

**The ecology of *Raoiella indica* (Hirst: Tenuipalpidae) in
India and Trinidad: Host plant relations and predator:
prey relationships**

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Abstract

Red Palm Mite, *Raoiella indica* (Acari:Tenuipalpidae) (RPM), an Old World species first recorded in India (1924), was reported historically on a small number of host species of Arecaceae (palms) throughout Asia and the Middle East. In 2004, the mite invaded the New World resulting in high population densities and apparent new host associations- including *Musa* spp. (bananas and plantains). Subsequently, RPM has become widely established in the tropical Americas. The aim of the work was to understand some of the factors underlying the differences in RPM population status between the two regions by comparing morphological and molecular characteristics, investigating Old World host relations and comparing predator–prey interactions in India and Trinidad. Field population densities on main host *Cocos nucifera* were significantly higher in Trinidad compared to India. Substantial RPM field densities and breeding colonies were confirmed on *Musa* spp. in Trinidad, but not India; although RPM was shown to survive and lay eggs on *Musa* spp. *in vitro* in India. Arthropod predator diversity in India was higher on *C. nucifera* and lower on *Musa* spp. compared to Trinidad. Surveys on both host species only recorded predatory mites in Trinidad, whereas predatory insects and mites were recorded in India. Although molecular studies were unsuccessful, morphological studies showed that, independent of host plant, RPM had significantly longer lateral setae in the Caribbean compared to India. Adventive range populations may have resulted from a genetic bottleneck upon introduction. Assays in Trinidad showed that RPM with droplets on dorsal setae were contacted and consumed by *Amblyseius largoensis* on fewer occasions than those without. Setae may also play a role in defence against *A. largoensis*. Studies confirmed differences in RPM severity and status on host plants between regions and suggest natural enemies play a role in maintaining a narrow host range in India.

Contents

Copyright declaration.....	2
Abstract	3
Contents	4
1.0 Introduction.....	8
2.0 <i>Raoiella indica</i> : A literature review.....	12
2.1 Mites, the Tenuipalpidae and the genus <i>Raoiella</i>	12
2.2 <i>Raoiella indica</i> : life cycle	13
2.3 Host plants of <i>Raoiella indica</i>	17
2.4 Biogeography	18
2.5 Predator relations	22
2.6 <i>Raoiella indica</i> - Population dynamics	27
2.7 Invasion ecology theories	28
2.8 Host association, host races and biotypes.	31
2.9 Questions relating to observations of invasive populations of RPM.....	33
2.9.1 Has RPM expanded its host range in the New World and if so, what are the factors behind this expansion?	33
2.9.2 How accurate are the reports relating to the increase in RPM population density between Old and New World regions? If accurate, what factors may be driving the differences?	34
3.0 The morphological and molecular characterisation and comparison of RPM from India and the Caribbean	36
3.1 Introduction.....	36
3.2 General approaches	38
3.2.1 Morphological comparison	38
3.2.2 Molecular techniques	40
3.3 Materials and Methods.....	42
3.3.1 Selection of populations.....	42
3.3.2 Collection of mites	42
3.3.3 Morphological measurements	43
3.3.4 Statistical analysis of morphological measurements	43
3.3.5 Molecular analysis	46
	4

3.3.6 DNA extraction.....	46
3.3.7 PCR methodology.....	47
3.3.8 Gel electrophoresis.....	47
3.3.9 Sequencing.....	47
3.3.10 Improved PCR methodology	48
3.4 Results.....	49
3.5 Discussion.....	72
4.0 Host plant relations in the Old World.....	77
4.1 Introduction.....	77
4.2 Methods.....	81
4.2.1 Hypothesis 1.....	81
4.2.2 Hypothesis 2.....	89
4.2.3 Analyses.....	90
4.3 Results.....	92
4.3.1 Hypothesis 1.....	92
4.3.2 Hypothesis 2.....	108
Survival and reproduction of RPM on natal host compared to alternative host	108
4.4 Discussion.....	115
5.0 A comparison of RPM and related predator complex between the adventive range (Trinidad) and naturalised range (India)	122
5.1 Introduction.....	122
5.1.1 An overview of RPM population studies to date	122
5.1.2 Review of natural enemies.....	126
5.2 Objectives	127
5.3 Materials and Methods.....	128
5.3.1 Study sites and seasons	128
5.3.2 Survey methodology	128
5.3.3 Predator diversity.....	129
5.3.4 Comparison of biological performance of RPM between Trinidad and India.....	129
5.3.5 Simple degree day model predicting RPM development in each range	130
5.3.6 Morphological comparison of <i>Amblyseius largoensis</i> between India and Trinidad	130

5.3.7 Statistical analysis.....	131
5.4 Results.....	131
5.4.1 Survey results: Baseline study comparing RPM population densities between India and Trinidad.....	131
5.4.2 Survey results: Predator density and diversity in India and Trinidad.....	134
5.4.3 Relationship between RPM densities on <i>C. nucifera</i> and <i>Musa</i> sp. found on the same site in Trinidad.....	135
5.4.4 Comparison of biological performance of RPM between Trinidad and India.....	136
5.4.5 Simple degree day model predicting RPM development in each range	139
5.4.6 Morphological comparison of <i>Amblyseius largoensis</i> between India and Trinidad	142
5.5 Discussion	144
6.0 The effect of RPM lateral setae and secreted droplets on predation by <i>Amblyseius largoensis</i>	152
6.1 Introduction.....	152
6.1.1 Mode of action of Phytoseiids	153
6.1.2 RPM defence.....	153
6.2 Baseline studies.....	154
6.2.1 Investigating and characterising the theoretical barrier to predation of RPM posed by long lateral setae on RPM specimens in Trinidad compared to India when compared to ‘attacking structures’ of <i>Amblyseius largoensis</i>	154
6.2.2 Investigating and characterising droplet expression between RPM cultured on different host plants and regions	160
6.3 A test of the effect of RPM lateral setae length and droplet expression on predation by <i>A. largoensis</i>	165
6.3.1 Methods.....	165
6.3.2 Results.....	168
6.4 Discussion	179
7.0 General discussion	185
7.1 Has RPM expanded its host range in the New World and if so, what are the factors behind this expansion?.....	186
7.2 How accurate are the reports relating to the increase in RPM population density between Old and New World regions? If robust, what factors may be driving the differences?	191
Conclusions.....	196

Acknowledgements.....	198
8.0 Bibliography	199
9.0 Appendix.....	219
Appendix 1 Fieldwork dates, locations and activities	219
Appendix 2 Host plant, country and location within country of RPM specimens collected and measured for the study	221
Appendix 3 Males specimens slide mounted for morphological ID.....	223
Appendix 4 Samples for molecular work	224
Appendix 5 Trinidad RPM sequence	226
Appendix 6 Density statistic results for RPM individuals and mean RPM measurements from Trinidad 2012.....	227
Appendix 7 Mean setae lengths of RPM from 4 different host plants in Kerala, India.....	228
Appendix 8 Mean seta lengths of RPM from four islands in the Caribbean (± 1 SE).....	229
Appendix 9 Mean seta lengths (μm) of RPM collected from <i>C. nucifera</i> and <i>Musa</i> spp. in the Caribbean (± 1 SE).....	230
Appendix 10 Mean setae lengths of RPM specimens from Caribbean and India.	231
Appendix 11 Molecular results.....	232
Appendix 12 Morphological features measured on <i>Amblyseius largoensis</i> specimens from India and Trinidad.....	233
Appendix 13 A preliminary study was set up to assess the egg consumption of <i>Amblyseius largoensis</i> between Trinidad and India.....	234
Appendix 14 <i>Amblyseius largoensis</i> method of attack	236
Appendix 15 Predatory Neuropteran consuming RPM mobile stages on leaflet in Trinidad.	240

1.0 Introduction

Raoiella indica (Acari:Tenuipalpidae) (Red Palm Mite-RPM) is a small mite, approximately 300µm long, which traditionally has been reported feeding on select members of the Arecaceae family. The first report of the mite was from India, in Coimbatore, (Hirst, 1924) on coconut (*Cocos nucifera*) (Arecales:Arecaceae) and it has since been reported throughout the Old World through Asia and the Middle East (CABI, 2007). Red Palm Mite has become of recent importance since it was detected in the Caribbean for the first time in 2004 (Flechtmann & Étienne, 2004), and then became widely established in its adventive range through the archipelago of Caribbean islands. Following this, RPM was reported in Venezuela (Vásquez et al., 2008), Florida, USA (Peña et al., 2009), Brazil (Navia et al., 2009), Mexico (Estrada-Venegas et al., 2010) and Colombia (Carrillo et al., 2011). The mite is a threat to the coconut industry and amenity trees as it feeds in great numbers on the underside of leaves through the stomata (Ochoa et al., 2011) on palms and other monocotyledon crops (particularly bananas) causing extensive yellowing to the leaves. This in turn causes not only a drop in the fruiting productivity of the plants and trees [over 50% yield loss in some locations (Carrillo et al., 2014)] but also affects the aesthetic value of the hosts which is important to the lucrative tourist industry in these countries.

Since the introduction into the New World, important differences in the nature of RPM infestations have been observed between the Old World and the adventive range. Population densities in the adventive range have been reportedly very high reaching around 4,000 per leaflet in some cases on coconut (Carrillo et al., 2010), compared to those reported on coconut in the Old World where approximately 300 RPM/leaflet have been reported (Taylor et al., 2012). In addition RPM has been reported apparently utilising a vastly wider host range than previously reported in the Old World, including the economically important *Musa* spp. (Zingiberales:Musaceae). These factors, along with its rapid dispersal throughout the New World indicate that RPM is a successful invader in the adventive range, however little is known about the factors that are driving the invasion. Prior to its introduction into the New World, the majority of research on the mite was carried out in India where it was a minor pest of coconut and betelnut plantations (*Areca catechu*) (Arecales:Arecaceae) and much literature related to the control of the mite using chemicals or its natural enemies (Daniel, 1983; Jalaluddin & Mohanasundaram, 1990; Jayaraj et al., 1991; Kanta et al., 1963; Nadarajan et al., 1980; Raju, 1983; Sarkar & Somchoudhury, 1988; Senapati & Biswas, 1990; Somchoudhury & Sarkar, 1987). Understanding more about the nature of the invasion and factors relating to the success of RPM in the adventive range will be beneficial, as outcomes will provide information in support of

management strategies to bring RPM populations under control.

The overall aims of this thesis were to investigate the differences in observations made between RPM populations in the Old and New World, in relation to host ranges and population densities, and to explore some of the main factors which may explain these differences such as increase in competitive ability and enemy release. Important gaps in knowledge about RPM host utilisation in the Old World were addressed, and comparative data were collected and/or analogous studies were undertaken in both regions where observations and studies were lacking in the current literature. Two countries were selected as focal study areas: India (Kerala) and Trinidad. These areas were selected as they both have established populations of RPM, and CABI has local collaborations which exist within both areas with access to experimental facilities. Work was undertaken during a series of short study trips between 2009-2014. The details of these study trips are given in Appendix 1.

The thesis is split into six sections (chapters 2-7). In chapter 2, literature relating to the current knowledge about the ecology and biology of RPM was reviewed; in addition to a brief overview of relevant subject matter in invasion ecology and host associations and biotypes in the Acari. Two broad questions were posed relating to the current situation with RPM. Firstly, has RPM expanded its host range in the New World and if so, what are the factors behind this expansion? And secondly, how accurate are the reports relating to the increase in RPM population density between Old and New World regions? And if accurate, what factors may be driving the differences?

In chapter 3, a morphological and molecular comparison of RPM collected from different host plants in India and the Caribbean was undertaken to characterise the populations in both countries. This study aimed to contribute to both the understanding of the biogeography of RPM, and explore host plant relations in morphological and molecular terms. Comparisons made between individuals from populations in both India and Trinidad on different host plants allowed for the potential existence of host plant races to be studied and geographical similarities and differences to be explored. Three hypotheses were proposed given the knowledge in the literature at the outset of the study. The first hypothesis stated that there will be molecular and/or morphological differences observed between hosts in India- particularly *C. nucifera* and *A. catechu*; the second that there will be no significant host associated molecular or morphological differences found between populations of RPM in the Caribbean between *Musa* spp. and *C. nucifera* and the third, that there will be no molecular or morphological differences between populations in the adventive range (Caribbean) and India. Results from this study formed baseline data used in the other studies reported later.

In chapter 4, the host plant relations of RPM in India were investigated by means of survival analyses and fecundity assessments along with field surveys. The results of this work were then used to interpret the observed host range in India and were compared to analogous published studies conducted in the New World. This chapter addressed important knowledge gaps about RPM host utilisation in India and allowed for more robust comparisons of host range to be made with observations in the adventive range. Results also raised further questions about the natural enemy associations of RPM in both regions. This chapter proposed two hypotheses. The first hypothesis stated that the host range will be wider than reported for RPM in the Old World; whereby colonies of RPM will be found on other palms/host plants which have been previously unreported. In contrast, the second hypothesis stated that RPM will have host specific races. The race found in Kerala is specific to the order Arecales (palms) and will not feed and reproduce plants from the order Zingiberales (*Musa* spp. and *Heliconia* spp.) or Pandanales (as previously reported in literature) (thus explaining the restricted reported host range).

In chapter 5, further, more detailed surveys on *C. nucifera* and *Musa* spp. were undertaken in both India and Trinidad and assessments of associated predators were made. These studies further explored and compared the relationship of RPM with *Musa* spp. and *C. nucifera*, compared and measured predator diversity and density and allowed for densities of RPM populations to be compared between the two countries and the two different hosts. Several hypotheses were proposed. These were firstly, that RPM populations will be significantly denser in the adventive range compared to the naturalised range on both *C. nucifera* and *Musa* spp.; secondly that there will be a relationship between RPM densities on *C. nucifera* and *Musa* spp. found on the same site- those with high population densities on *C. nucifera* will have higher RPM densities on *Musa* spp. The third hypothesis stated that predator diversity will be poorer in the adventive range (Trinidad) compared to the naturalised range (India). Factors which were hypothesised to drive observed density differences such as predator performance/biotype, performance of RPM and general climatic conditions were also explored in a series of baseline assessments.

In chapter 6, a more detailed investigation was made into the relationship RPM has with its predominant predator, the mite, *Amblyseius largoensis* (Acari:Phytoseiidae), with particular focus on the relationship with RPM lateral setae length and the presence and absence of droplets on the distal tips of these setae. These studies built on observations made in chapters 3, 4 and 5 relating to differences in lateral setae length between RPM from populations in both regions, the presence of droplets on RPM and the presence of *A. largoensis* in both regions. Experiments tested two hypotheses; firstly, that long lateral setae (c3, d3 and e3) will affect the efficacy of *A. largoensis* when

attacking adult female RPM- RPM with longer lateral setae will not be predated, RPM with shorter lateral setae will be predated. Secondly, droplets found at the distal end of the dorsal setae will enhance the defence of RPM against *A. largoensis* attack- RPM with droplets will be attacked on fewer occasions compared to those without droplets.

Lastly, in the final chapter (chapter 7), findings are summarised and an assessment of how they contribute to current knowledge about RPM is made. Remaining key gaps in knowledge about the RPM invasion are identified and discussed.

