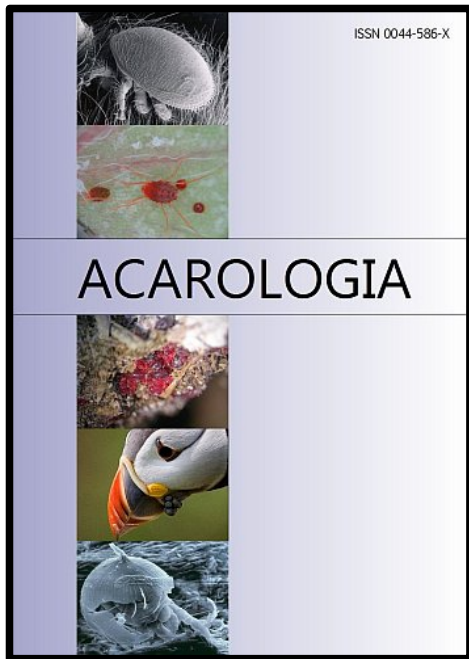




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POPULATION TRENDS OF THE RED PALM MITE, *RAOIELLA INDICA* HIRST (ACARI: TENUIPALPIDAE) AND ASSOCIATED ENTOMOPATHOGENIC FUNGI IN TRINIDAD, ANTIGUA, ST KITTS AND NEVIS AND DOMINICA

Yelitza COLMENAREZ¹, Dave MOORE², Perry POLAR³ and Carlos VASQUEZ⁴

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¹CABI Brazil. UNESP - Fazenda Experimental Lageado Fundação de Estudos e Pesquisas Agrícolas e Florestais. Rua José Barbosa de Barros, 1780, Botucatu - São Paulo, Brazil. y.colmenarez@cabi.org

²CABI UK-Biopesticide Division. Bakeham Lane, Edham, Surrey, TW 209TY. United Kingdom. d.moore@cabi.org

³CABI Caribbean and Latin America. Gordon Street, Curepe, Trinidad and Tobago. perrypolar@gmail.com

⁴Universidad Centroccidental Lisandro Alvarado. Decanato de Agronomía. Departamento de Ciencias Biológicas. Barquisimeto, estado Lara. Venezuela. carlosvasquez@ucla.edu.ve

ABSTRACT — A survey to determine population trends and entomopathogenic fungi associated with the red palm mite (RPM), *Raoiella indica*, was conducted in Trinidad, Antigua, St. Kitts and Nevis and Dominica. RPM population density was evaluated by sampling a total of ten coconut palms per site in Antigua, St. Kitts and Nevis, Dominica, and Trinidad (Manzanilla and Icos). Mites from the four islands were either surface sterilized or left unsterilized before being cultured on Tap Water Agar (TWA). A total of 318 fungal colonies were retrieved. A further 96 mites from Dominica were kept on sterile moist filter paper in a humidity chamber and a further 85 colonies were isolated. Based on morphological observations of all 403 isolates, a sample consisting of 32 colonies (8 %) was sent for identification at CABI-UK. Of the 27 fungi positively identified, 15 isolates belonged to the genera *Cladosporium*, three to *Simplicillium* spp., and one to *Penicillium*. Other fungi genera with limited or no entomopathogenic potential included: *Aspergillus*, *Cochliobolus*, *Fusarium*, *Pestalotiopsis* and *Pithomyces*. The results show a potential use of entomopathogenic fungi for population management of the red palm mite in the Caribbean region.

KEYWORDS — biological control; management; Caribbean region

INTRODUCTION

The red palm mite (RPM), *Raoiella indica* Hirst, is of Oriental origin (Dowling *et al.* 2012). It was first reported in the Caribbean region in 2004 (Flechtmann and Etienne 2004) and is currently widely distributed in most of the Caribbean islands (Kane *et al.* 2005, Rodrigues *et al.* 2007, de la Torre *et al.* 2010). Mainland colonization was verified in Venezuela (Vásquez *et al.* 2008), Florida (FDACS 2007), Mexico

(NAPPO 2009), and more recently in Brazil (Návia *et al.* 2011) and Colombia (Carrillo *et al.* 2011). The RPM can cause severe damage to plants of the Arecaceae family, especially coconut (*Cocos nucifera* L.), but also to Musaceae and others (Flechtmann and Etienne 2004, Flechtmann and Etienne 2005, Etienne and Flechtmann 2006, Carrillo *et al.* 2012a). Damage is caused by feeding through the stoma on the abaxial surface of the leaf, lamina altering guard cells

leading to yellowing, necrosis and death of infested leaves (Ochoa *et al.* 2011, Beard *et al.* 2012).

