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PEST MANAGEMENT

Infestation of *Raoiella indica* Hirst (Trombidiformes: Tenuipalpidae) on Host Plants of High Socio-Economic Importance for Tropical America

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Keywords

Arecaceae, Heliconiaceae, host range, Musaceae

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Abstract

The mite *Raoiella indica* Hirst was recently introduced into America, where it has shown amazing ability to disseminate and broaden its range of hosts. An experiment was conducted in Cancún, Mexico, to determine infestation levels of this mite on plants recorded as hosts: coconut palm (*Cocos nucifera*) of cultivars Pacific Tall and Malayan Dwarf, oil palm (*Elaeis guineensis*) hybrids Deli x Ghana and Deli x Nigeria, Dwarf Giant banana (*Musa acuminata*, AAA subgroup Cavendish), Horn plantain (*M. acuminata x Musa balbisiana,* AAB subgroup Plantain), lobster claw (*Heliconia bihai*), and red ginger (*Alpinia purpurata*). Nursery plants of these host species or cultivars were artificially infested with *R. indica* in February 2011. In the four replications of 10 plants, each plant was infested with 200 *R. indica* specimens, and the numbers of infesting mites were recorded for 6 months. A maximum of 18,000 specimens per plant were observed on coconut Pacific Tall and Malayan Dwarf, followed by lobster claw, with a maximum of 1000 specimens per plant. Infestations were minimal for the remaining plants. Mite numbers on all plants declined naturally during the rainy season. All plant materials sustained overlapping mite generations, indicating that they are true hosts. Complementarily, infestation level was determined in backyard bananas and plantains. Correlations of infestation with plant height, distance from coconuts, and exposure to direct sunlight were estimated. Both bananas and plantains were infested by *R. indica* even when situated far from infested coconut palms. A Spearman correlation was found between infestation and plant height, although it was significant only for Silk plantain.

Introduction

Coconut palm (*Cocos nucifera*), banana and plantain (*Musa* spp.), and oil palm (*Elaeis guineensis*) are economically and socially highly relevant for several countries in Latin America (FAOSTAT Food and Agriculture Organization of the United Nations 2015), some of which are among the most important producers worldwide (Table 1).

These plants, among others, are threatened by *Raoiella indica* Hirst, the red palm mite (RPM). Formerly distributed in

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Table 1 Latin American countries that are the main producers worldwide of coconut, banana (AAA genomic group), plantain (AAB genomic group), and oil palm (FAOSTAT Food and Agriculture Organization of the United Nations 2015).

Crop	Latin American producer-countries, in order of importance
Coconut palm	Brazil, Mexico, Jamaica
Plantain	Colombia, Peru, Cuba, Dominican Republic, Venezuela, Republic of Ecuador, Bolivia
Banana	Republic of Ecuador, Brazil, Guatemala, Mexico, Costa Rica, Colombia, Dominican Republic
Oil palm	Colombia, Honduras, Republic of Ecuador, Brazil, Guatemala, Costa Rica, Peru, Mexico

several countries around the Indian Ocean, the mite's western limit was in Israel and Egypt (Gerson *et al* 1983, Nagesha Chandra & ChannaBasavanna 1984). However, in 2004, it was detected in Martinique (Flechtmann & Etienne 2004). From there, it gradually dispersed through the neighboring islands and eventually arrived on mainland America, where it was detected in the USA (Smith & Dixon 2007), Venezuela (Vásquez *et al* 2008), Mexico (NAPPO North American Plant Protection Organization 2009), Brazil (Navia *et al* 2011), and Colombia (Carrillo *et al* 2011).

Early studies on RPM (Hirst 1924, Womersley 1943, Moutia 1958) included three species on its list of hosts: *C. nucifera*, *Phoenix dactylifera*, and *Dictyosperma album*, belonging to the Arecaceae family. Later, Daniel (1979) added *Areca catechu* as a host. However, after its arrival in the Americas, RPM proved to be a highly invasive pest, disseminating and thriving in the new areas on an increasingly broad range of hosts (Carrillo *et al* 2012, Fig 1).

Carrillo *et al* (2012) compiled a list of 91 plant species considered true hosts of RPM. A true host is defined as one that is infested by all developmental stages of the mite; multiple generations survive and reproduce on them and not settle just ephemerally. The authors discarded *Phaseolus vulgaris* and *Ocimum basilicum* from the list after demonstrating experimentally that they are not true hosts. They replaced the name *Acer* sp. for *Areca* sp. because *Acer* was an error in translation (Mitrofanov & Strunlova 1979, as was explained by Kane *et al* 2012). Finally, they eliminated *Eugenia* sp., *Eucalyptus* sp., *Olea* sp. and *Cassine transvaalensis* (cited by Kane & Ochoa 2006), which are parasitized by other species of the genus *Raoiella*. The list includes only monocots of six families. However, new records show that RPM continues to broaden its range of hosts, which now includes plants belonging to distant taxa: *Microcycas calocoma* and *Cycas* sp. (phylum Cycadophyta) (González & Ramos 2010); *Canna indica* (Cannaceae) and five species of Arecaceae (Gondim *et al* 2012).

