mortality of *R. indica*, whereas no mortality was observed with *B. thuringiensis*. Dicofol can still be used under particular situations in Cuba, e.g. in nurseries, but its widespread use is not recommended. Further studies are required to evaluate the actual impact of *A. largoensis* as a control agent of *R. indica*, and about its possible practical application by growers, using mass produced specimens.

Key words: Biology, control, pest management.

Introduction

From the first report of the red palm mite (RPM), *Raoiella indica* Hirst (Acari: Tenuipalpidae), in the Caribbean area (Etienne & Fletchmann, 2004), a surveillance mechanism for its early detection in Cuba was established. In 2008, it was found in the eastern part of Cuba, in Guantanamo and Santiago de Cuba, first on coconut (*Cocos nucifera* L.) and later on banana, arecanut (*Areca catechu* L.) and other ornamental palms (De la Torre *et al*., 2010).

Despite the fact that coconut is not an important crop in Cuba, there are many areas devoted to its production in Guantanamo, and it is very widely used as ornamental on most of the island. Banana is widely cultivated in Cuba, where production reaches over 280,000 tons a year (Statistics National Office, 2008). In addition, different species of palms are grown as ornamental plants in

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parks, gardens, touristic areas, beaches as well as in balconies and terraces. Thus, the occurrence of *R. indica* as an alien invasive species is an important threat to Cuba.

For this reason, a research program was carried out to determine important elements for the establishment of a management strategy for *R. indica* in Cuba, based on the knowledge of its local hosts, the biotic potential of the pest on its main hosts and the effect of materials for its potential emergency control.

Materials and Methods

Host plants

A survey was carried out in the Santiago de Cuba Province to find out the range of host plants of *R. indica* in this area. It was conducted monthly, from February 2009 to March 2010. We considered hosts only plants on which all stages of *R. indica* were found, indicating the natural colonization of the mite. Identification of the plant species attacked was done by Gabriel Garcés González, Natural Sciences Museum "Tomás Romay", Santiago de Cuba.

Development and reproduction

An experiment was carried out on coconut and banana leaves (*Musa acuminata* Colla Cavendish subgroup) under laboratory conditions (26.3 ± 1.3°C, 75 ± 4 % relative humidity and 14:10 daily photophase), at the Plant Protection Laboratory of Santiago de Cuba.

Females of *R. indica* were collected from coconut palms in La Marina, Santiago de Cuba. They were separated into two groups to start colonies on each of those plants. Each colony was maintained on a section of 30 cm^2 of coconut or banana leaf placed on a layer of wet cotton wool in a Petri dish (15 cm in diameter). The colonies were maintained for two months before starting the tests.

To study developmental time, each experimental unit consisted of a section of 3 cm^2 of coconut or banana leaf placed on a layer of wet cotton wool in a Petri dish (9 cm in diameter). Five-day-old females were placed in each unit, where they remained for six hours to oviposit, being subsequently transferred to other similar units. Upon hatching, each larva was transferred with a fine brush to a new separate experimental unit, similar to that just described. Mites were checked once a day to determine the duration of each developmental stage. This study was initiate with 56 mite eggs on coconut and 48 on banana leaves.

Females and males obtained in the study were then used to form couples, each of which was maintained in a unit similar to the ones on which they developed, counting daily the number of eggs laid during 20 days. Observations were conducted with 43 male-female pairs on coconut and 38 on banana, respectively. Throughout the study, the leaf substrate was replaced every four days.

Developmental times, male and female longevity and number of eggs laid by *R. indica* on coconut and banana leaves were compared by using Student´s t-test; data transformation was not necessary.

Seasonal occurrence

This study was carried out in a 2.5 ha banana (*Musa acuminata* Colla Cavendish subgroup) plantation at Carretera del Morro, km 3, Santiago de Cuba. The plot was established about one year before initiating the study, and the plants were spaced at 4 x 4 m. They received the cultural management practices established for banana plantation in Cuba, except for pesticide spraying, which was not done.