2.0 *Raoiella indica*: A literature review

2.1 Mites, the Tenuipalpidae and the genus *Raoiella*

Mites (Acari) are members of the sub-phylum Chelicerata (Walter & Proctor, 1999) and exploit many different niches. Phytophagous mites mainly feed on vascular tissues of higher plants and have adapted both physiologically and morphologically to do so (Krantz & Lindquist, 1979). Although acarine taxonomy is a much-debated area, recent papers suggest that the Acari can be split into 2 super-orders; the Parasitiformes and the Acariformes (Dabert et al., 2010). The phylogeny of the latter is also uncertain, but the most recent paper by Dabert et al. (2010) shows that the Acariformes may be split into 2 sub-orders; the Sarcoptiformes and the Trombidiformes. It is the Trombidiforme (=Prostigmata) order that contains all known acarine plant parasites which include the spider mites, false spider mites, bud mites gall mites etc (Walter & Proctor, 1999). To date approximately 50,000 species of Acari have been described (Dabert et al., 2010) and of those living on aerial parts of plants, certain members of the Prostigmata have evolved specialised mouthparts to enable feeding on the vascular tissues (Evans, 1992). The taxonomy of the Prostigmata is not resolved (Proctor, 1998), however it is agreed there are three main groups: the Anystina, Eleutherengona and the Eupodina. Within these groups are the Tetranychoidae which may be broken down into 5 families, all of which are phytophagous; these include the Allochaetophoridae, Linotetrantidae, Tenuipalpidae, Tetranychidae, and Tuckerellidae (Jeppson et al., 1975; Krantz & Lindquist, 1979; Naturae, 2009). *Raoiella indica* (Red Palm Mite- RPM) is a member of the Tenuipalpidae family.

The Tenuipalpidae (known as ‘False Spider Mites’) comprise of around 900 species (Gerson, 2008) including economically important pests such as *Brevipalpus* spp. (Acari:Tenuipalpidae) and RPM. The family was reviewed by Gerson (2008) and remain distinct from other Tetranychoid families by the absence of the thumb-claw complex and the lack of production of silk webbing (Zhang, 2003). Mites of this family are dorso-ventrally flattened, slow moving, between 200-400µm and are often brightly coloured (Sadana, 1997; Zhang, 2003). Tenuipalps may be found on various plant parts such as the leaf, fruit, under the bark, however the majority are found on the lower surface of the leaf where they feed and reproduce (Gerson, 2008). The host range of Tenuipalpid mites ranges from specialised to polyphagy and they are found to infest many different types of crops from trees to ornamentals, vegetables and medicinal crops (Sadana, 1997). The specialised members of the family form galls on branches or leaves (e.g. *Larvacarus transitans* on *Ziziphus* spp.-Ber branches) however the majority of Tenuipalps are polyphagous, for example *Brevipalpus phoenicis* and *Brevipalpus*

obovatus are both found on over 50 genera of ornamental plants (Zhang, 2003). Pritchard & Baker (1958) commented that Tenuipalpid mites have been spread throughout the world through commerce and that 'polyphagous species readily adjust to new hosts'.

To exploit their higher plant hosts, the Tetranychoidae have evolved specialised mouthparts. In some groups, chelicerae have evolved into a pair of long retractable stylets which form a piercing and sucking structure when used in unison. These stylets enable the mites to pierce into the plant cells such as the parenchyma, through epidermal cells and suck out their contents (Evans, 1992; Krantz & Lindquist, 1979) or insert and retract the stylet and feed upon plant juices released due to hydrostatic pressure from within the cell (Jeppson et al., 1975). Depending on the species of mite, different routes to the cells upon which they feed may be used. Inter-epidermal stylet insertion is used by *Panonychus ulmi* (Acari:Tetranychidae) whereas; intra-epidermal insertion is used by *Tetranychus mcdanieli* (Acari:Tetranychidae) (Evans, 1992). Tenuipalpid mites have long recurved stylet like chelicerae (Gerson, 2008) which when protracted act as a hollow tube which can pierce plant tissue. The chelicerae are housed within an eversible stylophore which provides the force required for penetration of plant tissues (Sadana, 1997). Red Palm Mite has been reported to insert its stylets through the stomatal opening of host plant leaves to feed on the tissues deep within the leaf (Ochoa et al., 2011).

2.2 *Raoiella indica*: life cycle

Red Palm Mite is considered the most economically important member of the genus *Raoiella* (Dowling et al., 2012). The adult female is approximately 300µm long (including palpi) (Figure 2.1) and the adult male is approximately 210µm long (including palpi) (Hirst, 1924). Zaher et al. (1969) observed that RPM were commonly found along the midrib of leaflets of *Phoenix dactylifera* (Arecales:Areceaceae) on both the upper and lower surfaces, however the mite is most commonly found on the lower surface of the leaflets. The numbers of eggs per colony can range between 108-330 and one female may produce between 1-6 eggs per day (Moutia, 1958). The sex ratios of colonies have been observed to fluctuate throughout different seasons. Populations studied in Mauritius had a 1:11 male:female sex ratio in April/May whereas in October/November, the sex ratio changed to 1:2 (Moutia, 1958). Colonies of RPM are easily identifiable as there is a mixture of cast skins, eggs, nymphs and adults. The mite does not produce webbing, thus is easily distinguishable from Tetranychidae found on leaves. Few studies have been carried out on the reproduction of RPM, however, RPM have been reported to undergo arrhenotokous parthenogenesis, where unfertilised eggs hatch to become males and fertilised eggs hatch to become female. Red Palm Mite are haplo-diploid whereby males have two chromosomes and females have four (Helle et al., 1980).

The lifecycle is split into five stages: egg, larva, protonymph, deutonymph and adult. Eggs are laid along the midrib or in depressions of the leaflet/leaf and measure around 118µm long (Zaher et al., 1969). The eggs are red/orange in appearance with a slender stipe (Kane et al., 2005) which often has a droplet of an unknown substance on the end (Vacante, 2016). On hatching, the larval mite starts feeding immediately, then goes through three instars where, prior to moulting the mite enters a quiescent stage. During moulting the mite, in common with other members of the genus *Raoiella*, insert stylets into the stoma of host plant material to anchor itself (Vacante, 2016). The length of the development time of the instars and incubation period of the egg are dependent on abiotic conditions such as temperature and humidity. Zaher et al. (1969) reported that between 23.7-27.8°C and 53.7-61.2% RH, the egg-adult time can be 21.3 days, Moutia (1958) reported an average of 22 days at 24.2°C and 33 days at 17.9°C. In the New World studies have shown that the development time of RPM (egg-adult) is 30.9 ± 3.4 days on coconut and 32.4 ± 4.6 on *Musa* spp. at $26.3 \pm 1.3^\circ\text{C}$, $75 \pm 4\%$ RH (Ramos-Lima et al., 2011). An overview of egg-adult development times collated from a review of the literature is shown in Table 2.1. These results show that egg to adult times in Mauritius and Venezuela were comparable in principle but there was a 5°C difference in assay temperatures. Assays conducted in Cuba on *C. nucifera* at 2°C above those in Mauritius showed a slower developmental time in comparison to those in Old World. There was evidence of differences in developmental time on different host plants within the same region (Grimán et al., 2015).

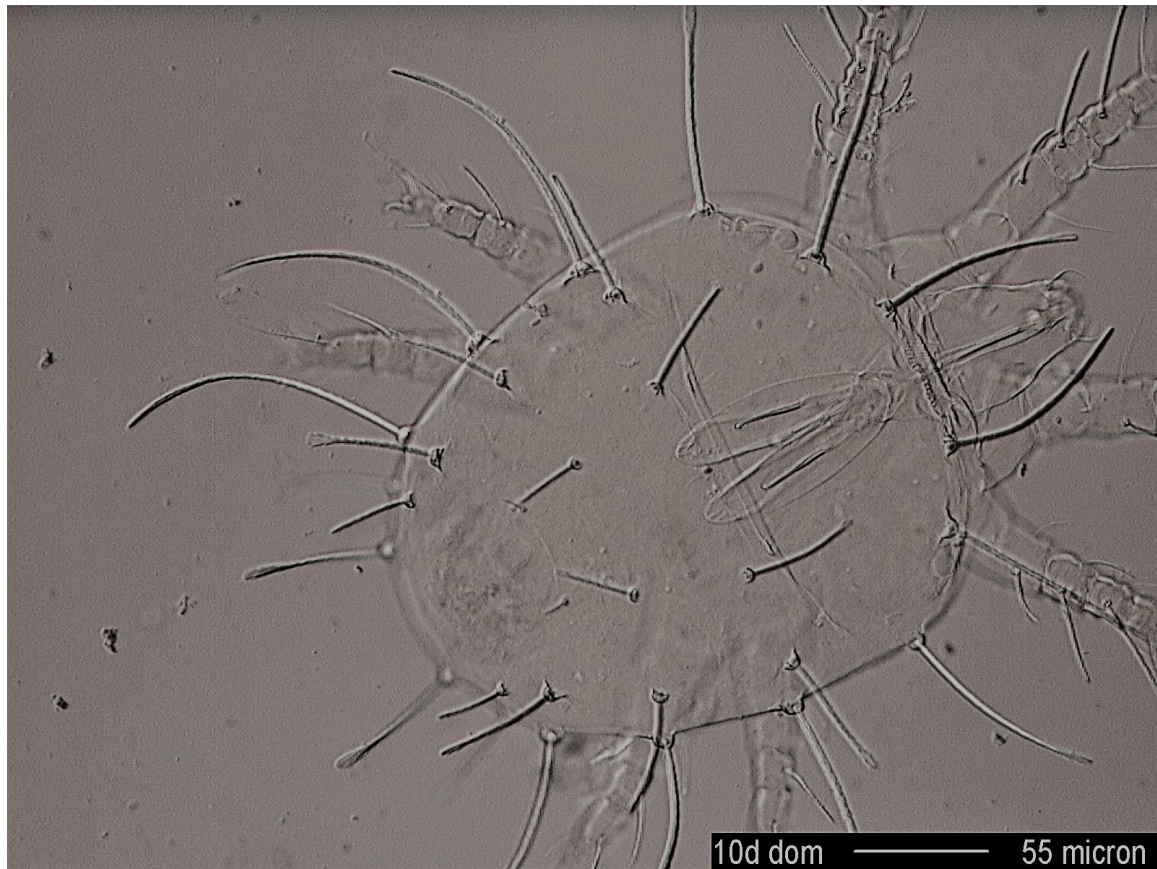


Figure 2.1 *Raoiella indica* adult female (slide mounted in Hoyers media).

The ecology of *Raoiella indica* (Hirst) (Acari:Tenuipalpidae) in India and Trinidad

Table 2.1 Developmental times and oviposition data of *Raoiella indica* on various host plants. Experiments were conducted in several different countries as shown in table.

	Moutia (1958)	Cocco & Hoy (2009)	Ramos- Lima et al. (2011)	Vasquez et al. (2015a)	Zaher et al. (1969)	Ramos- Lima et al. (2011)	Balza et al. (2015)				Grimà et al. (2015)			
Mean Temp (°C)	24.2	27.8-33.1	26.3	29.2	25.7	26.3	27.6	27.6	27.6	27.6	29.2	29.2	29.2	29.2
Host:	<i>Cocos nucifera</i>	<i>Cocos nucifera</i>	<i>Cocos nucifera</i>	<i>Cocos nucifera</i>	<i>Phoenix dactylifera</i>	<i>Musa spp.</i>	<i>Musa</i> spp. cv Manzano (AAB)	<i>Musa</i> spp. cv Topocho (ABB)	<i>Musa</i> spp. cv Plátano (AAB)	<i>Musa</i> spp. cv Guineo (AAA)	<i>Alpinia purpurata</i>	<i>Heliconia psittacorum</i>	<i>Ptychosperma macarthurii</i>	<i>Adonia merrilli</i>
Country:	Mauritius	USA (Florida)	Cuba	Venezuela	Egypt	Cuba	Venezuela	Venezuela	Venezuela	Venezuela	Venezuela	Venezuela	Venezuela	Venezuela
Mean durations (all given in days)														
Egg	5	6.2	8.2	6.1	6.1	8.2	5.9	6.8	6.1	6.2	7.8	7.4	8.0	7.4
Larva	7	12.1	8.1	5.5	5.7	8.5	6.5	5.9	2.3	3.2	9.0	9.2	7.7	5.9
Protonymph	5.5		7.7	4.5	5.4	7.7	4.4	3.5	-	-	7.3	7.6	6.7	4.7
Deutonymph	4.5		6.7	5.5	4.1	8.0	8.1	8.2	-	-	9.8	9.4	7.7	5.3
Total egg-adult	22	18.3	30.9	21.5	21.4	32.4	24.4	24.9	-	-	33.9	33.7	30.0	23.3
Mean adult female longevity	27		24.1	21.5	NA	14.8					9.9	9.7	10.0	13.3
Mean eggs/female/day (*calculated)	1.0	0.93*	1.9	1.3	NA	1.9					1.4	1.2	2.1	1.9
Total oviposition	28.1		12.6	16.6	NA	7.8					7.0	8.0	4.7	7.0

2.3 Host plants of *Raoiella indica*

Host plants of RPM reported prior to the introduction into the New World were *Cocos nucifera* (the coconut palm) (Hirst, 1924), *Areca catechu* (the betelnut or arecanut palm) (Daniel, 1983; Yadavbabu & Manjunatha, 2007) and *Phoenix dactylifera* (the date palm) (Sayed, 1942; Zaher et al., 1969). Other host species reported prior to the introduction of RPM into the Caribbean were *Dictyosperma alba* [sic] (Arecales:Areceae) in Mauritius (Moutia, 1958), *Ocimum basilicum* (Lamiales:Lamiaceae), *Phaseolus* sp. (Fabales:Fabaceae) and *Acer* sp. (Sapindales:Sapindaceae) (Carrillo et al., 2012a). The reports of RPM on *Acer* sp. have since been reported as a mistranslation of *Areca* sp. (Kane et al., 2012) and *Ocimum basilicum*, *Phaseolus* sp. were shown not to be viable hosts for RPM in experiments on potted plants in Florida (Carrillo et al., 2012a). Recent synonymisation of *R. indica* and the species *Raoiella pandanae* have also brought to light that two members of the Pandanaceae family as hosts to RPM in India (Mesa et al., 2009). The New World host range reported, is much wider than that reported in the Old World: in total 91 host plants have been reported with the majority comprising of members of the Areceae family (72), and the remaining comprising of members of the Heliconiaceae (5), Musaceae (6), Strelitziaceae (2), Zingiberaceae (4) and Pandanceae (2) (Carrillo et al., 2012a). Initial observations listed all hosts on which RPM was observed (whether breeding or just present) however more recent studies have confirmed that at least 27 of these are newly reported hosts on which RPM can breed, indicating at least a 30% expansion in reported host range (Carrillo et al., 2012a).

Red Palm Mite infestation may lead to noticeable damage symptoms on the leaflets of the host plants. In the Caribbean, the symptoms were initially thought to be those of lethal yellowing or nutrient deficiency, however, the cause was later found to be due to infestation by RPM (Kane et al., 2005). Red Palm Mite feeds on the lower surface of host plant leaves, causing yellow patches (Seshadri & Rawther, 1968) which has been hypothesised to be due to RPM feeding on sap and chlorophyll (Zaher et al., 1969). Fronds may appear yellow or bronze-orange from a distance. Peña et al. (2009) reported that damage symptoms appear as dark patches on the upper side of the leaflet, which leads to necrotic patches and that heavy infestations lead to death of young plants. The authors reported that in Trinidad 70% yield loss has been observed from coconut palms. Recent research has shown that RPM cause damage through insertions of its stylets through the stomata of the leaflet and feeds on the mesophyll cells (Ochoa et al., 2011). Scanning Electron Micrograph (SEM) photos taken by the authors show the mites stylets penetrating the stomata while other surrounding epidermal cells remain unpunctured. Research by Peña & Rodrigues (2010) has shown that plants heavily infested with RPM have a lower stomatal conductance than those which are RPM free, indicating that the mite is

affecting the transpiration of the leaflets.