Without underestimating the role that many palms and plants of other families play in local economies, we focus here on the relationship of RPM with the most important crops for tropical America: coconut palm, banana, plantain, and oil palm. The coconut palm appears in all host lists of RPM, and it is thus considered its most suitable host; however, none of the New World host lists specifies which coconut cultivar (Moutia 1958, Mendonça *et al* 2005, Etienne & Flechtmann 2006, Kane & Ochoa 2006, Peña *et al* 2006, Welbourn 2006, Cocco & Hoy 2009, Carrillo *et al* 2012), although Sarkar & Somchoudhury (1989) mentioned differential susceptibility to RPM of coconut cultivars in India.

Bananas and plantains did not appear as RPM hosts in early work; however, they were infested in the course of the invasion of this mite into America (Etienne & Flechtmann 2006, Kane & Ochoa 2006, Peña *et al* 2006, Welbourn 2006, de la Torre *et al* 2010, González & Ramos 2010, Vásquez *et al* 2008, Carrillo *et al* 2011, Vásquez & de Moraes 2013). In contrast, Cocco & Hoy (2009) established that RPM was unable to settle on several banana and plantain clones under quarantine conditions and, similarly, Carrillo *et al* (2012) did not find high RPM infestations on *Musa* spp.

Oil palm was not known as an RPM host in the Old World. It is included in the lists of Welbourn (2006) and Cocco & Hoy (2009), but Carrillo *et al* (2012) did not find infested oil palms in areas of Trinidad where this mite was present. Similarly, Gondim *et al* (2012) considered oil palm as an unfavorable host for RPM.

Research data so far have not determined with certainty how serious the risk associated with the presence of RPM is to banana, plantain, and oil palm in tropical America, nor do they determine differential infestations between Tall and Dwarf coconut. The capacity of RPM to colonize and sustain multiple generations on these five species and cultivars was tested in a nursery experiment complemented by field observations and expressed as RPM infestation levels. Two species of ornamental plants were also included: red ginger (*Alpinia purpurata*, Zingiberaceae) and lobster claw (*Heliconia bihai*, Heliconiaceae); these species are RPM hosts (Kane & Ochoa 2006, Welbourn 2006, respectively), and because they are intensely traded, they are possibly important vehicles of RPM dissemination.

Material and Methods

This study was carried out in Cancún and neighboring areas of RPM prevalence in Quintana Roo and Yucatán, Mexico. RPM had been detected in that area two years before (NAPPO North American Plant Protection Organization

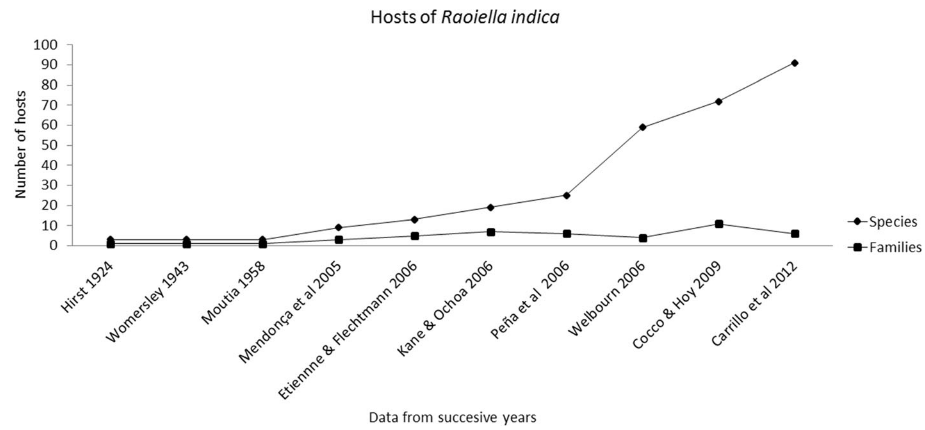

Fig 1 Number of recorded hosts of *Raoiella indica* in early observations and during its invasion of the American continent.

2009) and it was already widespread, mostly on coconut. Potted plants of the following species and cultivars were installed in a nursery (Vivero Kabah, 21°08′31″N, 86°50′30″ W): coconut palm cultivars Pacific Tall and Malayan Dwarf, oil palm hybrids Deli x Ghana and Deli x Nigeria, Dwarf Giant banana (*Musa acuminata* AAA subgroup Cavendish), Horn plantain, locally known as plátano Macho (*M. acuminata x Musa balbisiana* AAB subgroup Plantain), lobster claw, and red ginger. The age of the plants ranged from 2 months (*Musa* spp.) to 6–8 months (the remaining plants). The experimental design was randomized blocks in divided plots; there were four replications, each with 10 plants (Fig 2). In an adjacent section of the nursery, separated by a concrete wall (1.5 m high) and a double curtain of fine fabric (organdy, 3 m high, 30 threads/cm, 200 μm opening), the same number of plants was installed as a control treatment. Plants in both sections were close to each other, their crowns touching, and permitting mites to simply walk from plant to plant.