Between November 2009 and October 2010, leaf samples were collected once every ten days to evaluate the number of all developmental stages of *R. indica* and of associated phytoseiid mites. Each sample consisted of a section of about 10 cm^2 taken from a mature leaflet from each of twenty plants randomly distributed in the plot, and placed in a vinyl bag. The bags were placed in a plastic box to be transported to the laboratory, where they were maintained in a refrigerator for one day before being examined under a stereomicroscope.

All phytoseiids found were treated in lactic acid (50%) before being mounted in Hoyer´s medium for identification, which was done using the keys described by Muma *et al*. (1970) and Moraes *et* *al*. (1991). For the analysis, climatic parameters were taken from a weather station located about 500 m from the study area. Values of rainfall given correspond to the cumulative value for the ten days preceding each sampling date. A Principal Components Analysis (standardized data) was used to describe the relationships between population sizes of *R. indica* and the phytoseiid *Amblyseius largoensis* (Muma) and the climatic variables. This analysis, which includes climatic variables and mite populations in bilinear terms, provided a graphic analysis of the performance called Bi- plot. Professional Version 2.1 of INFO Stat (2009) was used for the analysis.

Effect of acaricides on *R. indica*

A commercial formulation of Dicofol 18% EC (Mitigan) at 0.05% ml (ai) and line 13 of *Bacillus thuringiensis* (10^8 spores. ml $^{-1}$) (LBt-13) were tested against *R. indica*. They were chosen for their respective mode of action; the first is a chemical contact acaricide, whereas the second is a biological acaricide widely available in Cuba, tested with good results against other mite species (Fernández-Larrea, 2007).

Discs (20 mm in diameter) were cut off from mature coconut leaves and infested each with 15 adult females (2–5-days-old) of *R. indica*. Six discs (three in each Petri dish lined with cotton wool soaked in distilled water) were used for each acaricide. The acaricides were applied to the discs using a hand sprayer. Distilled water was applied as a control treatment and Tween 80® at 0.1 % was added to all treatments. Mites on the treated discs were maintained at 24.4 ± 1.6^0C, 62 ± 8% RH and 14:10 daily photophase. Mortality was scored after one, two, three and seven days of the treatment.

Results and Discussion

Host plants

Colonies of *R. indica* were found on 21 plant species (Table 1). Eleven of them were Arecaceae, three Musaceae, two Heliconiaceae, two Zingiberaceae, two Cycadaceae and one Strelitziaceae. Most of those plants are widespread in Cuba, leading us to suppose that the mite may spread easily over wide areas of the country. Coconut and banana are by far the most economically important plants of that host list, but other plants are also important as ornamentals. Royal palm [*Roystonea regia* (Kunth) O.F.Cook] requires special attention as it is the national Cuban tree. Similarly, particular attention must be given to *Mycrocycas calocoma* (Miq.) A.D.C and *Cycas* sp., classified as living fossil plants with special value for Cuban biodiversity.

Most of the plants found to be colonized had already been reported as hosts in other parts of the New World (Peña *et al*., 2009; Hoy *et al*., 2008; Cocco & Hoy, 2009), except for *M. calocoma* and *Cycas* sp., reported as hosts for the first time.

TABLE 1. Plants colonized by *Raoiella indica* in Santiago de Cuba Province, Cuba. 2008–2009.

Family	Species	Family	Species
Arecaceae	*Areca catechu* L.	Cycadaceae	*Cycas* sp.
Arecaceae	*Coccothrinax barbadensis* (Lodd ex Mart.) Becc	Cycadaceae	*Microcycas calocoma* (Miq.)A.D.C
Arecaceae	*Coccothrinax miraguana* (Kunth) Becc.	Heliconiaceae	*Heliconia psittacorum* L.F.
Arecaceae	*Coccothrinax* sp.	Heliconiaceae	*Heliconea rostrata* Ruiz y Pavon
Arecaceae	*Cocos nucifera* L.	Musaceae	*Musa sapientum* L.
Arecaceae	*Livistona chinensis* (Jacq.) R.Br.	Musaceae	*Musa* spp.
Arecaceae	*Phoenix dactylifera* L.	Musaceae	*Musa* x *paradisiaca* L.
Arecaceae	*Ptychosperma elegans* (R.Br.) Blume	Strelitziaceae	*Strelitzia reginae* Banks et Dryard
Arecaceae	*Roystonea borinquena* O.F. Cook	Zingiberaceae	*Alpinia purpurata* (Vieill.) K.Schum
Arecaceae	*Roystonea regia* (Kunth) O.F.Cook	Zingiberaceae	*Etlingera elatior* (Jack.) R.M.Smith
Arecaceae	*Dypsis lutescens* (H. Wendl.) Beentje & J. Dransf.		