Most RPM population management strategies in the Eastern hemisphere have focused on chemical control (Senapati and Biswas 1990, Jayaraj *et al.* 1991). In the Neotropical region, several systemic insecticides (phosphamidon, monocrotophos, dimethoate, formothion and demeton-methyl) exhibited toxicity to larva, nymphs and adults (Rodrigues and Peña 2012).

Although RPM population management has relied on chemical compounds, there is some knowledge about its natural enemies, mainly in the eastern hemisphere (Peña *et al.* 2006). Recent information has shown that *Amblyseius largoensis* (Muma) is very common on coconut palms and thus is the primary predator associated with RPM (Carrillo *et al.* 2012b, Domingos *et al.* 2013) suggesting that it can play a role in controlling RPM (Carrillo *et al.* 2010).

Research has also been conducted on biological pesticides with variable results, since most of these obligate entomopathogenic fungi, often included in Entomophthorales, have narrow host ranges and often cause natural epizootics but are not easily grown *in vitro* (Chandler *et al.* 2000). These pathogens are not regularly used for inundative inoculation since mass production on living hosts is not cost effective (St. Leger and Screen 2001). Although several studies have demonstrated that a number of Entomophthorales, mainly *Hirsutella thompsonii* and *Neozygites floridana*, are specific to the Acari (Chandler *et al.* 2000), information related to those entomopathogenic fungi associated to RPM is still lacking. So far, it has been reported that a small portion of RPM was infected by a fungus, possibly *Hirsutella* spp. (Peña *et al.* 2006). In light of limited control by natural enemy populations, biological pesticides could be a potential option for RPM control in the Caribbean. The aim of the study was to examine the incidence of RPM cadavers demonstrating mycelial growth or other symptoms indicative of fungal attack (e.g. mummification); to isolate and identify the fungi involved; and to discuss the potential of further work with entomopathogenic fungi. Thus, surveys to identify the potential entomopathogenic fungi associ-

ated with RPM in Antigua, St. Kitts and Nevis and Dominica and Trinidad and Tobago coconut plantations were conducted in this study.

MATERIALS AND METHODS

Assessment of *Raoiella indica* populations

An evaluation of RPM population density was conducted in Antigua, St. Kitts and Nevis and Dominica by sampling a total of ten coconut palms per site. A total of 20 palm trees were sampled in Trinidad; ten each at Manzanilla and Icacos. Weather conditions in Antigua, St. Kitts and Nevis and Dominica made it difficult to sample tall plants (<20 feet), therefore sampling was restricted to shorter coconut palm trees.

Samples of pinnae were taken from coconut fronds of various ages. These were classified as young (taking the leaf at approximately 3 months post emergence), medium (approximately 5-6 months post emergence) and old (approximately 7-8 months post emergence). In the case of Trinidad, pinnae were taken from the 3rd, 5th and 7th fronds. Based on previous studies in Trinidad, only the 3rd and the 5th frond were taken in the other islands evaluated. Pinnae (5) were cut from the midrib at the base, near the middle and near the tip of the frond. These were placed in labeled plastic bags.

Two pinnae from each bag were randomly selected and cut into three pieces of equal length. The number of live RPM (motile forms) and eggs were counted using a 1 cm² cardboard cut-out, at five approximately equidistant points along each segment under stereomicroscope magnification. For Antigua, St. Kitts and Nevis and Dominica, five replications/segment, 15 replications/pinna, 90 replications/frond, and 180 replications/plant were run; giving a total of 1800 replications/area on each island. In the case of Trinidad, 90 replication/frond and 3 fronds/plant were run, resulting in 270 replications/plant and a total of 2700 replications/area.