Once in the nursery, all plants were sprayed with amitraz 1% (Mitac® CE 21.74%, Arysta Lifescience) to eliminate mites and insects on the plants. A lapse of 2 weeks was allowed for plants to acclimatize and for the pesticide to degrade and/or wash off, based on results from Kumral & Kovanci (2007), who found that amitraz loses its acaricide activity in times ranging from 1 to 12 days.

Plants were inoculated with RPM in February 2011. Small leaf pieces of coconut palm with mite colonies but without other apparent diseases or pests were excised and examined under a stereomicroscope to count specimens and to manually eliminate other mites and insects. Pieces carrying about 100 RPM specimens of mixed developmental stages were attached with clips to the leaves of the experimental potted plants, both abaxial surfaces in contact. One month later, plants were again infested with the same procedure. Plants were irrigated three times a week with a hose directed to the soil, trying not to hit the foliage with the water stream.

Plants in the control section received the same management, but they were not artificially infested. They were examined monthly to verify that they were still mite-free. After finding very low infestation of an undetermined mite (March 15, 2015), less than 10 mites per plant, they were treated with amitraz at the concentration above mentioned.

Every month from April 22 to September 20, 2011, the specimens of RPM on samples of both infested and control plants were counted. This time corresponded to the dry season, ending in June, with mean temperature of 26.4°C and total rainfall of 71 mm; and the beginning of the rainy season, starting in July, with mean temperature of 26.4°C and total rainfall of 238 mm (WunderMap 2015). One plant from each species and cultivar was randomly chosen; all its leaves were excised, dipped in a bottle of water mixed with liquid soap (1%) and shaken vigorously. Then, in small groups, leaves were placed on a column of two sieves of 2 mm (up) and 25 μm aperture (down) and hit by a strong jet of tap water to detach the mites, which were washed down from the lower sieve with 70% ethanol and recovered in plastic bottles. Eggs, larvae, nymphs, adult females, and adult males from each plant were counted separately under a stereomicroscope. The numbers of infesting mites from each successive sampling on the different hosts were compared with an ANOVA and Tukey's range test using the procedure PROC GLM (SAS 9.0) (SAS Institute 2002).

Observations of RPM infestation on banana and plantain were complemented by sampling backyard plants in the Municipalities of Benito Juárez, Quintana Roo, as well as Tizimín and Río Lagartos, Yucatán, during March–May 2012 and 2013. *Musa* spp. clones were identified by their phenotypes, following the criteria described by Contreras (1982). Complementarily and mostly when plant clones could not be morphologically identified, their identity was determined by sequence-related amplified polymorphism (SRAP) molecular markers, with known reference indexed plant material of the banana germplasm collection at the Uxmal-Experimental Site (20°24′27.72″N, 89°45′06.66″W) of the Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias (INIFAP), in Yucatan, Mexico (Youssef *et al* 2011, Valdez-Ojeda *et al* 2014).