Development and reproduction

No statistical differences were observed between coconut and banana in relation to the duration of egg, larva or protonymph stages (Table 2). Durations of deutonymph stage and total duration of the immature phase was barely significantly lower on coconut than on banana. Cocco & Hoy (2009) also found a shorter life cycle on coconut than on banana. Calculated mortality of each developmental stage was almost the same on both hosts, but calculated mortality of the whole immature phase was higher on banana: 11.2% and 27.8% for coconut and banana, respectively. The proportions of females of the offspring obtained in the study were very similar on both hosts, about 68% on coconut and 66% on banana.

TABLE 2. Duration of each immature stage and of the total immature phase (days ± standard deviation) of *Raoiella indica* on coconut and banana leaves.

Stage	Host Plant		T	p[1]
	Coconut	Banana		
Egg	8.2± 0.1	8.2±1.5	0.07	0.94
Larva	8.1±1.8	8.5±2.1	-1.04	0.30
Protonymph	7.7± 2.0	7.7±2.6	-0.03	0.98
Deutonymph	6.7±1.4a	8.0±1.3b	-5.04	0.05
Total	30.9±3.4a	32.4±4.6b	-1.96	0.05

[1]*p* value for t-test

Female longevity and total number of eggs laid were significantly higher on coconut (Table 3). However, no significant difference was found in relation to male longevity. Nageshachandra & ChannaBasavanna (1984) showed similar results for fecundity and longevity of females and males on coconut.

On both hosts, oviposition started one day after female emergence (Fig. 1). It decreased slightly from the first to the third day, increasing afterwards to a maximum on the fifth (coconut) or sixth days (banana), decreasing very steadily one or two days later, but slowly afterwards. In some days, the standard errors were zero or very slightly higher. Maximum daily oviposition rate was about 4.0 eggs/female on coconut and only 1.7 on banana. Oviposition rates on coconut and banana were lower than reported by Nageshachandra & ChannaBasavanna (1984) on coconut.

TABLE 3. Means of longevity (days ± standard deviation) and oviposition of *Raoiella indica* on coconut and banana leaves.

Variable	Host Plant	N	Mean[1] ± SD
Female longevity	Coconut	43	24.1 ± 8.0 a
	Banana	38	14.8 ± 4.2 b
Male longevity	Coconut	43	16.5 ± 6.1
	Banana	38	13.0 ± 4.7
Total number of eggs	Coconut	43	12.6 ± 4.3 a
	Banana	38	7.8 ± 2.6 b

[1]For each parameter, averages followed by different letters differ significantly (t-test; $p \leq 0.05$)

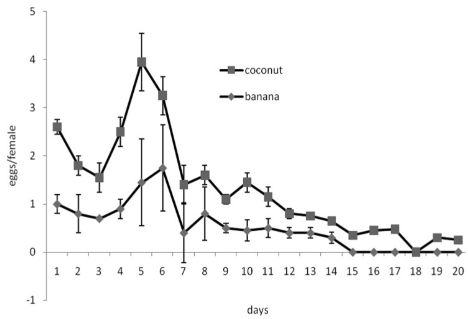

FIGURE 1. Mean daily oviposition of *Raoiella indica* on coconut and banana leaves over a period of 20 days (vertical bars indicate standard errors).