The means of motile forms and eggs counted on each island were compared using paired t-tests at the 99% level of significance. A square root transformation of the means was required before analysis

as the disparity in numbers between islands were highly significant.

Isolation of fungi associated with *Raoiella indica*

RPM colonies from fronds of ten coconut palms sampled from Trinidad (Manzanilla, Icacos), Antigua, St. Kitts and Nevis and Dominica, and from random plants from Cumana, Salibia and Siparia, were observed for mites with “abnormal” characteristics which could indicate entomopathogenic fungi (EPF) infection. These characteristics included unusual darkening and fungal spores observed on the cuticle of the mite.

A total of 902 mites from the four islands were selected and plated on TWA to encourage growth of pathogenic fungi. *Raoiella indica* females (432) from Trinidad (Cumana, Salibia, Icacos, Siparia, Manzanilla) were cultured on Tap Water Agar without sterilization, while females from Antigua (50), Dominica (250), and St. Kitts and Nevis (170) were sterilized with a drop of 95% ethanol and cultured on Tap Water Agar.

Some RPM females (96) from Dominica exhibiting “mummification” were placed on filter paper in glass vials, transferred to Petri dishes and either sterilized in 3% hydrogen peroxide or 95% ethanol for five seconds and rinsed in sterile distilled water or left unsterilized. Mites were placed on moistened sterile filter paper in Petri dishes which were placed in a humidity chamber to encourage sporulation.

These plates were examined after 5–6 days, clean isolates were given a unique ID (Annex 2) and upon observation of colony morphology, representative isolates of all morphs observed were cultured on Potato Carrot Agar (PCA). These isolates (32 in total) were selected as representative of the colony morphologies and sent to Bioservices Division in the UK lab for identification using a combination of morphological and molecular techniques observed.

RESULTS AND DISCUSSION

RPM Population Densities

Overall, the highest RPM population densities were encountered in Trinidad-Icacos (5.9 motile and 3.7

eggs per cm², respectively) and the lowest in Antigua (1.32 motile and 2.1 eggs per cm²) (Figure 1). The RPM population densities (motile forms in St. Kitts and Nevis and Dominica were found to be similar while egg number was significantly higher in S. Kitts. Information about the RPM population trends are still scarce in the Caribbean region, however, according to Roda *et al.* (2012), maximum RPM density values in the whole canopy ranged from 6 to 8 mites/cm² from 2006–2008 in Trinidad, being comparable to those determined in the present study. Although, Taylor *et al.* (2012) did not find any climatic factor such as temperature, humidity and rainfall to be significantly related to population growth, interactions between all climatic factors were evident on coconut. According to these authors, whether the regional differences in climatic factors are enough to affect RPM populations on their own remains to be proved but, the same relationship between lower humidity and higher temperature and RPM density was also reported by Nagesha-Chandra and Channabasavanna (1983) on coconut.

Fungi isolated and identified from RPM

Mite inspection resulted in 318 fungal colonies which were morphologically examined and recorded (Table 1). Five of the isolates could not be identified due to bacterial contamination. The identification of 23 of the isolates was accomplished by morphological keys with four isolates requiring molecular fingerprinting to confirm identification.

Of the morphologically identified isolates, specimens of the genera *Aspergillus*, *Cladosporium*, *Curvularia*, *Drechslera*, *Fusarium*, *Penicillium*, *Pestalotiopsis* and *Simplicillium* were obtained in all the countries evaluated (Figure 2). Of particular interest are the four isolates identified by molecular fingerprinting. Three of these were identified as *Simplicillium* sp. (isolate no. 2, 3 and 6) and they could constitute an undescribed taxon, being herein reported as pathogenic on insects. The fourth isolate was identified as a possible mite specific strain of *Penicillium* sp. (isolate no. 5) similar to a species described as *Paecilomyces parvisporus*. Current information about entomopathogenic fungi associated to RPM is lack-