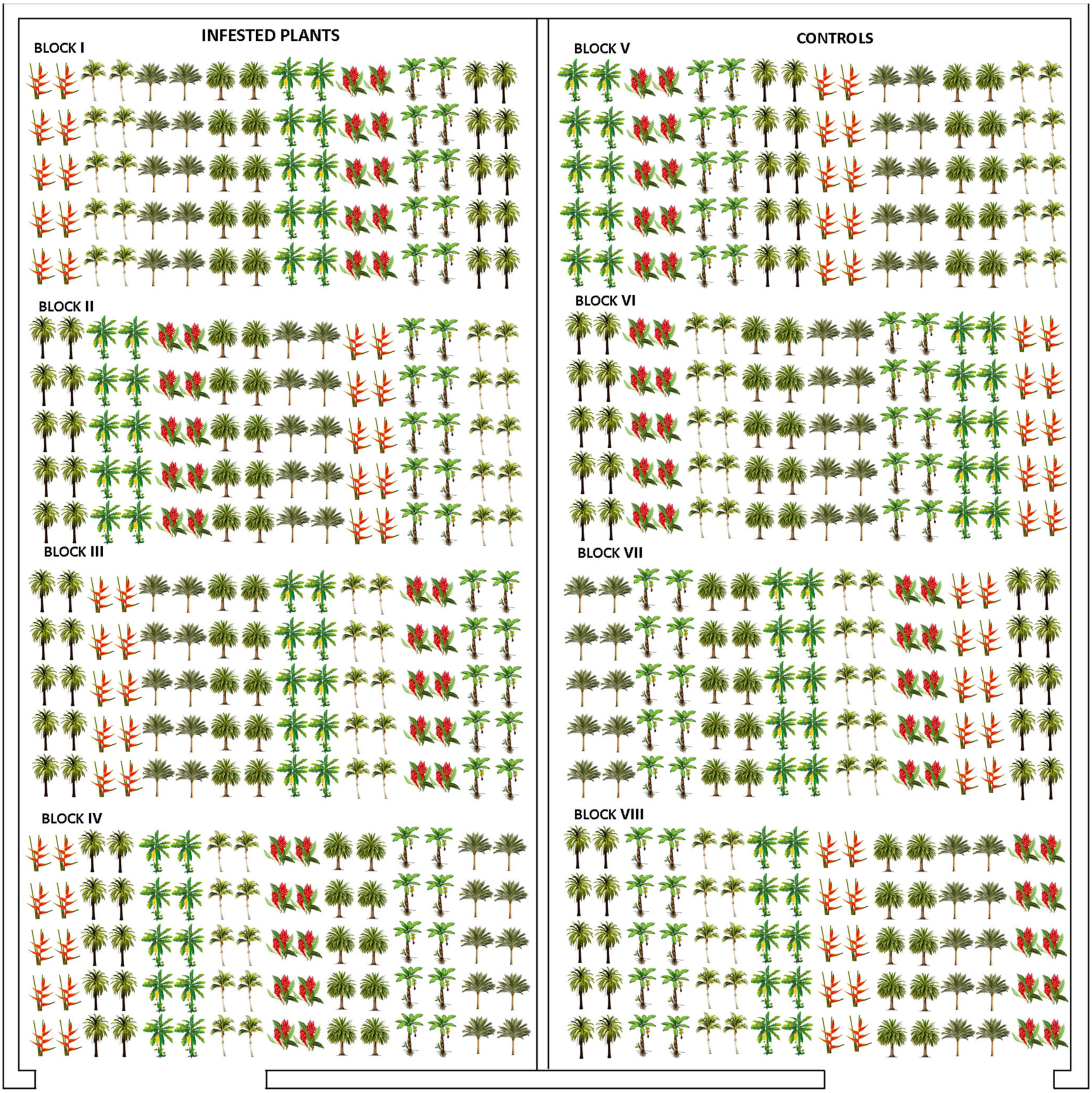

Fig 2 Distribution of treatments in the experiment of RPM infestation. Pacific Tall coconut palm. Malayan Dwarf coconut palm. Deli x Ghana oil palm. Deli x Nigeria oil palm. Dwarf Giant banana Horn plantain. Lobster claw Red ginger.

The DNA was extracted from the youngest cigar leaf of the collected plant materials, both unknown plants and indexed reference plants. The SRAP protocol was implemented using the tools of Khirshyat 1.0 (Youssef 2012) with six combinations (C1 to C6) of forward and reverse primers Me1-Em7, Me3-Em6, Me3-Em3; Me4-Em7, Me4-Em3; Me3-Em7, after, Li & Quiros (2001). For data analysis, SRAP fragments were scored considering presence (coded: 1) or absence (coded: 0); the similarity matrices based on Jaccard's similarity coefficient were used to construct an unweighted pair group with arithmetic average dendrogram (UPGMA) using NTSYSpc ver. 2.20 s (Exeter Software, Setauket, NY, USA). Statistical stability of the dendrogram branches was tested by bootstrap analysis with 1000 replicates using the Free Tree 0.9.1.50 software program.

Field observations on bananas and plantains and data from the test in the nursery suggested that young plants were barely infested by RPM. In contrast, plants under water stress bore high infestations. Rodrigues & Irish (2012) found that bananas growing near RPM-infested coconut palms sustained higher RPM numbers than plants that were farther. To explain the contradictory observations of RPM infestation on banana and plantain, possible association among infestation and plant age, water stress, and proximity to infested coconut was tested. To this end, backyard bananas and plantains were sampled. Measurements were taken of their height to the tip of the cigar leaf (m, a fact associated to age and availability of mature leaves), distance from infested coconut palms (m, an indicator of risk of infestation from those plants), exposure to sunlight (an indicator of potential water stress), and infestation level. Exposure to sunlight was estimated and described with the following scale: 1: full exposure, 2: partial shade, 3: shade from trees or tall buildings. Infestation level was graded with the following scale: 0 (mite-free), 1 (slight, sparse mites, small colonies, leaves without color changes), 2: moderate (large colonies mostly in lower leaves, chlorotic leaves), and 3: severe (large colonies, mites disseminated over most leaves except the youngest ones, severe chlorosis and burned aspect). Estimation of the infestation level was visual; decimals were used for intermediate levels. Infestation levels on the most frequently found plantain clones (Bluggoe, Horn, and Silk) were compared by the Kolmogorov-Smirnov test with PROC UNIVARIATE (SAS 9.0) (SAS Institute 2002). The Spearman correlation coefficients were estimated between infestation and plant height, distance from coconut palms, and exposure to sunlight using PROC CORR (SAS 9.0) (SAS Institute 2002).