Seasonal occurrence

Fig. 2 shows the average number of *R. indica* and *A. largoensis* mites on 10 cm^2 of banana leaf in relation to temperature, relative humidity and rainfall. In the first two months of the study (mid November to mid January), the population of *R. indica* was very low. However, in the subsequent three months (mid January – early April), it increased steadily, reaching a maximum average of 215

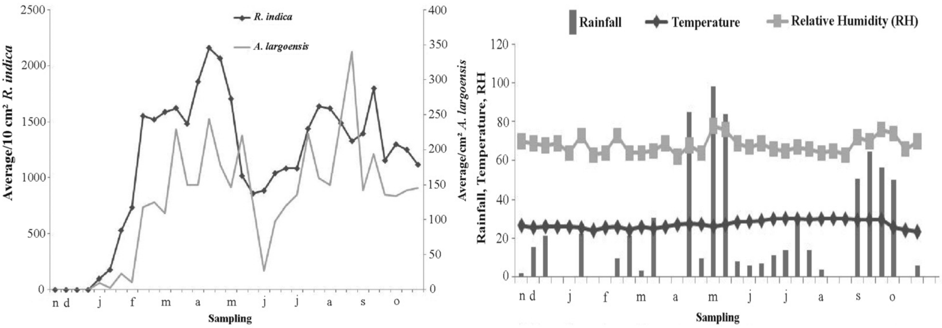

FIGURE 2. Average numbers of *Raoiella indica* and *Amblyseius largoensis* on 10 cm^2 of banana leaf in relationship to temperature (ºC), relative humidity (%) and rainfall (total precipitation for the ten days preceding the corresponding sampling date) at Santiago de Cuba (2008–2009).

mites/10 cm^2. Afterwards, the population level fluctuated between 100 and 150 *R. indica*/10 cm^2. The drop of the population level after the peak was achieved coincided with the relatively high rainfall that occurred in May (over 60 mm for two consecutive periods of ten days). Roughly, the population of *R. indica* tended to increase in months with low rainfall and to reduce in months with relatively high rainfall. Mean relative humidity and especially temperature showed light variations during the study.

Amblyseius largoensis was the only predatory mite species observed in association with *R. indica*. In general, the trends in population levels of *R. indica* and *A. largoensis* were similar, suggesting that the predator could be exerting some level of control of the pest. Despite the fact that *A. largoensis* was not able to keep the population of *R. indica* at low levels, the levels could be still higher in the absence of that predator.

These observations were corroborated and better explained by the Principal Components Analysis that had a cophenetic correlation value of 0.91, lending high reliability to the analysis (Table 4). In the first component (e^1), the best relationship (the higher values) was with the temperature (mean, maximum and minimun), being this relationship positive, indicating that when the temperature increased, *R. indica* population also increased. For a poikilotherm organism as *R. indica*, this result indicates that no other factor had effects sufficiently strong on that mite to modify this expected result. Other variables had lower values in this component. In the second component (e^2), the best relationships were with rainfall and relative humidity; the relationships were negative, indicating that the increase in those factors corresponded to a decrease in the population level of *R. indica*. This sort of relationship is best noticed in relation to rainfall in Fig. 2.

TABLE 4. Principal Components Analysis (standardized data) for the relationship between *Raoiella indica* and *Amblyseius largoensis* populations with the climatic variables.

	Auto-vectors	
Variables	**e1**	**e2**
R. indica	0.32	0.22
A. largoensis	0.39	0.2
Maximum temperature	0.5	-0.23
Mean temperature	0.59	-0.09
Minimum temperature	0.5	-0.14
Mean relative humidity	0.03	-0.61
Rainfall accumulated/10 days	0.14	-0.67

Cophenetic correlation= 0.914

The Biplot analysis supports these observation: the samples are displayed as points while variables are displayed either as vectors or linear axes. Points on the right hand side represent the samples with a lower population, for example, point seven (sample seven) corresponds to the lowest population level; conversely, points on the left correspond to the samples with higher levels; point 29 (sample 29) corresponds to the highest population level. The vectors show the relationship between the variables: the best realtionship was between *R. indica* and *A. largoensis* and among the temperatures, because the angle between them is lower than between others (Fig. 3).