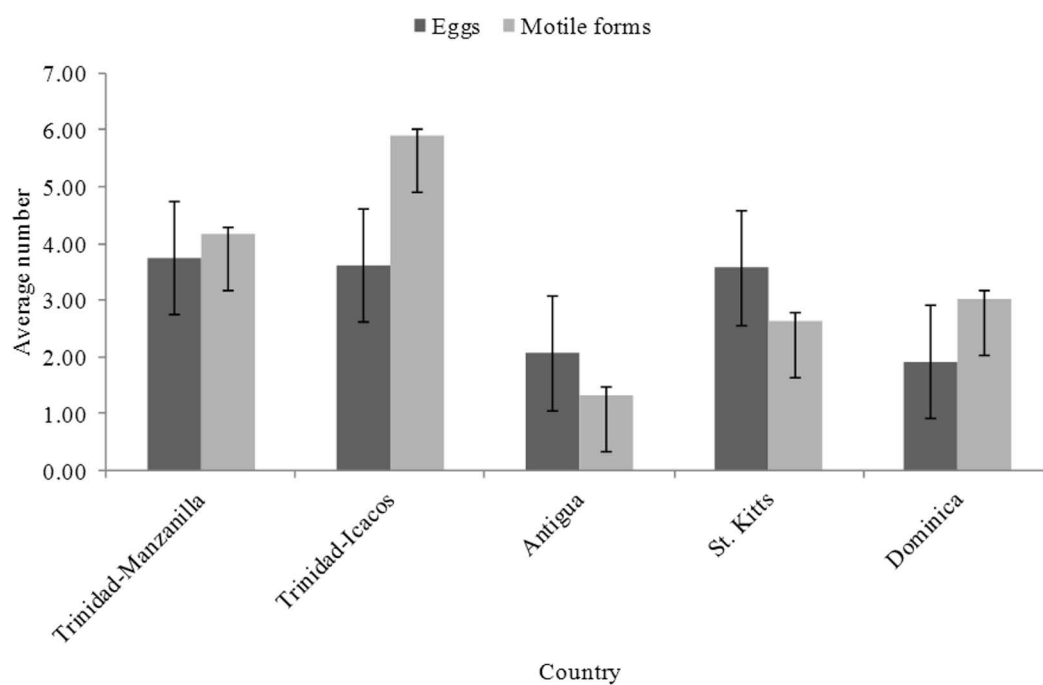


FIGURE 1: Average population of Red Palm Mite in the different islands evaluated.

TABLE 1: Number of fungal colonies recovered from *Raoiella indica* plated on TWA

Country	Number of mites plated out	Number of cadavers showing mycelial growth
Trinidad	432	250
Antigua	50	1
St Kitts and Nevis	250	48
Dominica	170	19
Total	902	318