Results and Discussion

RPM infestation level

All the plants in the control treatment remained uninfested by RPM or had very small infestations, not more than 10 specimens per plant, probably migratory mites proceeding from infested plants that were placed at a short distance but separated by a concrete wall and an additional barrier of a fine fabric (organdy). The physical barrier almost completely prevented infestation of the control plants, and early detection allowed control of all mites, not only RPM. Thus, the control plants were considered practically mite-free, and the increasing numbers of specimens on the infested plants were the result of RPM reproduction and not migration from plants surrounding the nursery. Although the presence of insects inside the experimental area could not be excluded, predatory mites or insects were not found throughout the study. Because the entire plants were destroyed to estimate RPM infestations, insects or other mites would be detected, if present. Species present in very low numbers could be overlooked but, in that case, it is assumed that their effect on RPM increase was negligible.

RPM infestations were notably higher on coconut palm where the fluctuations of mite numbers on the cultivars Pacific Tall and Malayan Dwarf were almost identical (Fig 3), indicating that they have very similar levels of susceptibility, while infestations were significantly lower on the remaining hosts (Tukey, $p < 0.05$). RPM attained almost 18,000 specimens per coconut plant in July, but naturally decreased to 500 specimens per plant in September, a fact associated with heavy rainfalls starting in June (WunderMap 2015). The same trend was observed with lower populations for other hosts although in lobster claw, a peak was observed in August. The above data show that both cultivars of coconut palm, Pacific Tall and Malayan Dwarf, are the most suitable hosts for RPM, with similar levels of susceptibility.

Following coconut palm, lobster claw presented the highest infestation, up to about 1000 specimens per plant, while infestations did not reach 100 specimens in the remaining plants. Although both lobster claw and red ginger are widely commercialized in Mexico and other countries, the former seems to be more important as a vehicle for RPM dissemination. Excluding coconut palm, no significant differences were found in mite numbers among hosts on any sampling date (Fig 3). Since the infested plants were close to each other, mites could easily migrate from one to another; thus, mites present on slightly infested plants could proceed totally or partially from migratory mites coming mostly from coconut. It is also possible that mites could migrate from other hosts to coconut but, because infestation levels on coconut were considerably higher, migration in this sense is assumed to have been minimal.

Oil palm had extremely low infestations. On hybrid Deli x Nigeria they attained a maximum of 21.75 total RPM adults per plant in June, while on the hybrid Deli x Ghana infestation had a maximum average of 7.75 adults in July. Welbourn (2006) included oil palm as an RPM host, whereas Carrillo *et al* (2012) did not find this mite on the same host near infested coconut palms in Port Spain, Trinidad. Our data suggest that the oil palm hybrids included in our research are not suitable hosts of RPM.

RPM infestations on bananas and plantains were very low; the largest number of mites on Horn plantain was 2.5 adults per plant, while on Dwarf Giant banana it was 8.5 adults per plant. Previous observations on *Musa* spp. are very discordant. No species of this genus appeared as RPM host for the Old World (Hirst 1924, Womersley 1943, Moutia 1958, Daniel 1979), but in the New World infestations on bananas and plantains have been repeatedly found (Etienne & Flechtmann 2006, Kane & Ochoa 2006, Welbourn 2006, de la Torre *et al* 2010, González & Ramos 2010, Vásquez & de Moraes 2013).