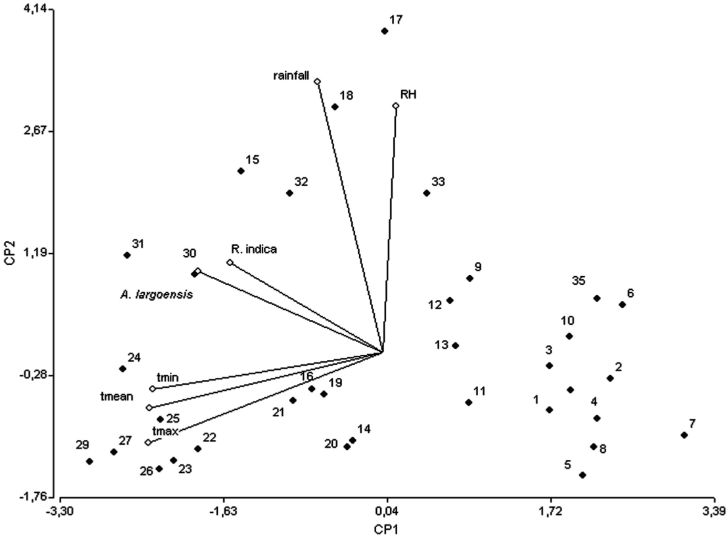

FIGURE 3. Biplot analysis: Relationship between *Raoiella indica* and *Ambylseius largoensis* populations with climatic variables.

Moutia (1958) determined that *R. indica* showed a negative correlation with rainfall and relative humidity but a positive correlation with temperature. Peña *et al*. (2009) observed that *A. largoensis* population increased with increasing population of *R. indica*, also observed in the present study.

Effect of acaricides on *R. indica*

All female *R. indica* were dead in the first evaluation after 24 h of the treatment; conversely, no effect of *B. thuringiensis* - line 13 (LBt13) on *R. indica* was observed (Table 5). Mendonça *et al*. (2005) reported that Monocrotophos (0.03 % i.a.), Dimethoato 30 CE (0.03 % i.a.), Phosphamidon 85 WSC, Methomyl 25 CE (0.005 %) Ethion (0.1 %) and Phosalone (0.007 %) provided the best control of *R. indica*, although Dicofol (2.5 mL.L^{1}, 300–350 g i.a.ha^{1}) was also recommended (ICARDA, 2005).

In summary, it seems that the long duration of the immature phase and the relatively low fecundity could in part explain why *R. indica* has not become an important pest in Cuba so far. Different environmental conditions could probably explain its damaging levels in other American countries, especially in the Caribbean (Kane *et al*., 2005; Kane & Ochoa, 2006); however, the predator *A. largoensis* could still account for part of the relatively low *Raoiella* population observed in Cuba. Laboratory studies have shown the ability of this predator to develop and reproduce on *R. indica* (Rodríguez *et al*., 2010), but its effectiveness as a control agent of the pest remains to be determined. A method for mass rearing *A. largoensis* was developed in Cuba by Rodríguez & Ramos (2003). If its efficiency as a control agent of *R. indica* is adequately demonstrated, then its practical use in Cuba may be feasible.

TABLE 5. Mean number of live adult females (± standard deviation) of *Raoiella indica* on discs of banana leaf (20 mm diameter) sprayed with a chemical, a biological product or water (control), at different periods after treatment.

Periods after spraying	Control	Dicofol	LBT-13
0*	15.0 ± 0.0	15. 00 ± 0.0	15. 0 ± 0.0
hours	16.1 ± 2.3	0.0	17.0± 3.6
48 hours	17.7± 2.7	-	18.0± 5.3
72 hours	21.1± 7.4	-	20.4 ± 7.7
7 days	28.3±11.0	-	28.6± 10.6

*Pre-treatment count

Dicofol is a well known product, banned in many countries but still in use in others, including Cuba. Although its widespread use is not recommended, it is allowed in Cuba, and can be used in nurseries, under emergency situations. LAPROSAV (Plant Protection Laboratory) has the responsibility to provide guidelines and options for an effective management of *R. indica* in Cuba, taking into consideration the presence of beneficial organisms, and to advise farmers how to act in each case. Additional research is being programmed for the development of a suitable management to be used in case *R. indica* becomes an important pest in Cuba.

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