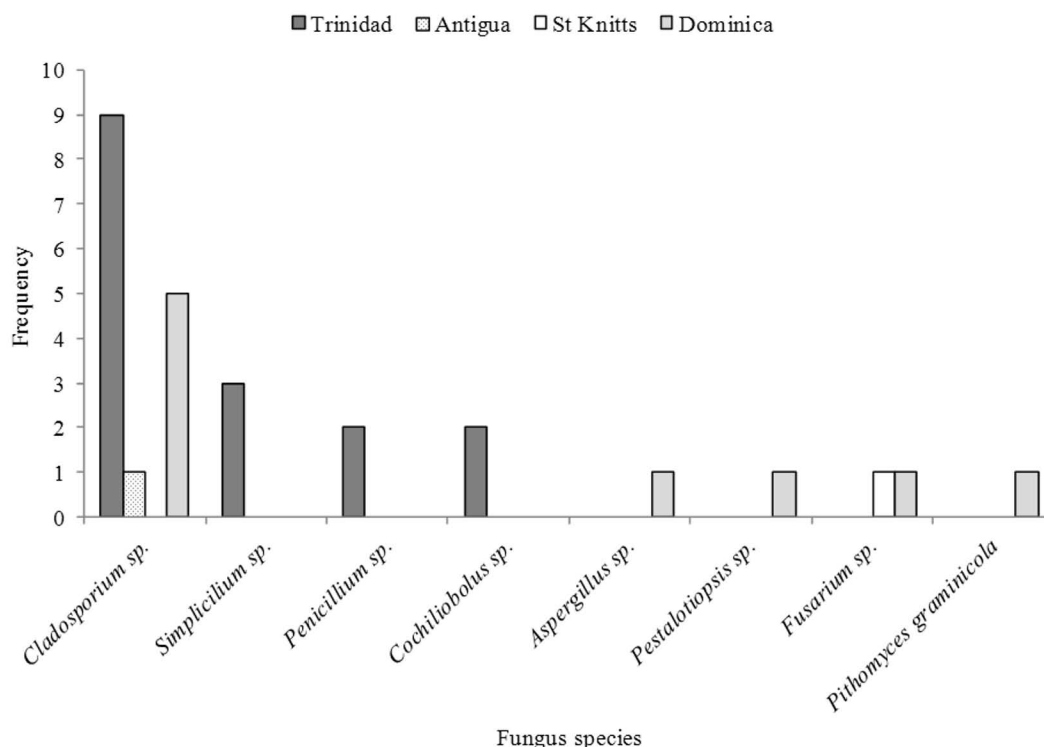


FIGURE 2: Identified material from selected samples in the different islands evaluated. NB. For reference, the population density (mites/cm²) from Trinidad-Icacos (10.69), Trinidad (5.79), Antigua (3.38), St. Kitts and Nevis (6.17) and Dominica (4.94).

ing, thus these four isolates could be considered to be of interest for future evaluation of their biological pesticide potential. So far, *Lecanicillium psalliotae* has been the only fungus species reported in association with RPM in Saint Lucia (ARSEF 2009).

From the total 8 genera reported, 4 were present in Trinidad, including the two genera of higher interest for further evaluations, namely *Simplicillium* and *Penicillium*. The three *Simplicillium* isolates were recovered from Trinidad-Icacos (2) and Trinidad-Siparia (1). The *Penicillium* isolates (2) were also found in Trinidad-Manzanilla. Although Zare and Gams (2001) erected the new genus *Simplicillium* to include only fungicolous species, some of them have been reported as insect pathogens. *Simplicillium lanosoniveum* has been obtained from the rubber tinged bug *Leptopharsa heveae* (Hemiptera: Tingidae), *Simplicillium lamellicola* from an unidentified mite (Acari: Oribatidae) and *Simplicillium* sp. from the whitefly *Bemisia tabaci* (Hemiptera:

Aleyrodidae) (ARSEF 2009). Polar *et al.* (2005) found *Simplicillium lamellicola* (ARSEF1367) to be pathogenic to ticks.

On the other hand, *Penicillium* species have also been isolated from insects including grasshoppers, *Zonocerus variegatus* (Balogun and Fagade 2004). Our strains are of some interest as they belong to a poorly known group with *Eupenicillium teleomorphs*, others of which have been found associated with ant mortality. From Trinidad two isolates, 5 and 7, were reported as *Penicillium*, with isolate 5 producing white colonies and it appears to be similar in morphology and the ITS sequence close to a soil species from Yunnan, recently described as *Pae-cilomyces parvisporus*. This isolate is of importance for further studies to test its pathogenicity to RPM. Conversely, isolate 7 produced green colonies and was markedly different from isolate number 5. It was not considered to be a potential invertebrate pathogen.

Previous studies have stated that species belonging to *Cladosporium*, *Fusarium*, *Penicillium*, *Aspergillus*, and many other genera are among the most commonly encountered growing on arthropod cadavers, and that at best they are facultative pathogens (Roberts and Humber 1981, Humber 1991). However, it is still unclear the extent to which those genera are pathogenic to arthropods or might be opportunistic secondary pathogens or saprobes that colonize available cadavers (Sosa-Gómez *et al.* 2010).

Fifteen(15) of the 27 cultures were identified as belonging to the genus *Cladosporium* (isolates no. 1, 8, 10, 11, 13, 14, 16, 17, 18, 21, 22, 23, 25, 29, 32). *Cladosporium* was isolated from RPM populations from three islands in this survey, the exception being St. Kitts and Nevis. Nine of the *Cladosporium* isolates were found in Trinidad-Icacos (4), Cumana (3), Siparia (1), Salibia (1). Five *Cladosporium* isolates were found in Dominica and only one in Antigua. There were two *Cochliobolus* isolates from Trinidad-Salibia (1) and Trinidad-Manzanilla (1). There was one *Aspergillus* isolate (isolate no. 19) and one *Pestalotiopsis* isolate (isolate no. 26) from Dominica, two *Fusarium* isolates (isolate 27, 30) from Dominica and St. Kitts and Nevis and one *Pithomyces graminicola* (isolate no. 31) from St. Kitts and Nevis.