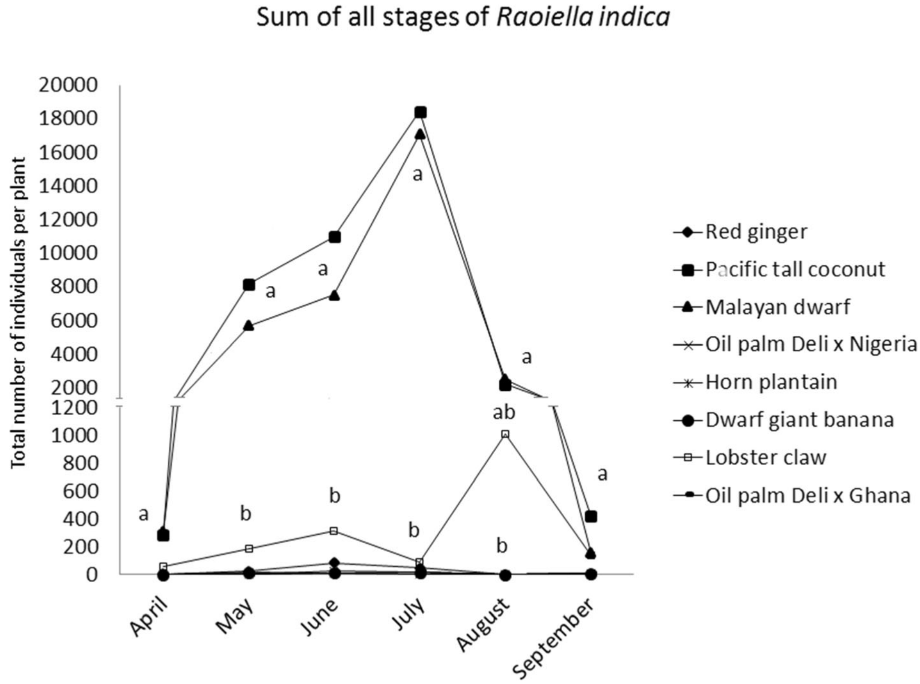

Fig 3 Population fluctuation of *Raoiella indica* on experimentally infested hosts. Cancún, Q. Roo, Mexico, April to September 2011. Values of a given sampling date marked with the same letter (a, b) are not significantly different (Tukey, $p > 0.05$).

Composition of developmental stages

Although RPM infestations were notably higher in coconut palm, all developmental stages of this mite coexisted on all crops, at least in some of the monthly records (Fig 4). This shows that all experimental species and cultivars sustained overlapping generations and they are therefore true hosts, as defined by Peña *et al* (2006) Carrillo *et al* (2012), Vásquez & de Moraes (2013). However, only on coconut palms (both cultivars) and lobster claw, was the number of immature stages, including eggs, higher than the number of adults, suggesting that RPM is capable of founding colonies, though small, on all hosts. An important population component, however, may be mites migrating from nearby coconut palms.

Infestation on backyard bananas and plantains

The observation areas do not have commercial banana or plantain plantations, so plants in backyards (also streets, empty lots, hotels, etc.) were observed. Seventy-seven plants of the following genotypes were observed (in parentheses, genomic group and number of specimens): Bluggoe, Bárbaro or Cuadrado, Bluggoe subgroup (ABB, 33), Horn or Macho, Plantain subgroup (AAB, 8), Silk or Manzano, Silk subgroup (AAB, 14), Dominico French-plantain type (AAB, 2), Morado or Red (AAA, 1), and Roatán banana (AAA, 2). Seventeen samples could not be identified either by morphological or molecular criteria, so they remained as unidentified. After UPGMA, a dendrogram of genetic similitude was constructed; banana and plantain samples were grouped together with reference plant material when the shared Jaccard coefficient was higher than 0.75 (Fig 5).

Contrasting with the reduced RPM infestations found in the nursery experiment, bananas and plantains found in the backyards presented varying infestation levels and at least some specimens had infestation levels close to 3 of the established scale (severe infestation, Fig 6) in all the observed clones. No significant differences were found among the clones Bluggoe, Horn, Silk, and unidentified ones (statistic D from Kolmogorov-Smirnov test = 2.23; $p > 0.0605$). Banana and plantain leaves looked burned, as described by Kane & Ochoa (2006), and Peña *et al* (2006); however, plants with similar symptoms but carrying low or even unapparent infestations were also found; so, leaf burning cannot be attributed exclusively to RPM.

Many have observed high RPM infestation levels in banana and plantain in the New World, including the authors of this work, leading to include at least some species or hybrids of the genus *Musa* as hosts of this mite. It is thus surprising that potted plants in the nursery presented very small infestations. This disagreement also appears in other studies; for example, Cocco & Hoy (2009) recorded reduced capacity of RPM to colonize several banana and plantain clones under quarantine conditions, while Carrillo *et al* (2012) observed very low RPM infestations on *Musa* sp. in Florida, USA.

Since the most abundant clones found in the backyards were Bluggoe, Horn, and Silk, they were selected to record the possible relation of their infestation (scale 0–3) with