It is important to note that prior to the introduction of RPM to the adventive range there were no reports in the literature of RPM on hosts from the Zingiberales (which include plants from Musaceae, Strelitziaceae and Zingiberaceae) and only a few reports have emerged on *Musa* spp. in the Old World since (Hountondji et al., 2010; Shailesh et al., 2014) posing important questions about the relationship RPM has with *Musa* spp. in both regions. Taylor et al. (2011) found only one small colony of RPM on *Musa* spp. in Kerala after extensive surveys indicating its rarity on this host in this area. Since the introduction of RPM to the New World, there have been many reports of plants from the Musaceae in this region which encompass *Musa* spp. harbouring high infestation levels of RPM (Carrillo et al., 2012a; Cocco & Hoy, 2009; González Reus & Ramos, 2010; Kane et al., 2012; Peña et al., 2006; Rodríguez et al., 2007; Vásquez et al., 2008; Étienne & Flechtmann, 2006). Rodrigues & Peña (2012) demonstrated that cultivar and proximity to *C. nucifera* palms played a role in RPM populations found on *Musa* spp.. Balza et al. (2015) also demonstrated cultivar related differences in RPM performance on *Musa* spp. and suggested that anatomical features and chemical composition (mainly phenols) contributed to these differences. Otero-Colina et al. (2016) demonstrated differing infestation levels of RPM on various hosts with infestation levels always higher on *C. nucifera* plants (authors studied *C. nucifera* cv Pacific Tall and Malayan Dwarf, *Elaeis guineensis* (oil palm) cv Deli x Ghana and Deli x Nigeria, *Musa* spp. cv Dwarf Giant and Horn plantain, *Heliconia bihai* (lobster claw), and *Alpinia purpurata* (red ginger)). Performance data shown in Table 2.1 suggests that developmental rates may differ depending on the host plant on which RPM is reared. Recent studies have shown that the thickness of leaf cuticle and abaxial epidermis, stomatal density and the concentration of secondary metabolites may all play a role in resistance against RPM (Balza et al., 2015). In Florida, laboratory studies by Cocco & Hoy (2009) found difficulty in rearing successful colonies of RPM on banana leaf discs, even when colonies of RPM were collected from *Musa* spp. in the field in Florida.

2.4 Biogeography

Red Palm Mite was first recorded in Coimbatore, India in 1924 (Hirst, 1924), then later reported in Egypt on *Phoenix dactylifera* in 1942 (Taher Sayed, 1942). It has since been reported in many countries across Asia, the Middle East, Africa and the Americas (Table 2.2). The area of origin however is unknown. Molecular studies have pointed towards the Middle East as the area of origin of the mite (Dowling et al., 2012) which would link in to the origins of the date palm *Phoenix dactylifera* which is thought to originate from South West Asia (Arabian Peninsula to South Pakistan) (Morton, 1987; WCSP, 2009). The hypothesis stated that the mite originated in the Middle East and spread

eastwards to India, from where it spread to Mauritius and Reunion along trade routes, on infested plant material (Dowling et al., 2012). From an examination of the origins of reported host plants however, it has also been hypothesised that the mite originated in South-East Asia perhaps in the floristic province of Malesia (encompassing the Malay peninsula, Indonesia, New Guinea and The Philippines) (Taylor, 2009).

Figure 2.2 is an updated version of Taylor (2009) which uses the most up to date host plant list, and indicates that regions spreading from India, south-east towards Australia are still a focal zone for the area of origin of *Raoiella indica* based on reported host plants. Beard et al. (2012) reignited the discussion into the origins of the genus through extensive surveys in Australia where several new members of the *Raoiella* genus have been identified and have been recorded feeding on dicotyledon hosts. The centre of diversity for the *Raoiella* genus had been hypothesised to be Australia due to the abundance of members of the genus discovered there supporting the Melanesian origin of RPM, however it is now hypothesised that this was due to rapid evolution on introduction to this region and the area of origin is now hypothesised to be in Africa or the Middle East (Dowling et al., 2012). The most eastern reports of RPM are in the Philippines where the mite has been recently reported as a pest [the pest was reported as *Rarosiella cocosae*; (Rimando,1996), however, was recorded as a new synonymy for RPM by Mesa et al. (2009)]. Molecular work has shown these populations to probably have originated from those which spread to India (Dowling et al., 2012). It is likely that populations of RPM have been established in India for some time given the reports dating back to 1924, thus in this thesis India is considered to be part of the naturalised range of RPM. In 2004, RPM was reported in the Caribbean for the first time on the island of Martinique (Flechtmann & Étienne, 2004) and is thought to have been introduced along trade routes from the island of Reunion (Dowling et al., 2012). Red Palm Mite has since spread rapidly through the islands and has now been reported in much of the Caribbean and South and Central America (Table 2.2).



Figure 2.2 Areas of origin of reported host plants of RPM in the Caribbean in the Old World; green areas and numbers indicate the number of host plants which are native to that country- darker green = more host plant species originate from a country, light green = fewer host plants have originated in that country. Species with an area of origin in the New World were not included. Data obtained from Jones (1995), Uhl & Dransfield (1987), WCSP (2009) and Carrillo et al. (2012a)

Table 2.2 Distribution of RPM

Country	Author
Antigua	Beard et al. (2012)
Aruba	Beard et al. (2012)
Barbados	Beard et al. (2012)
Benin	Zannou et al. (2010)
Brazil	Masaro et al. (2010); Navia et al. (2010b)
Cuba	Rodrigues et al. (2007)
Dominica	Kane et al. (2005)
Dominican Republic	Welbourn (2006)
Egypt	Taher Sayed (1942); Zaher et al. (1969)
Granada	Welbourn (2006)
Guadeloupe	Flechtmann & Étienne (2005)
Haiti	Welbourn (2006)
Kenya	Zannou et al. (2010)
India	Hirst (1924)
Iran	Arbabi et al. (2002)
Iraq	Beard et al. (2012)
Israel	Beard et al. (2012)
Jamaica	Welbourn (2006)
Martinique	Flechtmann & Étienne (2005)
Mauritius	Moutia (1958)
Mexico	Estrada-Venegas et al. (2010)
Namibia	Giliomee & Ueckermann (2016)
Oman	Elwan (2000)
Pakistan	Mansoor ul & Shamshad (2000)
Panama	Beard et al. (2012)

Country	Author
Philippines	Rimando (1996)
Puerto Rico	Rodrigues et al. (2007)
Reunion	Ueckermann (2004)
Saint Lucia	Kane et al. (2005)
Saint Kitts and Nevis	Moore, pers. comms 2009.
Saint Martin	Beard et al. (2012)
Saint Vincent and Saint Thomas	Beard et al. (2012)
Saudi Arabia	Soliman & Al-Yousif (1979)
Sri Lanka	CABI (2007)
Sudan	Taher Sayed (1942)
Thailand	Beard et al. (2012)
Trinidad and Tobago	Kane et al. (2005)
Tunisia	Zouba & Raeesi (2010)
Turks and Caicos Island	Beard et al. (2012)
United Arab Emirates	Gassouma (2004)
USA	Peña et al. (2009)
Venezuela	Vasquez & Moraes (2013)
US Virgin Islands	Welbourn (2006)

2.5 Predator relations

Much work has been carried out on the natural enemy complex of RPM. Tables 2.3 and 2.4 give an overview of the predators reported globally in association with RPM. In India, where the majority of studies have been undertaken, the natural enemy complex is comprised of predatory arthropods including predatory mites from the Phytoseiidae and insects from the orders Coleoptera, Hemiptera, Neuroptera and Diptera. Somchoudhury & Sarkar (1987) reported that the predators most commonly associated with RPM in West Bengal were *Oligota* sp. (Coleoptera:Staphylinidae), *Phytoseius* sp. (Acari:Phytoseiidae) and *Amblyseius* sp. (Acari:Phytoseiidae). Numbers of *Oligota* sp. and *Phytoseius* sp. both showed positive correlations with RPM number, whereas *Amblyseius* sp. had a significant

negative correlation. Other studies in India have shown that *Stethorus keralicus* Kapur (Coleoptera:Coccinellidae) and *Amblyseius channabasavanni* Gupta & Daniel (Acari:Phytoseiidae) were major predators of RPM on *A. catechu* in Kerala, India. Other predators reported included two genera of Cecidomyiidae (Diptera), Chrysopidae (Neuroptera) and Anthocoridae (Hemiptera) (Daniel, 1983). Daniel (1976) showed that *S. keralicus* was a voracious predator of all stages of RPM consuming 2.41-6.46 mites/h over a period of 24 h and as a larva, the predator consumed on average 281.5 mobile RPM and 40.6 eggs during development. *Amblyseius channabasavanni* has also been reported to be strongly associated with RPM populations and was able to feed on up to 11-40 eggs per day or 6-13 adult female RPM per day (Daniel, 1983), however further work to clarify the taxonomy of this species is required. Taylor et al. (2012) collected specimens from *Amblyseius tamatavensis* species group (Acari:Phytoseiidae) (which comprises *A. channabasavanni* and *Amblyseius tamatavensis*) associated with RPM in Kerala, India, and specimens identified as *A. tamatavensis* using a key from Denmark & Muma (1989) but were also consistent with the type description of *A. channabasavanni* (Gupta, 1978). Descriptions of *A. channabasavanni* by Denmark & Muma (1989) and Gupta (1978) were not consistent, so reports of *A. channabasavanni* may indeed be *A. tamatavensis*- a species found associated with coconuts in many different countries. *Amblyseius raoiellus* (Acari:Phytoseiidae) was described by Denmark & Muma (1989) from specimens recorded feeding on RPM in Kannara, India in 1975, however this is the only report of this species. Moutia (1958) reported *Typhlodromus (Amblyseius) caudatus* (Acari:Phytoseiidae) was an important predator of RPM eggs in Mauritius, and observed that it rarely attacked the nymphal and adult stages of RPM. More recently, it has been hypothesised that this species, could be a mis-identification of *Amblyseius largoensis*, a predator which has been reported in association with RPM in several of the Old and New World countries (Carrillo et al., 2012c). *Amblyseius largoensis* has been reported associated with RPM populations by several authors in the New World (Bowman & Hoy, 2012; Carrillo et al., 2010; Domingos et al., 2013; González et al., 2013; Hoy, 2012; Peña et al., 2009; Ramos-Lima et al., 2011; Rodríguez et al., 2010) and the Old World including Mauritius, La Reunion, India and Thailand (Bowman & Hoy, 2012; Domingos et al., 2013; Hoy, 2012; Moraes et al., 2012; Silva et al., 2014; Taylor et al., 2012). *Amblyseius largoensis* has been reported on many different host plants throughout many regions of the world, and there is evidence that some populations differ slightly morphologically in terms of seta length, however specimens from different locations have shown to be the same species when molecular work has been carried out (Navia et al., 2014).

The ecology of *Raoiella indica* (Hirst) (Acari:Tenuipalpidae) in India and Trinidad

Table 2.3 Acarine predators associated with RPM throughout the Old and New World

Family	Species	Location/Country	Host plant (s)
Phytoseiidae	<i>Amblyseius channabasavanni</i>	Karnataka, India (Daniel, 1983; Gupta, 1978)	<i>Areca catechu</i>
	<i>Amblyseius largoensis</i>	Mauritius (Moutia, 1958) , Kerala, India (Taylor et al., 2012), Benin, Tanzania (Zannou et al., 2010), USA (Carrillo & Peña, 2010), Cuba (Ramos-Lima et al., 2011), Brazil (Navia et al., 2010), Trinidad and Tobago (Peña et al., 2009)	<i>Cocos nucifera</i>
	<i>Amblyseius longispinus</i>	India (Gupta, 2003)	Not specified
	<i>Amblyseius raoiellus</i>	Karnataka, India (Denmark & Muma, 1989; Gupta, 2003)	Not specified
	<i>Phytoseius</i> sp.	West Bengal, India (Somchoudhury & Sarkar, 1987)	<i>Cocos nucifera</i>
	<i>Typhlodromus caudatus</i>	Mauritius (Moutia, 1958)	<i>Cocos nucifera</i>
Bdellidae	<i>Bdella</i> sp.	Trinidad and Tobago (Peña et al., 2009)	<i>Cocos nucifera</i>
	<i>Bdella distincta</i>	Florida, USA (Carrillo et al., 2010)	<i>Cocos nucifera</i>
Chyletidae	<i>Cheletomimus</i> sp.	Trinidad and Tobago (Peña et al., 2009)	<i>Cocos nucifera</i>
Stigmaeidae		India (Daniel, 1983)	<i>Areca catechu</i>

The ecology of *Raoiella indica* (Hirst) (Acari:Tenuipalpidae) in India and Trinidad

Table 2.4 Insect predators associated with RPM throughout the New and Old World.

Order	Family	Species	Location/Country	Host plant
Coleoptera	Coccinellidae	<i>Jauravia soror</i>	Karnataka, India (Daniel, 1983)	<i>Areca catechu</i>
		<i>Spilocaria bisettata</i>	Karnataka, India (Daniel, 1983)	<i>Areca catechu</i>
		<i>Stethorus keralicus</i>	Kerala, India (Daniel, 1976; 1983; Kapur, 1961)	<i>Areca catechu</i>
		<i>Stethorus parcampunctatus</i>	Mysore, India (Puttarudriah & Channabasavanna, 1953)	<i>Areca catechu</i>
		<i>Stethorus pauperculus</i>	Shimoga, India (Yadavbabu & Manjunatha, 2007)	<i>Areca catechu</i>
		<i>Stethorus tetranychii</i>	Tharikare; India (Daniel, 1983)	<i>Areca catechu</i>
		<i>Stethorus utilis</i>	Florida, USA (Carrillo et al., 2010)	<i>Cocos nucifera</i>
	Dermestidae	<i>Aspectes indicus</i>	Karnataka, India (Daniel, 1983)	<i>Areca catechu</i>
	Nitulidae	<i>Cybocephalus semiflavus</i>	Karnataka, India (Daniel, 1983)	<i>Areca catechu</i>
Diptera	Staphylinidae	<i>Oilgota</i> sp.	West Bengal, India; Shimoga, India (Somchoudhury & Sarkar, 1987; Yadavbabu & Manjunatha, 2007)	<i>Cocos nucifera</i> ; <i>Areca catechu</i>
	Cecidomyiidae	<i>Arthrocnodax</i> sp.	Karnataka, India (Daniel, 1983)	<i>Areca catechu</i>
		<i>Feltiella</i> sp.	Karnataka, India (Daniel, 1983)	<i>Areca catechu</i>
		unknown sp.	Trinidad and Tobago (Peña et al., 2009)	<i>Cocos nucifera</i>
Neuroptera	Chrysopidae	<i>Chrysopa</i> sp.	Karnataka, India (Daniel, 1983)	<i>Areca catechu</i>
		<i>Chrysoperla</i> sp.	Trinidad and Tobago (Peña et al., 2009)	<i>Cocos nucifera</i>

The ecology of *Raoiella indica* (Hirst) (Acari:Tenuipalpidae) in India and Trinidad