Cladosporium is one of the largest, most heterogeneous genera of hyphomycetes, comprising more than 772 names (Crous *et al.* 2007), and including endophytic, fungicolous, human pathogenic, phytopathogenic and saprobic species. However, there are a few records of *Cladosporium* isolated from insects and mites, and they are unlikely to be pathogenic to those organisms. *Cladosporium* sp. has been isolated from whitefly (Homoptera: Aleyrodidae), aphid *Brevicoryne brassicae* (Homoptera: Aphididae), butterfly *Orachrysops ariadne* (Lepidoptera: Lycaenidae), scale (Homoptera: Coccidae), leafhopper (Homoptera: Cicadellidae) and a mite, *Tetranychus urticae* (Acari: Tetranychidae) (ARSEF 2009). Moreover, nymphs of whiteflies shown to be more susceptible to *Cladosporium* species than other life stages (Aldeghairi *et al.* 2013, Abdel-Baky 2000). Members of this genus are of

widespread distribution and are commonly isolated from a wide range of plant species as well as from soil. Anamorphic species linked to this genus belonging to both *Curvularia* (isolate 15) and *Drechslera* (isolate 12) were isolated. *Curvularia* species as facultative parasites are among microorganisms being used for controlling weeds (de Luna *et al.*, 2002). Although, it is still unclear its role as entomopathogenic agents, some *Curvularia* species have been isolated from the spittlebug, *Zulia carbonaria* (Homoptera: Cercopidae) (ARSEF 2009). Members of *Drechslera* are known to cause some of the most devastating disease epidemic in plants in modern times (Strobel *et al.* 1988) and there are no reports of *Drechslera* being isolated from arthropods from the ARSEF collection (ARSEF 2009). Similarly, there are no reports of *Pestalotiopsis* being isolated from arthropods (ARSEF 2009).

Regarding *Fusarium*, application of *Fusarium semitectum* dust water formulation in combination with monocrotophos (0.05%) was able to suppress the population of *Polyphagotarsonemus latus* in *Capicum annum* (Mikunthan and Manjunatha 2006a) and the combination of *F. semitectum* at 4.20×10^9 spores/ml and dimethoate at 0.03% recorded the highest mortality on *Myzus persicae* (Manjunatha *et al.* 2009). Furthermore, *F. semitectum*, in general, expressed its selectivity against sucking pests and proved its eco-friendly characteristics to beneficial organisms in Karnataka, India, demonstrating that this novel fungus can be well incorporated as a viable tactic into integrated pest management programs (Mikunthan and Manjunatha 2006b).

In general, the fungi with the greatest potential for pathogenicity on the RPM was not found on Antigua, the island which had the lowest mite population density with the mean number of eggs being close to double that of the number of adults. Conversely, these fungi were found in Trinidad where the highest mite population density was encountered. However, the mite population density at Manzanilla-Trinidad, where *Penicillium* isolate (greatest pathogenic potential) was found is not significantly different from the densities found in St. Kitts and Nevis and Dominica, although the fungus was not found on the latter islands. Contrary to

expectations, two of the *Simplicillium* isolates were found at Icacos where the highest RPM population density was encountered.

These results suggest that the presence of fungi with entomopathogenic potential is not the only factor determining RPM population densities in the Caribbean. Thus, role of other biotic and abiotic factors regulating the RPM populations should be considered. Furthermore, the population densities and composition of natural enemy complexes found in association with RPM colonies on all islands needs further investigation. That being said, the discovery of *Simplicillium* and *Penicillium* isolates found in association with RPM populations is of high potential for further use in pest management programs.