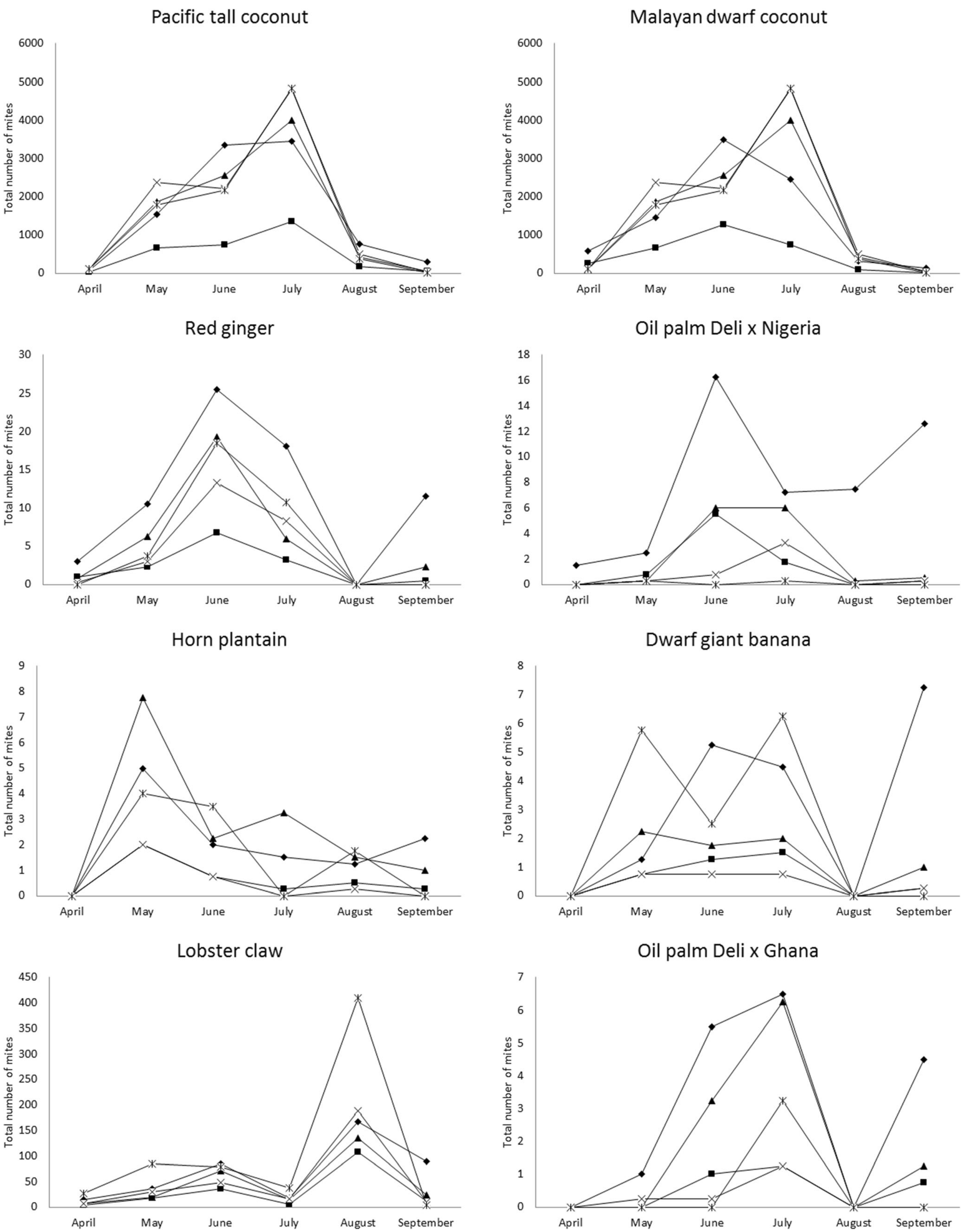

Fig 4 Population fluctuation of developmental stages of *Raoiella indica* on experimentally infested plants. Cancún, Q. Roo, Mexico, April to September 2011. females males nymphs larvae eggs.