Order	Family	Species	Location/Country	Host plant
		<i>Ceraeochrysa claveri</i>	Florida, USA (Carrillo et al., 2010)	<i>Cocos nucifera</i>
Thysanoptera	Phlaeothripidae	<i>Aleurodothrips fasciapennis</i>	Florida, USA; Trinidad and Tobago (Carrillo & Peña, 2010; Pena et al., 2009)	<i>Cocos nucifera</i>

2.6 *Raoiella indica*- Population dynamics

The population dynamics of RPM in the Old World have been studied by several authors on both coconut and arecanut (Chandra & Channabasavanna, 1984; Daniel, 1983; Moutia, 1958; Prabheena & Ramani, 2014; Sarkar & Somchoudhury, 1989; Taylor et al., 2012; Yadavbabu & Manjunatha, 2007). Populations tend to peak in the hot dry summers of India (Prabheena & Ramani, 2014; Taylor et al., 2012) and crash with the onset of the monsoon or rainy season (Chandra & Channabasavanna, 1984; Moutia, 1958; Prabheena & Ramani, 2014; Taylor et al., 2012). Abiotic factors affecting RPM numbers were studied in West Bengal (Sarkar & Somchoudhury, 1989) and it was established that temperature had the most influence on mite populations on coconut, and rainfall had little effect. Other authors have emphasised the importance of rainfall in the decrease of RPM numbers at the onset of the monsoon season (Moutia, 1958; Prabheena & Ramani, 2014; Taylor et al., 2012). Recent studies published in Brazil have shown that oviposition and development of RPM on *C. nucifera* is higher on well irrigated plants than those under a decreased irrigation regime (Villasmil et al., 2014), thus population decrease in line with rainfall may be due to physical effects i.e. dislodging of RPM or increase in epizootics, rather than associated with plant health. Chandra & Channabasavanna (1984) carried out detailed studies on how climate parameters affected all life-stages of RPM and they found that the build-up of populations was associated with lower humidity, higher temperature and longer sunshine hours. High humidity and rainfall have been associated with low population densities of RPM for egg, larval and nymphal stages of RPM, although not significantly so with the adult stage. In Kerala India, high RPM population densities have been associated with higher temperatures. Taylor et al. (2012) demonstrated that RPM populations were denser in areas which were significantly hotter and drier. Other factors associated with the build-up of populations of RPM are nitrogen and moisture content of the leaves. Trees grown in conditions of poor drainage, irrigation and low mineral and organic matter are particularly affected by infestations of RPM (Devasahayam & Nair, 1982; Sathiamma, 1996) and plants grown in well irrigated shaded nurseries tend to have low mite infestations (Ponnuswami, 1967). More recently, Grimàn et al. (2015) showed that the performance of RPM in terms of development time was not significantly affected by fertilization regime, however there was evidence of reduced longevity and oviposition rates of RPM on fertilised *C. nucifera* plants in Venezuela.

Population dynamics in the New World have been studied to a lesser extent in relation to abiotic factors, but more so in relation to native predators. Peña et al. (2009) studied populations of RPM in relation to the native predator complex on *C. nucifera* in Florida, USA and reported peaks in populations of up to 4,000 mites per leaflet. Their study showed that, between January 2008-June

2008, population densities were lower in January and February (just over 1000 RPM per pinna in West Palm Beach and around 100/pinna in Broward county) and peaked in March in West Palm Beach, Florida (4000/pinna) and April (1600/pinna) and June (1600/pinna) in Broward county, Florida. In analogous studies carried out in Puerto Rico between November 2007-August 2008, population densities were observed to peak at around 2,000 mites per pinna in March 2008, however populations for the other monthly periods observed remained around 500 RPM/ pinna. Duncan et al. (2010) working as part of the same team, reported that infestations were higher in the first four months after initial infestation in Florida, however, population densities have shown a negative trend in density since July 2008. Studies in Cuba have recorded population densities on *Musa* spp. and *C. nucifera*; an exponential increase in populations was observed in the dry season peaking around April, then dropping off in relation to high rainfall in May (Ramos-Lima et al., 2011). Studies in Puerto Rico (Rodrigues et al., 2010) have shown that rainfall negatively impacts on populations of RPM and entomopathogens have been isolated during this season.

2.7 Invasion ecology theories

The introduction and establishment of an organism from a native to non-native range has become increasingly common over the 200 years or so with an increase in global travel (Mack et al., 2000). A small proportion of the introduced species survive, establish and become invasive. An organism is considered invasive if it has an adverse impact in the invasive range (IUCN, 2017) and this could be through impacts to local biodiversity, agriculture, nutrient cycling etc. (Mack et al., 2000). Catford et al. (2012) defined an invasive species as being an introduced species which “*sustains self-replacing populations over several life cycles, spreads considerable distance from its site of introduction and often reaches very large numbers*”. Red Palm Mites are regarded as a highly invasive (Dowling et al., 2012). As mentioned in section 2.2, since its introduction into the New World, high population densities have been reported in its invasive range (Peña et al., 2009) and the mite has spread rapidly through several countries in the Caribbean and South America (Flechtmann & Étienne, 2004; 2005; Gondim Júnior et al., 2012; González et al., 2013; Peña et al., 2009; Rodrigues et al., 2007; Rodríguez et al., 2007; Vasquez & Moraes, 2013; Vásquez et al., 2008; Étienne & Flechtmann, 2006) and has increased its host range by at least 30% (Carrillo et al., 2012a).

The causes and impacts of biological invasions have been widely studied. Lowry et al. (2013) conducted a systematic review of causes and impacts of biological invasions and compiled a list of 17 different broad hypotheses which have been tested by authors relating to causes or factors underpinning biological invasions. Hypotheses which could be relevant to the successful invasion of

RPM included Community species richness hypothesis (Elton, 1958), Evolution in general, Evolution of Increased Competitive Ability EICA (Blossey & Notzold, 1995), Enemy Release Hypothesis (ERH) (Keane & Crawley, 2002), Inherent superiority and Preadaptation to climate. Colautti et al. (2006) also highlighted that in many cases, propagule pressure is an important factor related to the establishment and invasiveness of many invasive species.

The community species richness hypothesis states success of invasion is affected by species richness in the invasive range. Elton (1958) hypothesised that communities would be invasion resistant if they had high species diversity i.e. it would be unlikely there would be a vacant niche for the organism to occupy. Shea & Chesson (2002) presented papers which both support and conflict this and stated that species diversity could be affected by several factors such as latitude, climate, soils and supply rates of resources in the system.

Evolution in general may be related to the success of invasive species. Lee (2002) stated that the genetic structures of invading populations may be altered by genetic drift or natural selection, leading to populations with modified tolerances or behaviours. There may be selection pressures in a new environment which lead to adaptations manifesting in changes to morphology, physiology or phenology of the species. Often successful invaders are derived from a small founder population which may reduce the genetic diversity of an invasive, and such genetic bottlenecks may contribute towards the evolutionary diversification of an invasive from its source populations (Vellend et al., 2007). Work investigating the biogeography of RPM has found that RPM found in UAE and Iran differ slightly from those found in India, and of those found in India, it is those from the areca palm which have identical COI sequences (DNA sequences commonly used for molecular barcoding) as those found in the New World (Dowling et al., 2012). Morphologically, differences in RPM size were reported between specimens from UAE and Oman and those found in Trinidad. The authors hypothesise that there are perhaps two haplotypes of RPM. The Middle Eastern haplotype appeared to be the most primitive which has then spread through the Old World and then to the New World. Whether the populations in the adventive range have been subjected to selection pressures causing diversification is unclear. Molecular evidence suggests that invasive RPM in the adventive range are analogous to those collected from *A. catechu* in India, however given the reported differences in population densities it is important to investigate whether there are some conferred evolutionary advantages in the invasive population compared to the populations in the Old World.

The Evolution of Increased Competitive Ability EICA theory (Blossey & Notzold, 1995) states that a species found in its introduced range will, given identical conditions, produce 'more biomass' than

those which are found within the native range i.e. for RPM one could equate this to a higher fecundity. The driver behind this is hypothesised to be that an individual may invest less in defence against predators and more into reproduction when these pressures are removed. This links with the Enemy Release Hypothesis (Keane & Crawley, 2002), which states that an invasive species may flourish when released from control by natural enemies found in its native range either by absence of adapted predators or lack of efficiency of predators in the invasive range. The Enemy Release Hypothesis assumes that the natural enemies in the native range are important regulators of the organism's population and that natural enemies impact more on populations of native rather exotic organisms. As a result of the lack of regulation of natural enemies, it is hypothesised the organism can afford to invest less in defence and more in traits such as reproduction and growth, thus leading to increase in populations. Bernays & Graham (1988) stated that "*generalist natural enemies, especially predators of herbivores, may be the dominant factor in the evolution of a narrow host range*" which could suggest that RPM are kept within a narrow host range in India through the action of generalist predators (see section 2.5). Many of the underlying assumptions relating to classical biological control programmes centre on ERH. As discussed in section 2.4, the area of origin of RPM is not yet known, therefore the native natural enemy complex is yet to be established.

Preadaptation to the climate is likely to aid the success of an invasion, however does this contribute any further knowledge to the success of an invasion in terms of higher pest densities? Evolution in general is a possible factor to explain invasion success, however, populations from the Caribbean have been shown to be analogous genetically with some populations from India (Dowling et al., 2012) therefore these factors could potentially be ruled out.

Finally, propagule pressure has been highlighted as an important factor behind the invasiveness of species (Colautti et al., 2006), whereby the numbers of individuals or introduction occasions may be related to the success of an invasive species. Repeated introductions can increase the chance of establishment, as the probability of encountering ideal conditions can increase with the number of introduction events (Wittmann et al., 2014).

To explore effectively the factors which underpin the success of invasive species, it is important to consider information relating to native populations and habitats and comparative approaches between the native and invasive ranges may be useful (Guo, 2006). Given that the native range for RPM is unknown, this thesis focuses on RPM in India where RPM was first reported, and can be considered naturalised.

2.8 Host association, host races and biotypes.

Section 2.3 described the increase in host species utilisation of RPM since its introduction into the adventive range. The observations to date report new associations with *Musa* spp. in the adventive range which raises important questions about why these observations have been made. Is this evidence of host range expansion, lack of reporting of associations in the naturalised range or are there host races or biotypes of RPM in existence where one race exploits only hosts from the Arecales and the other race exploits hosts from the Arecales and Zingiberales? Magalhães et al. (2007) suggested that several characteristics may predispose a mite towards host specialisation. Philotrapy, (meaning a tendency to stay at the natal site), low dispersal ability, high intimacy with the host environment, host associated mating and high reproductive rates are among those which encourage this. Red Palm Mite forms distinct colonies where a ring of cast skins can be seen around the egg laying site, indicating a tendency to stay near the natal site (at least during development). In addition, the mite does not form webbing associated with 'ballooning' aerial dispersal associated with some species of Tetranychidae, and RPM has the potential to form dense colonies indicating high reproductive rates. These traits would indicate that RPM has the potential to form host associated races.

Claridge & Den Hollander (1983) outlined the concept of biotypes in relation to insect pests of agriculture. The authors stated that in general there are two approaches to describing biotypes- the first being a general term which aggregates either individuals or populations of the same species according to their biological characteristics such as virulence on particular hosts, and the second being a more specific genetic based aggregation where specific genetic markers are related to virulence on a particular host. They highlighted that the terminology to describe differences between discrete allopatric populations can be confused, with terms such as population, race, subspecies and species applied. Diehl & Bush (1984) classified biotypes into five separate categories- a) non-genetic polyphenisms, b) polymorphic and polygenic variation within populations, c) geographic races, d) host races and e) species. Race when applied to a geographically defined population indicates that individuals from defined populations may be differentiated from other discrete allopatric populations through morphology, virulence, behaviour etc. Morphological variation has been observed between populations of *Aceria anthocoptes* (Acari:Eriophyidae) and was attributed to seasonal variation (Magud et al., 2007) and geographic morphological races have been observed for *Aceria guerreronis* (Acari:Eriophyidae) (Navia et al., 2009).

The term host race may be applied to sympatric populations which may be found on different host plants in the same locality. Host race is a term which has been used to describe discrete populations of

acarine pests which have distinct host use and genetic divergence from sympatric populations of the same species, but still have appreciable gene flow (Drès & Mallet, 2002; Skoracka et al., 2002). Host associated differences which arise due to environmental conditions (and no separation of gene pools) can be regarded as phenotypic plasticity (Skoracka et al., 2002). Host associations of mites may however be dynamic; underlying genetic variation in a population of herbivores may allow species to survive on or accept a new host (Fry, 1989; Gould, 1979); for example, *Tetranychus urticae* Koch (Acari:Tetranychidae) has been observed to adapt to hosts which were initially unfavourable in less than 10 generations (Fry, 1989). Changes in host specificity may occur when the host plants to which an organism has been exposed changes, or if there are changes in competition and predation (Tallamy, 1999). Host races perform better on their own host compared to other hosts present in the same environment and may occur as a result of ‘adaptation combined with restricted gene flow among populations inhabiting different hosts’ (Magalhães et al., 2007). Factors which may encourage host race formation include a difference in the lifespan of the host and parasitic organism; where long-lived hosts favour the development of host races. Host races have been described for several species using different methods; for example, using methods to observe genetic variation between populations from different host plants [*T. urticae* (Gotoh et al., 1993), *Tetranychus kanzawai*; (Nishimura et al., 2005), *Eotetranychus carpini* (Malagnini et al., 2012)], host plant preference (Gotoh et al., 1993), host switch experiments (Malagnini et al., 2012) and mate choice (Gotoh et al., 1993). Morphological analyses have also been used to assess host associated variation, for example morphological variation between populations of *Abacarus hystrix* (Acari:Eriophyoideae) collected from different hosts was attributed to phenotypic plasticity caused by different environments on different host plants (Skoracka et al., 2002).

With regards to the current observations relating to RPM, the broadened host range highlights the lack of knowledge existing about RPM host plant relations. The new associations reported on Zingiberales were unreported prior to the introduction in the adventive range, thus the question of whether two host races exist is raised: Are populations in India a race that is specific to the host plant order Arecales and populations elsewhere may be specific to the order Zingiberales? Were the multiple introductions into the adventive range if this is the case? Additionally, given the molecular differences observed between RPM collected from *C. nucifera* and *A. catechu* in India (Dowling et al. 2012), are there two distinct races which are either host associated or geographic within India?

2.9 Questions relating to observations of invasive populations of RPM

The literature review has shown that although there has been much work studying RPM in the adventive range and its naturalised range, there are gaps in knowledge relating to host plant associations and the reason as to why RPM is found in such high densities in the adventive range compared to its naturalised range. The following section asks two main question, and explains how this thesis aims to contribute towards the body of knowledge relating to them.