The population of RPM in Trinidad was higher than in the other islands. The pest is widespread in Trinidad and most of the varieties of coconut used in the country show to be susceptible to it. In Dominica, the lower population or absence of the pest in the material evaluated lead us to believe that there is some level of tolerance to RPM in some of the palms. It is interesting to verify that although the RPM was first reported in Dominica in 2005, the population did not develop as fast as it did in Trinidad. Further studies are needed in order to verify if there is such level of tolerance in those plants and to study other possible factors that may restrict the development of the population.

The most desirable situation in prospecting for entomopathogens of the RPM would be obtaining colonies of mites in which dead individuals display mycelial growth. It would then be a relatively simple procedure to sterilize the outer surface of the organism (as the fungi grows inside) and culture on media or directly transfer some mycelia from a cadaver to a medium followed by purification of the isolate.

Preliminary examination of a considerable number of pinnae in Trinidad showed no obvious sporulating mite cadavers. Consequently, a different strategy was adopted by examining the colonies of RPM closely and observing individuals which looked "abnormal". This strategy allowed for a greater number of palms to be observed in a shorter period. No signs of obvious abnormal mite colonies

were observed in Trinidad, Antigua or St. Kitts and Nevis. On the last island sampled, Dominica, the final two trees examined presented mummified mites that were clearly different from any other seen from previous samples.

Of the 27 fungal isolates identified, three belonging to *Simplicillium* sp., representing a possible undescribed taxon, and *Penicillium* sp. (isolate no. 5) were of the most interest for future evaluation of their potential as biological pesticides. *Simplicillium* isolates, formerly identified as species of *Verticillium*, have been used in commercial biological pesticides for the control of whiteflies, thrips and aphids (Butt *et al.* 2001). Based on preliminary molecular analysis, *Penicillium* sp. (isolate no. 5) appears to belong to a strongly insect-associated clade. Members of this group have not been used yet in biological pesticide production but could be investigated further.

Some of the remaining fungi from the survey have potential entomopathogenic properties but do not belong to the most favoured genera for biological pesticide development (*i.e.* *Beauveria*, *Metarhizium*). Chandler *et al.* (2000) state that it is unclear whether the fungi commonly used in biological pesticide development are opportunists or have evolved a degree of specialization for the Acari. A number of isolates of *H. thompsonii* Fisher and *N. floridana* are specific to the Acari (Chandler *et al.* 2000) but were not isolated in this study.

The overwhelming numbers of cultures positively identified as *Cladosporium* sp. (15/28) may indicate an association of the fungus with the mite environment (possibly saprophytic) since *Cladosporium* sp. is not known to be entomopathogenic. The majority of the 318 fungal cultures obtained are also likely to be *Cladosporium* based on initial morphology. The fungus observed by Hosein (2008) was subsequently identified and found to be *Cladosporium* sp., hence this study confirms his findings. The mummified cadavers from Dominica also revealed the presence of *Cladosporium*. This indicates that *Cladosporium* may be ubiquitous in the environment of the RPM.

The RPM are delicate organisms, so their cadavers decompose and lose shape very quickly. If killed

by EPF and the environmental conditions are appropriate, some cadavers may retain their shape to an extent as they become, in effect, mummified. It is likely that *Cladosporium* sp., acting as a saprophyte, may have infested dead or dying mites (which may or may not have been previously attacked by a pathogen). The mycelium observed from the cadavers, often at leg segments, may have given the appearance of death by entomopathogenic fungi.

Pithomyces graminicola, a pathogen which causes leaf diseases in small grain cereals and major yield losses throughout the world, was unidentified in St. Kitts and Nevis. It is not known whether this pathogen has been previously reported in the country.

Of the four islands surveyed in this study, Trinidad showed the higher mite population and also the higher occurrence of entomopathogenic fungi infections. However, it seems that the presence of these entomopathogenic fungi is not exerting any control yet on the population of RPM. Placed in the context that the report from Trinidad is one of few studies to date from the Caribbean, it would appear that the level of control exerted by the entomopathogenic fungus is low. This may just reflect true pathogenesis through to sporulation from cadavers; many mites may have been killed by fungus, which failed to grow through. Thus further studies are necessary in order to evaluate the level of pathogenicity of the fungi reported in this study. Future research should also include the evaluation of known effective biopesticides against RPM.

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