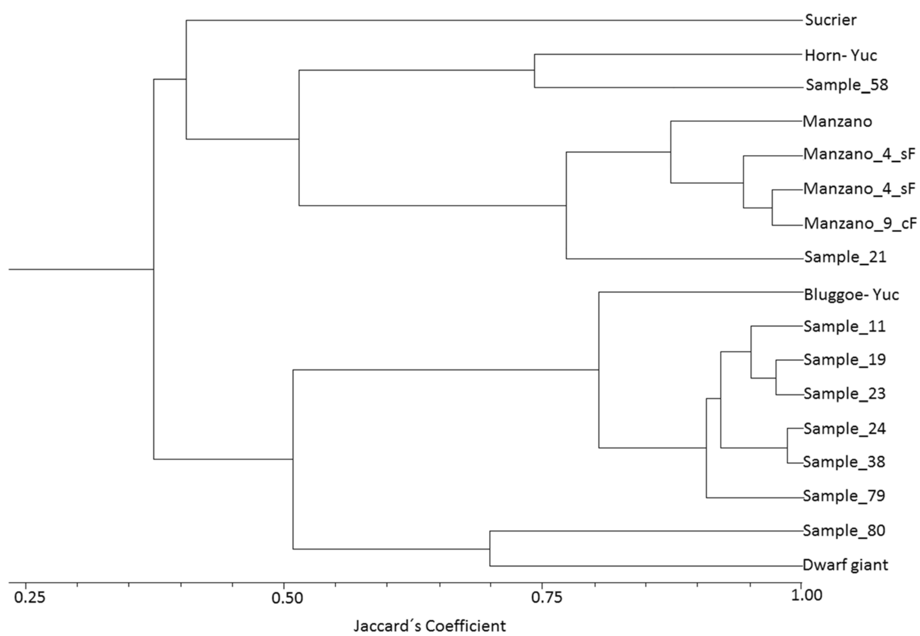

Fig 5 Dendrogram of genetic similitude formed by cluster analysis of unweighted pair-grouping method with arithmetic averages (UPGMA), according to the Jaccard's coefficient, for 17 genotypes of banana and plantain proceeding from the Bank of Germplasm, Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias, Uxmal, Yucatán, Mexico, and field samples. Unnamed samples were identified as follows: 58, Horn plantain; 21, Silk plantain; 11, 19, 23, 24, 38, and 79, Bluggoe plantain; 80, close to Dwarf Giant.

plant height (m), distance from infested coconut palms (m) and exposure to sunlight (scale 1–3). Trends expressed by correlations are shown in Table 2.

As a general trend, taller plants were more seriously infested by RPM; however, a significant relation was found only for Silk plantain. Rodríguez *et al* (2007) observed that this mite attains higher infestations on old coconut palm basal leaves. In our research, the highest infestations also occurred in the lowest (and oldest) banana and plantain leaves, while the youngest leaves were regularly mite-free. Plant height, associated with availability of mature leaves, might explain why in the nursery experiment, no high infestations were observed, similar to results of Cocco & Hoy (2009).

Banana and plantain plants growing close to RPM-infested coconut palms presented slightly higher infestation levels than more distant plants. However, the relation between infestation and distance from coconut palms was not significant for any cultivar. Rodrigues & Irish (2012) observed significantly higher RPM infestations in *Musa* spp. when these plants were surrounded by infested coconut palms. They suggested that such infestations may be due, at least partially, to continuous immigration of mites from surrounding coconut palms. The results of our research do not support that hypothesis since at least some bananas and plantains situated far from coconut palms were seriously infested and overlapping mite generations were observed on them.

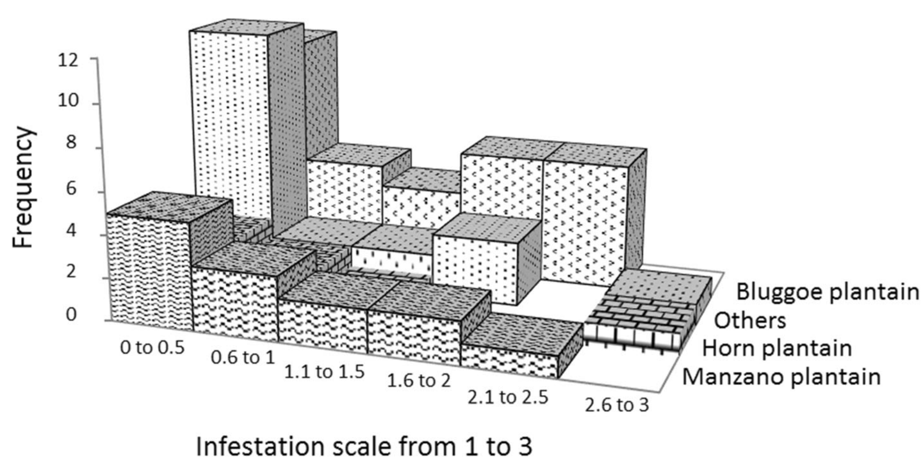

Fig 6 *Raoiella indica* infestation levels on backyard and street bananas and plantains of different genotypes. Cancún, Quintana Roo, and El Cuyo, Yucatán, Mexico, 2012 and 2013. 0: negative; 1: slight; 2: moderate; 3: severe.

Table 2 Spearman's correlation coefficients between the scale of infestation and plant height, distance of plants from infested coconut palms, and exposure to sunlight in Bluggoe, Horn, and Silk plantains (SAS, 9.0) (SAS Institute 2002).

Musa clones	Spearman's coefficients and associated probabilities between RPM infestation and the variables below		
	Plant height (m)	Distance from nearest infested coconut (m)	Scale of exposure to sunlight
Bluggoe plantain	0.0912 $p > 0.6196$	0.01911 $p > 0.9173$	−0.1629 $p > 0.3730$
Horn plantain	−0.0496 $p > 0.9070$	0.1732 $p > 0.6817$	0.4543 $p > 0.2581$
Silk plantain	0.6813 $p > 0.0052$	−0.1022 $p > 0.9712$	−0.0959 $p > 0.7337$

High infestations more likely resulted from reproduction of mites previously settled on those plants.

Intuitively, it was observed that bananas and plantains subjected to stress presented higher infestations. The degree of exposure to sunlight was used as an indicator of moisture deficit stress. However, no significant correlation was found between infestation and exposure to sunlight.

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