2.9.1 Has RPM expanded its host range in the New World and if so, what are the factors behind this expansion?

The expansion in host range is one of the indicators of RPM success reported in the literature to date with at least a 30% increase in new breeding host associations reported in the New World and an increase in reports of RPM from only seven hosts prior to its invasion in the New World to 91 hosts post invasion (Carrillo et al., 2012a). This host range expansion appears to be very significant, however some hosts recorded to date represent records of plants on which RPM has been recorded but not found to be breeding on, and some records are on hosts which are not typical RPM hosts i.e. they are not members of the Arecales or Zingiberales. Thus, the actual number of breeding hosts may be smaller than this and work needs to be undertaken to clarify these records. Studies have been carried out in the adventive range exploring the host associations of RPM (Balza et al., 2015; Carrillo et al., 2012a; Cocco & Hoy, 2009), however little work has explored the extent of host associations of RPM in the naturalised range in India. Such studies would clarify whether the increase in numbers of host reported is a true host range expansion or whether records are unreported in the naturalised range. The reports of RPM on *Musa* spp. in the adventive range have been of particular interest as historically *Musa* spp. have not been recorded as hosts in the Old World, and breeding colonies have been widely reported on *Musa* spp. in the Caribbean (Cocco & Hoy, 2009; Peña et al., 2009; Ramos-Lima et al., 2011; Rodrigues & Irish, 2012). This suggests that in India, there could be an element of local varietal resistance, difference in agronomic practices, or that the mite is under strong natural control in its naturalised range. Whereas more recently much work has centred on the relationship of RPM with *Musa* spp. in the New World (Balza et al., 2015; Cocco & Hoy, 2009; González Reyes & Ramos, 2010; Otero-Colina et al., 2016; Rodrigues & Irish, 2012; Rodrigues & Peña, 2012) more work to clarify the relation of RPM with *Musa* spp. and other host plants is required in the Old World where so few host records have been reported in comparison to the invasive range.

Overall, it is important to verify the host range observations using a scientific approach so the

underlying factors which may be related to these differences can be explored. Underlying drivers for host range expansion could be related to increased exposure to novel hosts/cultivars, a lack of reporting of actual host range in the native range or the presence of a more aggressive/different RPM race in the New World. Additionally, it may be that natural enemies which are found on bananas in India that are not found in the New World.

To provide more information about these observed differences, in the first experimental chapter (chapter 3), focus was on any morphological or molecular differences which may be present between RPM populations collected from different host plants in India and Trinidad. Differences were explored at both a host level and a regional level to investigate whether there was evidence of host or geographical races of RPM. In the second experimental chapter (chapter 4), RPM host plant relations were explored in Kerala, India, paying particular attention to the relationship RPM has with *Musa* spp. This study aimed to complement work that had been carried out in the adventive range and to provide insight into the relationship RPM has with both hosts from Arecales and Zingiberales in Kerala, India, filling important gaps in current knowledge about Old World host plant relations.

2.9.2 How accurate are the reports relating to the increase in RPM population density between Old and New World regions? If accurate, what factors may be driving the differences?

Observations reported in the literature are that RPM could reach population densities of up to 4,000 RPM per leaflet on *C. nucifera* in the New World, however such population densities have not been recorded in the Old World on *C. nucifera*. Invasion ecology literature suggests that reasons for increased population densities in the invasive compared to native ranges may be explained by one or more theories (see section 2.7), however important comparative studies of RPM density are lacking to confirm these observations, as a variety of population estimation techniques have been applied in each region (see chapter 5). Once confirmed, factors influencing these density differences should be investigated i.e. relations with natural enemies, performance between regions i.e. survival, development, fecundity etc, and morphology between regions etc. Additional data may help to explore factors which may be associated with the population density increase. Efforts to bring populations of RPM under control in the invasive range have investigated the possibility of classical biological control through exploring the possibility of the introduction of co-evolved natural enemies (Bowman & Hoy, 2012; Hoy, 2012; Navia et al., 2014).

To explore the reported differences in densities, the third experimental chapter (chapter 5), used analogous survey techniques in each region to assess the population densities of RPM. During the

same surveys, densities of related predators were observed, along with an assessment of predator diversity. To provide more information relating to the similarities and differences between both RPM and predator populations in each region, the morphologies of individuals of both RPM (chapter 3) and analogous predators (chapter 5) from populations on both *C. nucifera* and *Musa* spp. in India were compared with those in Trinidad. In the final experimental chapter (chapter 6), the relationship between RPM and its most commonly collected associated predator *A. largoensis* was examined. This was to explore whether differences in RPM morphology between regions could potentially influence the efficacy of the predator. In chapter 7 the implications of findings from these studies were discussed in relation to the current situation with RPM and latest research.

3.0 The morphological and molecular characterisation and comparison of RPM from India and the Caribbean

3.1 Introduction

Molecular and morphological studies are often undertaken to understand the nature of different populations of mites of the same species. For example, comparisons between populations of a species can contribute to the understanding of the biogeography of an invasive pest (Navia et al., 2005; Navia et al., 2009; Zhang & Jacobson, 2000), help to differentiate between host races of the same species (Gotoh et al., 1993) or contribute to the understanding of populations for the purposes of classical biological control (Bowman & Hoy, 2012; Navia et al., 2014). For RPM, there are questions relating to the host plant relations of the mite due to its apparent host range expansion in the adventive range (Carrillo et al., 2012a). The current molecular data (Dowling et al., 2012) and recent synonymisations of different *Raoiella* species to RPM (Mesa et al., 2009) raise questions about the status of the taxonomy of separate populations of the mite.

Since its discovery in the Caribbean, the taxonomy of *Raoiella indica* and related species have been studied (Beard et al., 2012; Mesa et al., 2009) and results have shown that *R. indica* is a suspected senior synonym for *R. camur* Chaudhuri and Akbar, *R. empedos* Chaudhuri and Akbar, *R. neotricus* Chaudhuri and Akbar, *R. obelias* Hasan and Akbar, *R. pandanae* Mohanasundaram, *R. phoenica* Meyer and *R. rahii* Akbar and Chaudhuri and a synonym of *Rarosiella cocosae* (Rimando), *Raoiella phoenica* (Meyer) and *Raoiella pandanae* (Mohanasundaram). The synonymisation/suspected synonymisation of nine separate species of *Raoiella* to *R. indica* indicates that there may be polymorphic characteristics in populations at the intra-species level, which are not different enough to warrant differentiation at the species level. Of the nine synonymisations, the type specimens were collected from plants of the Arecaceae, including *Phoenix dactylifera* (*R. camur*, *R. empedos*, *R. neotricus*, *R. obelias*, *R. phoenica*, *R. rahii*) and *Cocos nucifera* (*Rarosiella cocosae*) and one from the Pandanaceae- *Pandanus* sp. (*R. pandanae*). The intraspecific variation and different host associations of the synonymies raise the question about whether there are host associated morphological differences.

Phylo-geographic work published by Dowling et al. (2012) suggested that in the Old World there was evidence of intraspecific genetic variation within *R. indica* between specimens collected from the same area within a country from different host plants, (*Areca catechu* Bangalore, India and *Cocos*

nucifera, Bangalore India); between different host plants in different locations (*Areca catechu* Bangalore, India, *Cocos nucifera*, Bangalore India, *Phoenix dactylifera* UAE/Iran) and molecular similarities between RPM collected from different host plants from different countries (*A. catechu* from India and *C. nucifera* The Philippines). They stated there may be two haplotypes of *R. indica* – one originating in the Middle East and all other *R. indica* specimens in their study (India and Philippines). The differences between the two haplotypes may be sufficient to represent a new species of *Raoiella* with 2.7-3.6% divergence between specimens from the Middle East and all others in the study, compared to between 0-1.2% between other *R. indica* specimens. They hypothesised that the origins of RPM may be in Africa as the molecular results indicated that specimens from Middle East and South Africa were the two most basal elements of the clades of *R. indica* examined. The specimens from *A. catechu* in India were hypothesised to have been the point of origin for the spread of specimens onto *C. nucifera* in the Philippines and also onto *C. nucifera* in La Reunion; and populations from La Reunion were hypothesised to be the source population for RPM reported in the adventive range (Dowling et al., 2008).

Although the phylogenetic studies by Dowling et al. (2008) and Dowling et al. (2012) have indicated there is molecular variation between different specimens of RPM, there are no morphological or molecular studies published to date to further examine this-specifically those investigating the intra-specific variation at a host, local (i.e. different sites within the same region) and regional level (i.e. India and Caribbean). With the level of intraspecific variation of specimens within the genus *R. indica*, it is important to further examine the morphological and molecular characteristics of RPM populations from both the Old World and the adventive range to understand the nature of the populations in both regions. In addition, for the purposes of biological control programmes it is important to survey the area of origin of the mite for natural enemies and given the current molecular evidence, there could be two molecular biotypes of RPM in existence in India- one on coconut and one on areca.

The approach of this chapter is to examine in more detail the morphology and molecular attributes of RPM collected from different host plants and sites within India to see if there are firstly any observable differences and secondly if these differences are related to host or location. Secondly, this study will be replicated in the adventive range to observe the level of variation within the adventive range and thirdly, results from both regions will be compared to investigate whether there are any inter-regional differences.

Based on the concepts discussed above, three hypotheses are suggested:

1. There will be molecular and/or morphological differences observed between RPM collected from different hosts in India- particularly *C. nucifera* and *A. catechu*. This hypothesis is based on molecular findings by Dowling et al. (2012) where specimens from *A. catechu* and *C. nucifera* in India were shown to have genetic differences.
2. Based on the current molecular knowledge of RPM in the adventive range (Dowling et al. 2008), there will be no significant host associated molecular or morphological differences found between populations of RPM in the Caribbean between *Musa* spp. and *C. nucifera*.
3. There will be no molecular/morphological differences between populations in the adventive range (Caribbean) and India. This is based on findings by Dowling et al. (2012) which showed populations of RPM ex *A. catechu* were akin to those in the adventive range.

3.2 General approaches

3.2.1 Morphological comparison

The Tenuipalpidae are part of the super family Tetranychoidae, which includes the families Tetranychidae (Spider mites), Tukanellidae, Linotetrantidae and Allochaetophoridae. Zhang & Jacobson (2000) looked at variation among two species of *Tetranychus* using characters such as number of setae on tibia and distance between setae and length of setae. The number of setae on the tibia was used as a diagnostic aid to separate the two species. Pappas et al. (2001) reviewed the literature and showed that herbivores may differ morphologically and also in life history traits from host to host. Differences could manifest as difference in body size of larvae or adult, in mandible size or shape (their study organism was *Gallerucella nymphaeae*, Coleoptera:Chrysomelidae). Skoracka et al. (2002) and Magud et al. (2007) both studied morphological differences between populations of Eryiophyid mites, Delfinado-Baker & Houck (1989) measured morphological variation of *Varroa jacobsoni* (Acari:Varroidae), an ectoparasite of honey bees (*Apis* sp.) from two different hosts. Body length, the length of and distance between scapular setae and the width of the genital plate were studied by all three authors, however, the morphology of each family of mites is different and certain traits are more diagnostic in certain families. Traits which were successful in separating different populations included body elongation (Magud et al., 2007; Skoracka et al., 2002), length of setae (Magud et al., 2007; Navia et al., 2009; Skoracka et al., 2002), body size (Magud et al., 2007; Skoracka et al., 2002); segment length (Navia et al., 2009) and position of setae (Navia et al., 2009).

For Tenuipalpidae, dorsal setae are an important diagnostic trait and ventral setae are not often used

for classification. All species of Tenuipalps have the dorsal setae v2, sc1 and sc2 setae and these are known as ‘stable setae’ on the propodosomal section of the mite (Figure 3.1). From the review by Mesa et al. (2009) which revised the descriptions of the Tenuipalpidae, setae c3, d3, e3, h2, and h1 are also considered stable on the opisthosomal region of the mite. The majority of Tenuipalps have three pairs of dorso-central setae also known as c1, d1, and e1 and RPM has all three, although e1 is often snapped (pers. obs.). Recently a lucid key has been compiled to differentiate the *Raoiella* spp. of the world (Beard et al., 2012). The key focusses on key traits to differentiate species of *Raoiella*. The principle features to identify RPM from other members of the genus are:

1. RPM is in the only member of the *Raoiella* genus to have an elongate, filiform, finely tapered h2 seta (non-spatulate).
2. Seta f2 is always shorter than f3 (although this is true for 15 other *Raoiella* species)
3. Seta h1 is always longer than h2 (although this is true for 15 other *Raoiella* species)
4. Seta e1 and d1 have a weakly spatulate tip
5. RPM do not have setae on coxae 3 and 4
6. RPM has 4 setae on femur II
7. RPM has 3 setae on genua I-II
8. RPM has a companion seta longer than a solenidion on tarsus I and II
9. Tibiae I and II dorsal setae are setiform with tapered tip
10. The palp tibiotarsus has one solenidion, and a tapered setiform eupathidium and one dorsal seta.



Figure 3.1 Picture of *Raoiella indica* with dorsal setae labelled.

For the current study of morphological differences between populations of RPM collected from different host plants and India and the Caribbean, it was proposed that a selection of characters were to be measured. Figure 3.1 shows the chaetotaxy of RPM. In the *Raoiella* genus key (Mesa et al., 2009), an important diagnostic to arrive at identification is the length of the h2 seta, along with d2, e2 and e3. Through preliminary studies however, the h2 seta was difficult to measure as it was so fine (it is not possible to see in Figure 3.1), and the terminal point could not be determined. In accordance with measurements taken in previous studies of this kind with other mite species, total body length and width were also considered. Distance between setae was not measured as the bodies of mites were soft and this measurement was not a reliable measure.

3.2.2 Molecular techniques

For the study of RPM populations to date, Dowling et al. (2012) used cytochrome oxidase I primers and domains 3-5 of 28S rDNA. Their investigation found differences between populations of RPM on *A. catechu* and *C. nucifera* from plants in the same area in India, and differences between *R. indica*

populations between India, Philippines and UAE/Oman. Internal Transcribed Spacer 2 (ITS2) and COI markers are commonly used to look at divergence in the mite genome. Navajas et al. (1998) showed a homogeneity between the ITS2 region of 30 *Tetranychus urticae* individuals, whereas the COI marker showed much more divergence. They postulated the rate of substitution for these areas of the genome were different. This conservation is not the same for all species of Tetranychidae however, and higher levels of variability were detected in *Mononychellus progresivus* (Acari:Tetranychidae) (cited in Navajas et al., 1998) a monophagous mite. Navia et al. (2005) carried out a phylo-geographic analysis of *Aceria guerreronis* (Acari:Eriophyidae) using mt DNA 16S ribosomal sequences, ITS sequences and COI sequences. Data from the three sequences showed that populations could be divided into distinct clusters geographically. Both the ITS and mitochondrial sequences showed that there was diversity in populations between different geographical locations. Navia et al. (2014) used ITS region and 12S mitochondrial rRNA to compare populations of *Amblyseius largoensis* (a predator of RPM).

For the purpose of looking for the development of host plant races, much work has been carried out. Magalhães et al. (2007) stated that there have been 12 studies on host race formation in Acari and these are heavily biased towards *Tetranychus* genus. They mention that F_{st} (fixation index) which measures variance in allele frequencies in populations is commonly used to measure genetic differentiation between populations. The methodologies employed to carry out analysis for host race formation were allozyme, AFLP (Amplified Fragment Length Polymorphisms), mtDNA and microsatellites. Of these techniques, microsatellites were most commonly used. Allozyme analysis is a form of protein electrophoresis, however its drawback can be that live or frozen material must be used and that only a small amount of genetic variation is revealed (Navajas & Fenton, 2000). Studies using allozyme techniques were more commonplace in the late 1990's and the more recent studies have looked at AFLP and microsatellites. Amplified fragment length polymorphisms were studied by Weeks et al. (2000), who highlighted that they can be applied to any organism without any prior knowledge of the sequence information. The major disadvantage of the AFLP technique is that for population studies, one cannot detect the presence of heterozygote from homozygotes, however, Weeks et al. (2000) were able to demonstrate genetic differences between populations of *T. urticae* on different host plants. They also investigated different populations of *Brevipalpus phoenicis* and found many different genotypes at different locations. Their results indicated that the AFLP technique allowed them to differentiate between clonal genotypes on a finer scale- which is not possible with RFLP and allozymes. The advantage of working with a system such as RPM is that they exhibit haplo-diploid reproduction, which would allow allele frequency to be determined from the haploid males (Weeks et al., 2000).

From the review of literature, the best technique to study the intra-species variation of RPM will be to sequence the COI, ITS and 12S regions initially and if no differences are found to use AFLP analysis.

3.3 Materials and Methods

3.3.1 Selection of populations

The populations in the Caribbean were expected to be fairly homogeneous at each site and from different host plants, as previous studies by Dowling et al. (2008) have shown little genetic variation between RPM from different locations in the adventive range and from those collected from *Musa* spp. and *C. nucifera*. It was therefore preferable to sample from a number of geographically distinct sites. Populations from host plants on four different islands (Trinidad, Dominica, Antigua and St. Kitts) were collected and several locations were sampled on each island. The majority of samples were collected from *C. nucifera*, however, where possible *Musa* spp. were sampled also. Collections were made in late May 2009 across all islands (by Dr. Dave Moore), and 6th-15th April 2012 and 28th October 2014 in Trinidad only. Locations were recorded using a handheld GPS unit and mapped (Figure 3.2).

In India, mites were collected from *A. catechu*, *C. nucifera*, *Phoenix roebelenii* (Arecaceae:Arecales) and *Musa* spp. from the Vadakkencherry and Kannara districts in Kerala (districts are roughly 14 miles apart). Sites 1-11 were geographically distinct sites within a 3-mile radius of Vadakkencherry. Collections were made during March in two different field seasons (6th-19th March 2010, 6th-15th March 2012). Colonies of mites on *Musa* spp. were not found, however individuals were collected when found on *Musa* spp. and stored in 80% alcohol for morphological comparisons. The list of samples used for analysis is given in Appendix 2 and a map of sample sites in Figure 3.3.

3.3.2 Collection of mites

Mites were stored in different strengths of alcohol for molecular [95% ethanol as per Dowling et al. (2012); Jeyaprakash & Hoy (2002)] and morphological work [80% ethanol to preserve morphological characters (Rowley et al., 2007)]. Red Palm Mite specimens, were collected into sterile bijoux vials (Caribbean) or Eppendorf tubes (India) for molecular assessments (autoclaved at 121psi for 15mins) and non-sterile tubes for morphological assessments. Mite populations were collected from the underside of host plant leaves on lower fronds/leaves of host plants. As many mites as possible were collected for both molecular and morphological work from each survey site. For molecular work,

gloves were worn for collection. For later collections, some mites were collected for DNA analysis using Whatman FTA paper. Collections were made as per manufacturer instructions in both Trinidad and Kerala, India. Samples from India were transported under a Material Transfer Agreement (MTA) agreed with the Indian government.

3.3.3 Morphological measurements

Approximately 2ml of alcohol containing mites were removed from the bijoux vial/Eppendorf tube and transferred into a watch glass with a plastic Pasteur pipette. Adult female specimens were removed and mounted using either Hoyers media (for initial slides prepared) [recipe from Helle & Sabelis, (1985)] or Heinze media (pers. comms Dr. Donald Griffiths). Life stages were confirmed using guidelines provided by Beard et al. (2012). A multivariate ANOVA showed there to be no significant difference between samples mounted in Heinze and Hoyers media ($F=2.04$, $p>0.05$). Slides were heated on a slide heater and left to clear. As specimens were stored in alcohol, measurements were not taken immediately as samples were likely to imbibe water from the mounting media. Initial photographs of the cleared specimens were taken using a Nikon Eclipse E600 Microscope camera at x20. A scale bar was added to the photograph automatically when the photograph was taken. The photograph was then loaded into Digimizer Image Analysis software V 3.7.1.0 (MedCalc Software), where the scale bar was measured to convert measurements into micrometres. Later specimens were measured using Pro Image Insight software. Measurements taken were body length (including rostrum), width at Sc2, length of v2, c1, d1, c2, d2, e2, f2, c3, d3, e3, f3. A list of all samples measured is given in Appendix 2.

As there were differences observed between adult female RPM between India and the Caribbean, slides were prepared of male specimens from both regions and a simplified list of measurements were made (Length, width, length c3, d3, e3 and h1) (Appendix 3).

To ensure that the density of mite colonies did not affect setae length, densities of colonies were noted during collections made in 2012 in Trinidad. A linear regression was made against colony densities and both raw and averaged setae length measurements to check for any significant relationships. Body length and width were also regressed against colony density.

3.3.4 Statistical analysis of morphological measurements

In total 12 traits were measured on 197 RPM adult female specimens. All measurements were combined into one vector and a Multivariate Analysis Of Variance (MANOVA) was carried out.

Separate analyses were made for Indian and Caribbean specimens to determine if there were within country differences between hosts/collection sites, then the whole dataset was analysed to determine inter-region differences. Samples were considered significantly different where $p < 0.05$. Where no significant interactions found, these terms were removed from the analysis. Linear discriminant analysis (LDA) and principle components analysis (PCA) was carried out on these data to determine which character combinations were able to classify individuals as per host, site and country. The prcomp programme in R (R, 2016) was used for the PCA and graphics were displayed using the ggplot2 package. PrComp was used as this is the preferred method for numerical accuracy (Crawley, 2007). Plots were drawn using 68% data ellipses as described by Friendly (2006). The ellipses visualise variation against a multivariate null hypothesis. The projections are proportional to the standard deviation. Hierarchical Cluster Analysis was performed using Euclidian distances. Data for all 12 measurements were turned into a dissimilarity matrix, then, Euclidian distances were calculated for each RPM specimen. These distances were used construct a dendogram [method described by Crawley (2007)]. A Tree model was then fitted to these data to classify mites into separate groups. All statistics were carried out using R 3.2.4 (R, 2016).

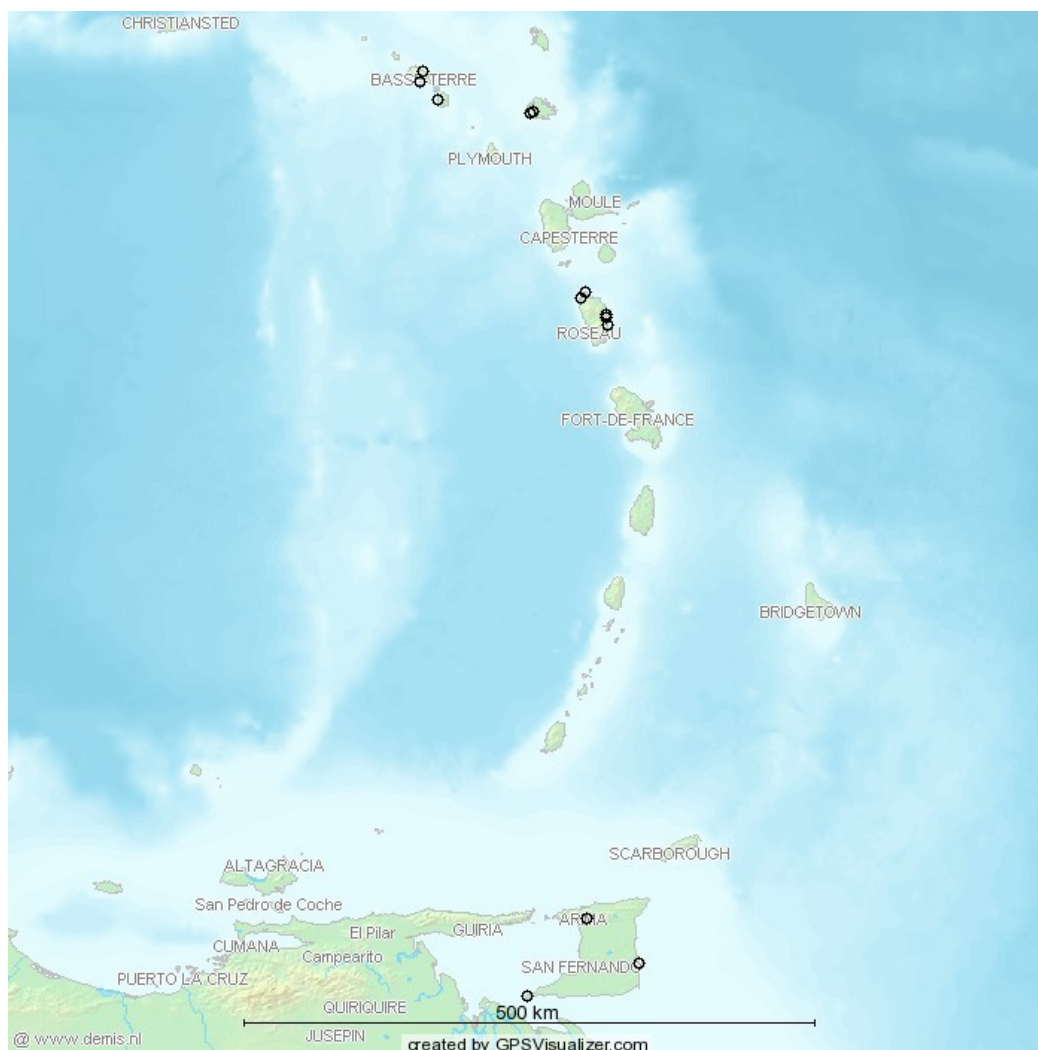


Figure 3.2 Overview of collection sites in the Caribbean.

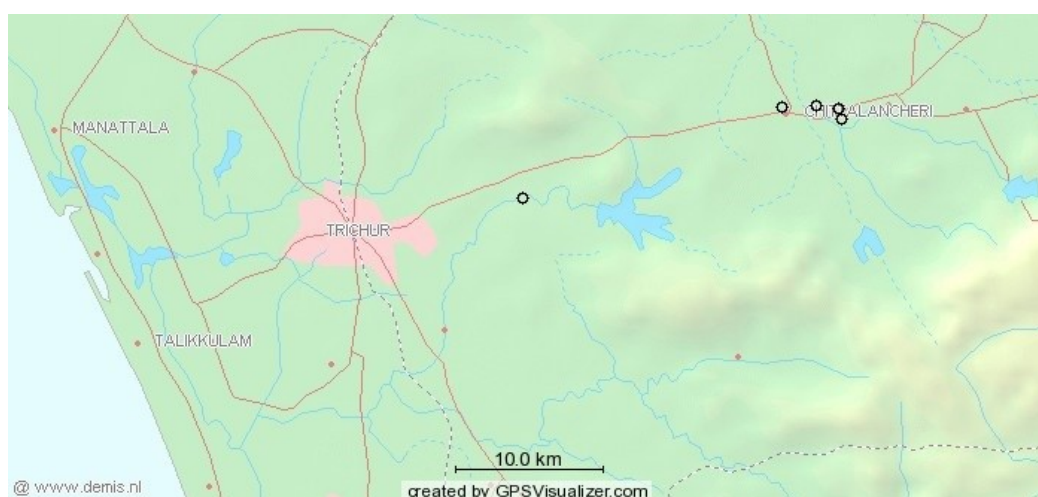


Figure 3.3 Collection sites within Kerala, excluding those from Kunnamkulam. Not all sites had GPS locations recorded, therefore number of sampling locations are higher than those shown.

3.3.5 Molecular analysis

Molecular work was repeated on several occasions as DNA extraction from samples proved difficult. Appendix 4 shows samples selected for molecular analysis. Samples were chosen as they represented RPM collected from a diverse geographic location and also a number of different host plants. It was noted in India, two cultivars of coconut were grown within the region surveyed, thus both were tested. It is not known which cultivar the coconut palms were from the Caribbean.

3.3.6 DNA extraction

Samples were removed from alcohol using a sterile Pasteur pipette and placed on a watch glass wiped with industrial methylated spirit (IMS). A needle was flamed and an RPM individual was removed by stabbing it. Dowling et al. (2012) stated that by rupturing the cuticle this facilitated DNA extraction. The adult female was washed in sterile water on a sterile cavity slide then the mite was left to dry. The mite was removed using the needle, then a single mite was placed in 10µl of Microlysis plus, in a sterile 0.2ml PCR tube (DNA/RNA free) (samples 1-18). For later assays, multiple mites were transferred into the Microlysis Plus (samples 23-43). A micropestle was used to push the mite into the liquid and the mite was then squashed to allow the Microlysis Plus to penetrate the exoskeleton. Samples were placed at 65°C for between 19-24h and then transferred to a Mastercycler PCR machine (Eppendorf) and was run on the cycle: 65°C for 30min, 96°C for 2 min, 65°C for 4min, 96°C for 1 min, 65°C for 1min, 96°C for 30sec and then held at 20°C.

As the first set of samples (1-18) yielded no results when amplified using PCR (COI primers), further samples were collected on Whatman FTA paper (samples 19-22;44-49). Samples collected on Whatman FTA paper were cut from the FTA papers using a Harris uni-core 200 cutter. A single disc was placed inside a PCR clean PCR tube (samples 19-22) or in later assays, multiple discs (samples 44-49). Into each tube, 200µl of FTA purification reagent was added for five min, then the liquid was discarded (using a new tip for each tube). Following this, to each tube, a further 200µl of purification reagent was added and left for five min. This again was discarded, then 200µl of TE1 was added to each tube (10mM Tris-HCl, 0.1mM EDTA, pH 8.0) for five min. This was done twice. Discs were then dried in open tubes prior to analysis. Following drying, 20µl of PCR mastermix was added to tubes, and tubes were then spun at 2500rpm to ensure all liquid was at the bottom of tubes. PCR was conducted as per PCR general methods.

3.3.7 PCR methodology

Each reaction comprised of 1µl of template DNA/one disc/multiple discs of FTA paper (see Appendix 4), 10µl Megamix Gold, 0.5µl of 6pmol primers (HCOI and LCOI) and 9µl of sterile water. A validation PCR run was set up using samples 1 and 13 at different concentrations of template DNA (1µl, 2µl and 3µl; 1/10, 1/100), however this yielded no DNA. PCR cycle was 95°C for 5min, [95°C for 30 sec, 52°C for 30 sec, 72°C for 1 min] repeated 35 times, 72°C for 10min, and then held at 10°C. Where there were no bands, a nested PCR was run by using 1ul of reaction mix in the same reaction mix recipe as above. Amplified samples were cleaned up using 15µl of amplified DNA and 15µl of MicroClean-Microzone.

3.3.8 Gel electrophoresis

All PCR and nested PCR products were visualised using gel electrophoresis (apart from those measured using the Qubit-see below). Agarose gel was prepared using 1.5g BioGene.com HiPure Low FFO agarose powder and was dissolved into 100ml 0.5X TBE plus 5µl of SafeView nucleic acid stain (NBS Biologicals, Cambridgeshire, UK). Gel was poured into a Flow Gen midi tank with two rows of 20 x spacing combs and allowed to set. To the tank, 475ml of 0.5TBE and 19µl of SafeView nucleic acid stain were added and combs were removed. To the wells, 4µl of PCR product were added along with 4 x 7µl of 100Bp ladder. Tanks were activated using voltage -12S, mA500 for a maximum of 3h. Once completed gels were visualised under UV light and photographs taken.

3.3.9 Sequencing

Sequencing was undertaken by the molecular biology department at CABI. Purified PCR products sequenced using a Primus 96 plus thermal cycler (MWG-BIOTECH AG, Germany) using BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems). Sequencing conditions were: 96°C for 1 min followed by 25 cycles of 20 s at 96°C, 10 s at 50°C, 4 min at 60°C (ramp rate: 1°C s⁻¹). Excess unincorporated BigDye was removed with DyeEx 2.0 affinity columns (Qiagen Ltd., UK) according to the manufacturer's instructions and the sequencing reaction products were suspended in HiDi Formamide (Applied Biosystems, UK). These products were separated on a capillary array 3130 Genetic Analyser (Applied Biosystems, UK). Sequence trace files were first assessed for quality using Sequencing Analysis Software v5.2 Patch 2 (Applied Biosystems, UK) and exported as text files (Appendix 5). The text file was entered into Mega5 sequencing software to compare to existing *R. indica* sequences.

3.3.10 Improved PCR methodology

From samples 1 – 49 only one band of DNA was returned using the standard COI PCR method using nested PCR (Tr1A Tree1 Trinidad). Modifications were therefore made to the methodology. For samples 23-43 (where multiple mite samples were used), DNA extracted from the Microlysis Plus method was quantified using a Qubit 3.0 Fluorometer to check that DNA extraction was successful. To prepare samples, 1µl of microlysis product was added to 9µl of sterile water and samples were vortexed, then spun using a micro-centrifuge. The Qubit working solution was prepared by mixing 199µl of Qubit buffer with 1µl for each sample (in bulk), then this was aliquoted in 190µl into Qubit reaction tubes. To this, the 10µl of template DNA and sterile water were added and left to stand for 2 min. DNA was then quantified using standard amounts. Results showed that DNA extraction was successful, however the extraction only yielded low amount of DNA (between 0.0216-0.216ng) which is much lower than the level required for standard PCR.

PCR methods were then adjusted in line with recommendations for low levels of DNA. Gavazaj et al. (2012) worked with low concentrations of human genomic DNA and found that a minimum concentration of 0.4 ng/µl of template DNA in a 25µl reaction mix was required to yield a DNA profile. Increasing the amount of template DNA in the reaction mix was hypothesised to yield a successful PCR amplification. Dowling et al. (2012) used 4µl of template DNA in their PCR reactions, therefore further PCR's were amended to follow this. Dowling et al. (2012) also extended the number of cycles from 35 to 40, therefore the same method was followed.

For the final PCR, 5µl of template DNA was mixed with 10µl Mastermix, 0.5µl of 6pmol primers (HCOI and LCOI) and 5µl of sterile water. PCR cycle was 95°C for 5min, [95°C for 45 sec, 53°C for 30 sec, 72°C for 1 min and 10 sec] repeated 40 times, 72°C for 10min, and then held at 10°C. Amplicon DNA was visualised using a Tape Station for selected samples (Agilent Technologies). Samples were prepared by adding 1µl of template DNA into 3µl of sample buffer. Samples were mixed for 1 min then spun to remove air bubbles, then loaded into the Tape station. The Tape station gave negative readings for PCR amplification, therefore a modified nested PCR was run using the samples. For this assay, 5µl of PCR product was added to 10µl Mastermix, 0.5µl of 6pmol primers (HCOI and LCOI) and 5µl of sterile water. PCR cycle was 95°C for 5min, [95°C for 45 sec, 53°C for 30 sec, 72°C for 1 min and 10 sec] repeated 40 times, 72°C for 10min, and then held at 10°C. DNA was visualised using gel electrophoresis.

3.4 Results

The baseline study to check that setae length was not affected by density showed there was no relationship between them (see Appendix 6). There was however a significant relationship observed between length of RPM and density of colonies ($F= 5.15$ on 1 and 13 d.f., $p= 0.04$), however not width, thus length was not deemed to be a reliable indicator of differences between populations. The function `lm.influence` was used in R to investigate the influence of outliers to test the robustness of the model output, points 5 and 12 were found to be influential, therefore analysis was repeated without them. The relationship was still significant ($R^2=0.33$, $F= 6.81$ on 1 and 11 d.f, $p= 0.024$; Figure 3.4).

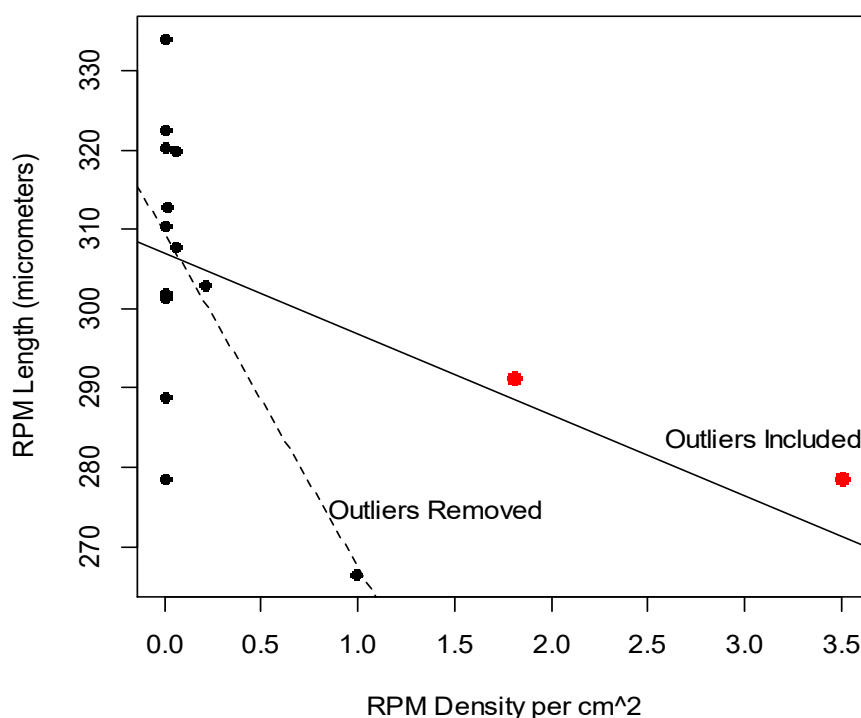


Figure 3.4 RPM density per square centimetre regressed against mean RPM length (μm) (with outliers: $R^2=0.28$, $F= 5.15$ on 1 and 13 d.f., $p= 0.04$; without outliers: $R^2=0.33$, $F= 6.81$ on 1 and 11 d.f., $p= 0.024$). Red points indicate outliers identified using `influence.measures` in R.

The first study conducted investigated the morphology of *R. indica* collected from different host plants within Kerala, India, with the hypothesis that there will be significant differences in the morphology of RPM collected from *C. nucifera* and *A. catechu*. A two-way MANOVA showed there was no significant interaction between host and site for all measurements, apart from seta e2 ($F(2,52) = 5.79$, $p=0.02$), therefore this term was removed for all setae apart from e2 in subsequent analysis. The minimal adequate model showed there to be a significant difference in lengths of e2, c1, c2, v2 and f3 between hosts ($F(3,52)= 6.59$ $p<0.001$; $F(3,54)=7.75$ $p<0.001$; $F(3,54)=4.16$, $p=0.010$;

$F(3,54)=11.65$ $p<0.001$, $F(3,54)=7.31$ $p<0.001$ respectively; Figure 3.5, Appendix 7) and significant differences in lengths of e2, c1, c2, v2, f2 and f3 between sites ($F(10,52)=2.88$ $p<0.01$; $F(10,54)=2.02$, $p=0.05$; $F(10,54)=2.73$ $p=0.009$; $F(10,54)=3.99$ $p<0.001$; $F(10,54)=2.38$ $p=0.02$; $F(10,54)=5.98$ $p<0.001$ respectively; Table 3.1). Tukey HSD post hoc tests from individual two-way ANOVA's on e2, c1, c2, v2 and f3 showed that in all cases these setae were significantly longer on specimens collected from *P. roebelenii* than those collected on *A. catechu* ($n=8$ and $n=24$ respectively; ($p<0.001$, $p<0.01$, $p=0.01$, $p<0.001$, $p<0.01$ respectively) and compared to specimens collected from *C. nucifera* ($n=34$), these setae were significantly longer in all cases apart from setae c1 and f3 ($p<0.001$, $p=0.24$, $p<0.01$, $p=0.01$, $p=0.44$ respectively). None of the setae lengths were significantly different between those collected on *Musa* spp. and *P. roebelenii*, however sample size was only $n=2$ for *Musa* spp. To further investigate if this was associated with host difference or site difference, an analysis of all RPM from the site (site I4) where *P. roebelenii* populations were found was carried out ($n=8$ for *P. roebelenii* derived RPM and $n=5$ for *C. nucifera* derived RPM). Results showed that there was a significant difference in the length of e2 and c2 setae ($F(1,11)=7.38$ $p=0.02$ and $F(1,11)=6.68$, $p=0.03$ respectively) with the e2 and c2 seta significantly longer on specimens found on *P. roebelenii* than on *C. nucifera* ($44.4\mu\text{m}\pm 1.35$ compared to $38.8\mu\text{m}\pm 1.47$ and $46.5\mu\text{m}\pm 0.96$ and $42.1\mu\text{m}\pm 1.52$ respectively). When compared to all sites, RPM collected on this site (I4) had setae in the upper quartile of all measurements for 7 out of the 11 setae measurements made, indicating that setae length in general was longer on this site than the mean.

With regards to a direct comparison between *A. catechu* and all other hosts, setae c1 ($F(3,54)=7.8$ $p<0.0001$ for the model, $p<0.04$ Tukey HSD), v2 ($p<0.02$ -excluding *Musa* sp.) and f3 ($F(3,54)=11.7$, $p<0.0001$ for the model, $p<0.05$ -excluding *Musa* sp. Tukey HSD) were found to be significantly shorter on *A. catechu*, however there were no significant differences between the lengths of all other setae on specimens collected from different hosts apart from e2 between *A. catechu* and *P. roebelenii*.

As there was often only one host type per site, the site analysis was rerun with only the coconut data so significant differences between site may be assessed. Overall the MANOVA showed significant differences between the lengths of setae c2 ($F(8,25)=2.63$, $p=0.03$), e2 ($F(8,25)=3.25$, $p=0.01$), c1 ($F(8,25)=3.37$, $p=0.008$), v2 ($F(8,25)=2.82$, $p=0.02$) and f3 ($F(8,25)=3.89$, $p=0.004$) between different sites. For *A. catechu*, significant differences were found between lengths of f2 ($F(3,20)=3.63$, $p=0.03$) and v2 ($F(3,20)=3.55$, $p=0.03$).

When a PCA was applied to these data from India, there was a strong positive loading on Principle Component 1 (PC1) (Figure 3.7), with the most influential measurement shown to be v2 (0.39).

Principle Component 1 accounted for 41.4% of the variation within the samples. There was a negative loading on PC 2 for setae e3, c3, d3 and f3- all lateral setae; and a strong positive loading on PC 2 for d1, d2, c2 and e2- all dorso-lateral setae. There was a strong outlier and inspection of the data showed this was a specimen from coconut on wind-trap site 1. This specimen had longer e2 and d2 setae (45.9 μ m and 50.9 μ m respectively compared to the overall mean of 38.31 μ m and 37.71 μ m). Also, it had much shorter lateral setae- d3- 60.5 μ m, e3- 58.3 μ m and c3- 65.6 μ m compared to the group mean of 93.85 μ m, 99.72 μ m and 91.3 μ m. Figure 3.6 shows that there is no distinction between samples when labelled according to host or site.

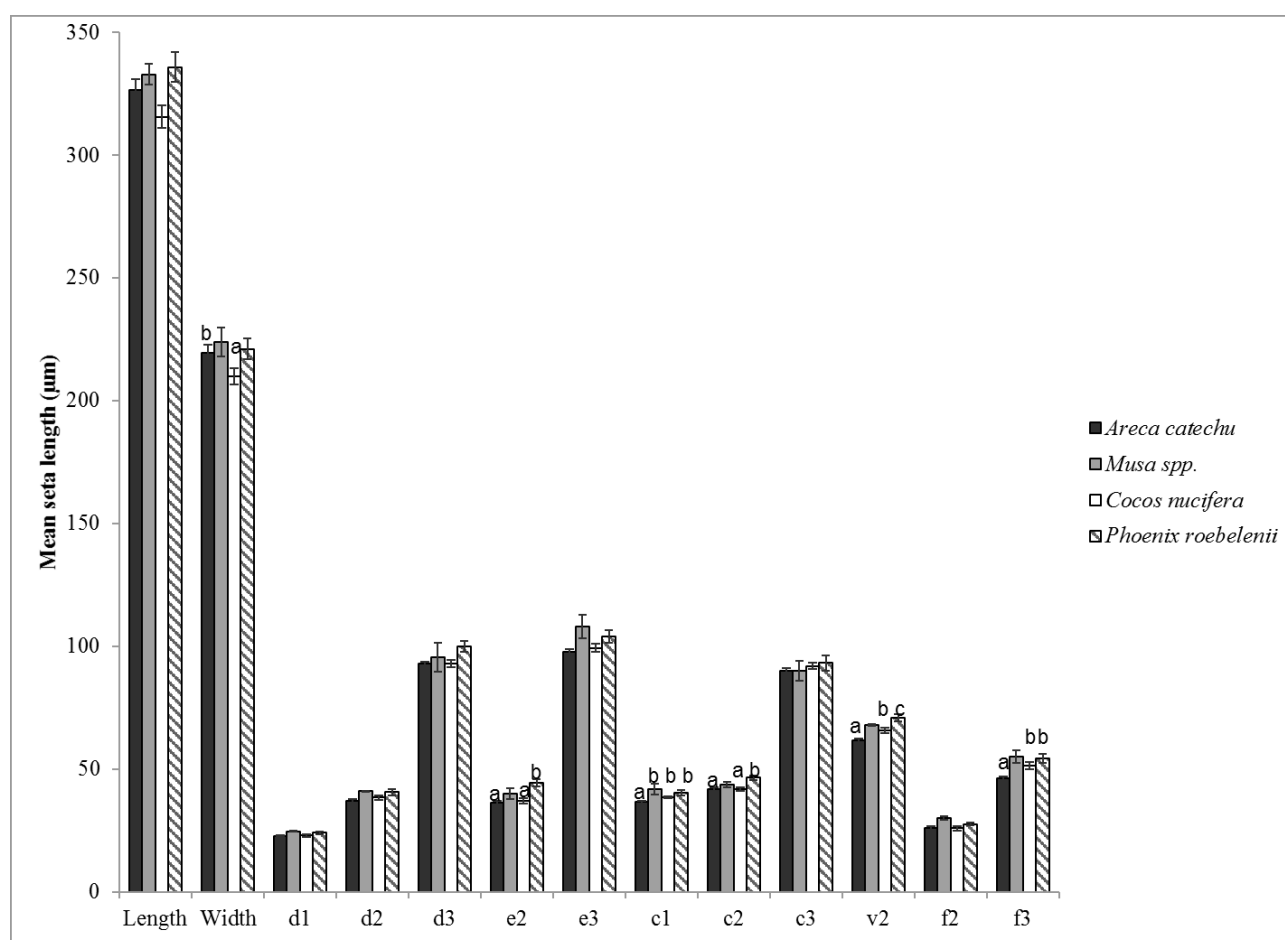


Figure 3.5 Lengths of dorsal setae, body width and body length (μ m; $\pm$ 1SE) of RPM collected from different host plants in India. n=34 for *Cocos nucifera*, n=2 for *Musa spp.*, n=24 for *Areca catechu* and n=8 for *Phoenix roebelenii*. Significant differences are marked with different letters, where letters are not shown, no significant differences were found. Figure of results is found in Appendix 7.

Table 3.1 Mean lengths of setae (μm) measured on Indian RPM specimens between different sites ($\pm 1\text{SE}$). Significant differences marked using different letters. Site names are prefixed with the letter I, and hosts plant species on each site are given as C= *Cocos nucifera*, A= *Areca catechu*, M = *Musa* spp., P=*Phoenix roebelenii*. There was no significant interaction between host and site thus samples from different host plants were analysed together for each site.

	I1 (C,n=14, M, n=1)	I11 (C,n=1)	I13 (A,n=3; C, n=2)	I2 (C,n=2)	I20 (C, n=3)	I3 (C, n=2; A, n=11)	I4 (C, n=5, P, n=8)	I4b (C, n=3)	IB (A, n=9)	IK (A,n=2, M, n=1)	IPAL (C,n=1)
Length	312.4 $\pm$ 5.64 ^{ab}	304.2 $\pm$ NA ^{ab}	308.9 $\pm$ 9.88 ^{ab}	290.5 $\pm$ 5.57 ^a	350.2 $\pm$ 52.60 ^b	329.9 $\pm$ 5.91 ^{ab}	332.8 $\pm$ 6.64 ^{ab}	335.5 $\pm$ 3.71 ^{ab}	330.4 $\pm$ 8.01 ^{ab}	306.9 $\pm$ 15.17 ^{ab}	280.7 $\pm$ NA ^{ab}
Width	207.0 $\pm$ 4.42 ^{abd}	199.5 $\pm$ NA ^{abd}	191.8 $\pm$ 3.91 ^d	191.5 $\pm$ 1.37 ^{abd}	235.4 $\pm$ 0.59 ^c	227.4 $\pm$ 2.83 ^{ac}	222.7 $\pm$ 4.42 ^{acd}	212.9 $\pm$ 4.35 ^{abcd}	220.8 $\pm$ 5.62 ^{abcd}	196.8 $\pm$ 10.57 ^{bcd}	184.6 $\pm$ NA ^{abcd}
d1	23.2 $\pm$ 0.91 ^a	23.4 $\pm$ NA ^a	21.9 $\pm$ 0.98 ^a	23.4 $\pm$ 1.70 ^a	23.0 $\pm$ 0.54 ^a	23.2 $\pm$ 0.43 ^a	24.1 $\pm$ 0.38 ^a	23.5 $\pm$ 0.52 ^a	21.7 $\pm$ 0.64 ^a	22.7 $\pm$ 0.95 ^a	20.9 $\pm$ NA ^a
d2	36.6 $\pm$ 1.50 ^a	41.2 $\pm$ NA ^a	41.1 $\pm$ 2.01 ^a	42.4 $\pm$ 6.78 ^a	38.3 $\pm$ 0.33 ^a	36.5 $\pm$ 0.69 ^a	39.1 $\pm$ 1.15 ^a	44.5 $\pm$ 0.32 ^a	37.0 $\pm$ 1.25 ^a	38.6 $\pm$ 1.76 ^a	38.0 $\pm$ NA ^a
d3	89.7 $\pm$ 2.66 ^a	111.8 $\pm$ NA	96.6 $\pm$ 2.05 ^a	96.8 $\pm$ 3.86 ^a	92.3 $\pm$ 2.76 ^a	91.9 $\pm$ 0.96 ^a	98.5 $\pm$ 1.52 ^a	97.1 $\pm$ 6.32 ^a	93.3 $\pm$ 1.97 ^a	90.8 $\pm$ 0.64 ^a	89.5 $\pm$ NA ^a
e2	35.5 $\pm$ 1.64 ^a	38.5 $\pm$ NA ^{ab}	34.9 $\pm$ 1.69 ^a	30.2 $\pm$ 1.69 ^a	38.1 $\pm$ 2.05 ^{ab}	36.0 $\pm$ 1.37 ^a	42.3 $\pm$ 1.25 ^a	49.6 $\pm$ 2.80 ^b	37.1 $\pm$ 1.480 ^a	39.6 $\pm$ 1.90 ^a	30.0 $\pm$ NA ^a
e3	97.5 $\pm$ 3.20 ^a	109.0 $\pm$ NA ^a	99.3 $\pm$ 1.04 ^a	96.5 $\pm$ 1.39 ^a	103.7 $\pm$ 4.85 ^a	97.8 $\pm$ 1.48 ^a	103.2 $\pm$ 1.75 ^a	102.8 $\pm$ 2.09 ^a	98.3 $\pm$ 2.68 ^a	99.5 $\pm$ 2.71 ^a	104.8 $\pm$ NA ^a
c1	38.10.65 ^a	38.1 $\pm$ NA	38.1 $\pm$ 1.52 ^{ab}	38.4 $\pm$ 1.91 ^{ab}	37.1 $\pm$ 0.52 ^{ab}	36.5 $\pm$ 0.79 ^a	39.7 $\pm$ 0.76 ^{ab}	43.51.09 ^b	37.5 $\pm$ 0.74 ^{ab}	37.9 $\pm$ 1.70 ^{ab}	35.3 $\pm$ NA ^{ab}
c2	41.1 $\pm$ 1.04 ^a	45.9 $\pm$ NA ^{ab}	40.4 $\pm$ 2.04 ^a	39.5 $\pm$ 2.57 ^a	41.4 $\pm$ 0.74 ^{ab}	41.2 $\pm$ 0.75 ^a	44.8 $\pm$ 1.00 ^a	49.8 $\pm$ 2.63 ^b	44.1 $\pm$ 1.42 ^{ab}	41.3 $\pm$ 0.83 ^{ab}	35.1 $\pm$ NA ^a
c3	89.0 $\pm$ 2.19 ^a	107.3 $\pm$ NA ^a	87.05 $\pm$ 3.66 ^a	90.3 $\pm$ 3.34 ^a	90.4 $\pm$ 2.59 ^a	93.5 $\pm$ 1.10 ^a	98.8 $\pm$ 2.15 ^a	89.5 $\pm$ 0.36 ^a	90.4 $\pm$ 1.59 ^a	93.3 $\pm$ 0.87 ^a	100.8 $\pm$ NA ^a
v2	63.5 $\pm$ 1.12 ^a	74.4 $\pm$ NA ^{ab}	61.6 $\pm$ 2.27 ^{ab}	60.5 $\pm$ 5.54 ^a	64.3 $\pm$ 2.33 ^{ab}	61.5 $\pm$ 1.28 ^{ab}	70.7 $\pm$ 1.01 ^{ab}	73.6 $\pm$ 0.10 ^b	64.9 $\pm$ 1.11 ^{ab}	63.2 $\pm$ 2.95 ^{ab}	66.4 $\pm$ NA ^{ab}
f2	25.3 $\pm$ 1.07 ^a	26.0 $\pm$ NA ^{ab}	27.2 $\pm$ 1.50 ^{ab}	23.3 $\pm$ 1.52 ^a	27.1 $\pm$ 1.18 ^{ab}	26.3 $\pm$ 0.70 ^{ab}	27.1 $\pm$ 0.73 ^{ab}	33.4 $\pm$ 5.03 ^b	25.1 $\pm$ 1.12 ^a	23.9 $\pm$ 3.57 ^{ab}	26.1 $\pm$ NA ^{ab}
f3	45.6 $\pm$ 2.11 ^a	69.8 $\pm$ NA ^b	51.8 $\pm$ 2.43 ^b	60.8 $\pm$ 1.51 ^b	54.3 $\pm$ 1.92 ^{ab}	46.4 $\pm$ 1.16 ^a	53.1 $\pm$ 1.20 ^a	61.2 $\pm$ 1.69 ^b	46.7 $\pm$ 1.15 ^{ab}	49.4 $\pm$ 4.19 ^{ab}	45.7 $\pm$ NA ^{ab}

A linear discriminate analysis showed no evidence of a trend towards distinction between RPM populations on *A. catechu*, *C. nucifera*, *P. roebelenii* and *Musa* spp. populations (Figure 3.7). Function 1 (LD1) accounted for 60.44% of the variance in the data. The top three traits with most influence in function 1 (according to structure matrix) were length of c1 (0.178), v2 (0.133) and d1 (0.111) respectively. *Phoenix roebelenii* populations were less easy to distinguish using this technique. The LDA correctly predicted host origin on 69% of occasions. Figure 3.8 showed that if an LDA was applied to data for setae lengths e2, c1, c2, v2 and f3 there was still no clear distinction in populations. A hierarchical cluster analysis was not able to distinguish between populations from different hosts based on the setae length measurements (Figure 3.9).

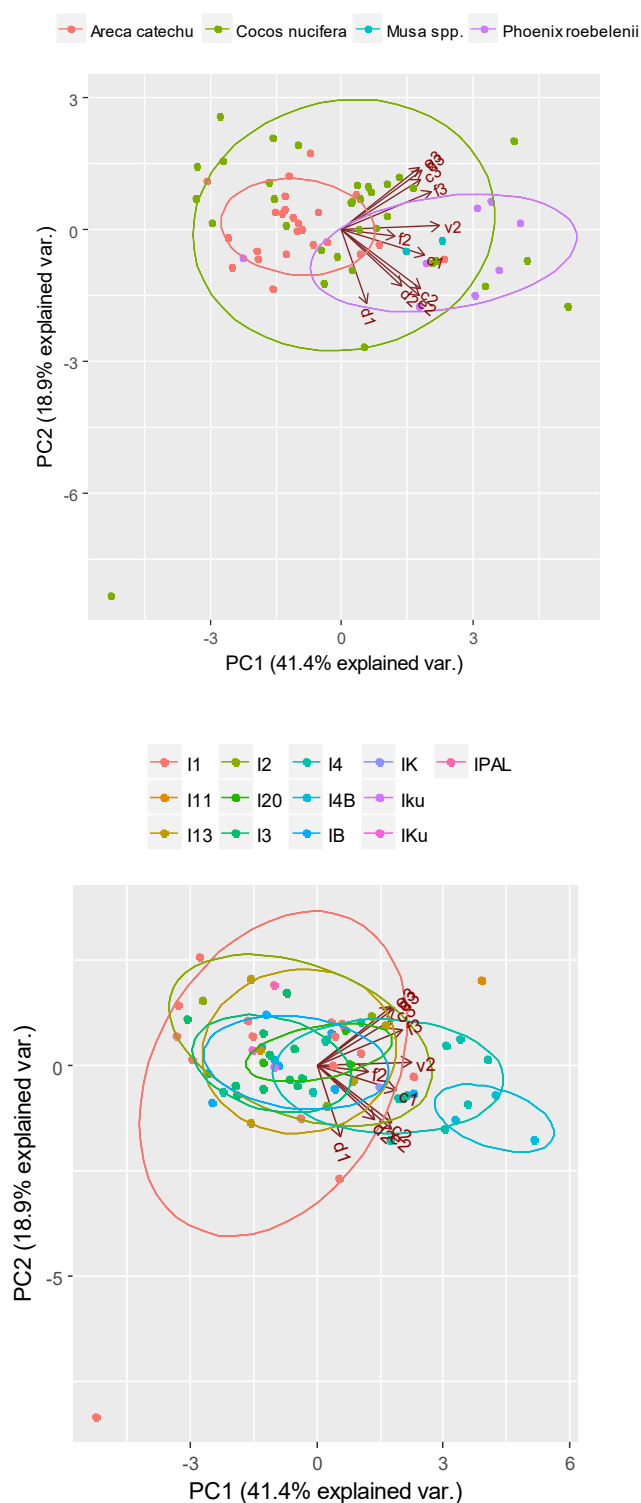


Figure 3.6 Principle component analysis bi-plot from RPM in India categorised using host species (top) and site (bottom) (using ggbiplot in R). Ellipses are normal contour lines drawn using ellipse.prob where the probability is set to a default of 68% for each group (Friendly, 2006).

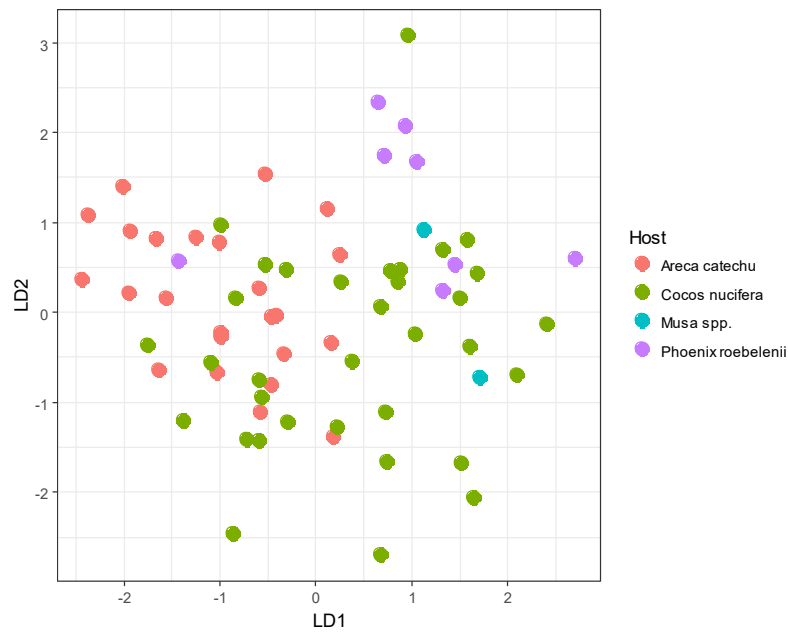


Figure 3.7 A linear discriminant analysis of RPM dorsal setae lengths in India, using host for categorisation.

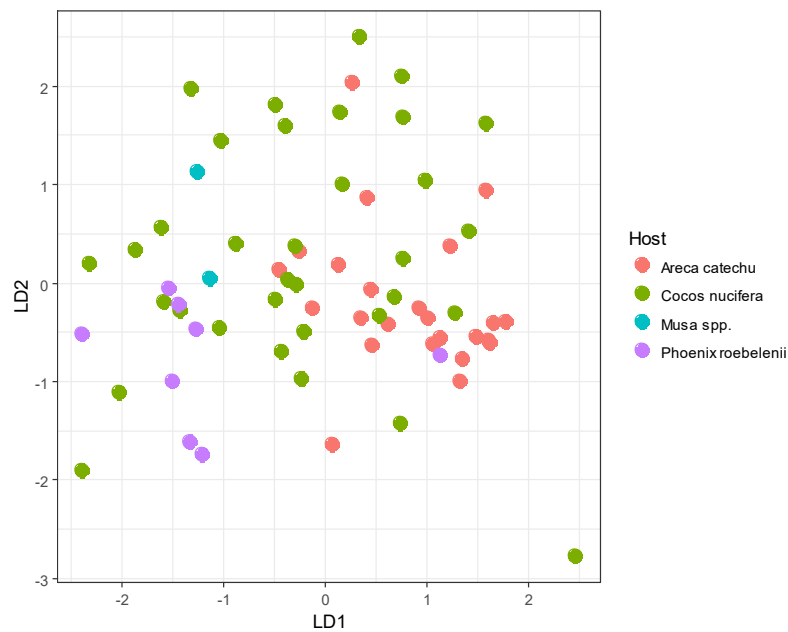


Figure 3.8 A linear discriminant analysis of RPM conducted using e2, c1, c2, v2 and f3 dorsal setae lengths, categorised using host

A LDA between sites showed that LD1 accounted for 47.6% of the variation in the samples and the most influential measurements between sites were v2, f3, d2, d1 and e2. The model predicted the correct site from measurements on 69% of occasions.

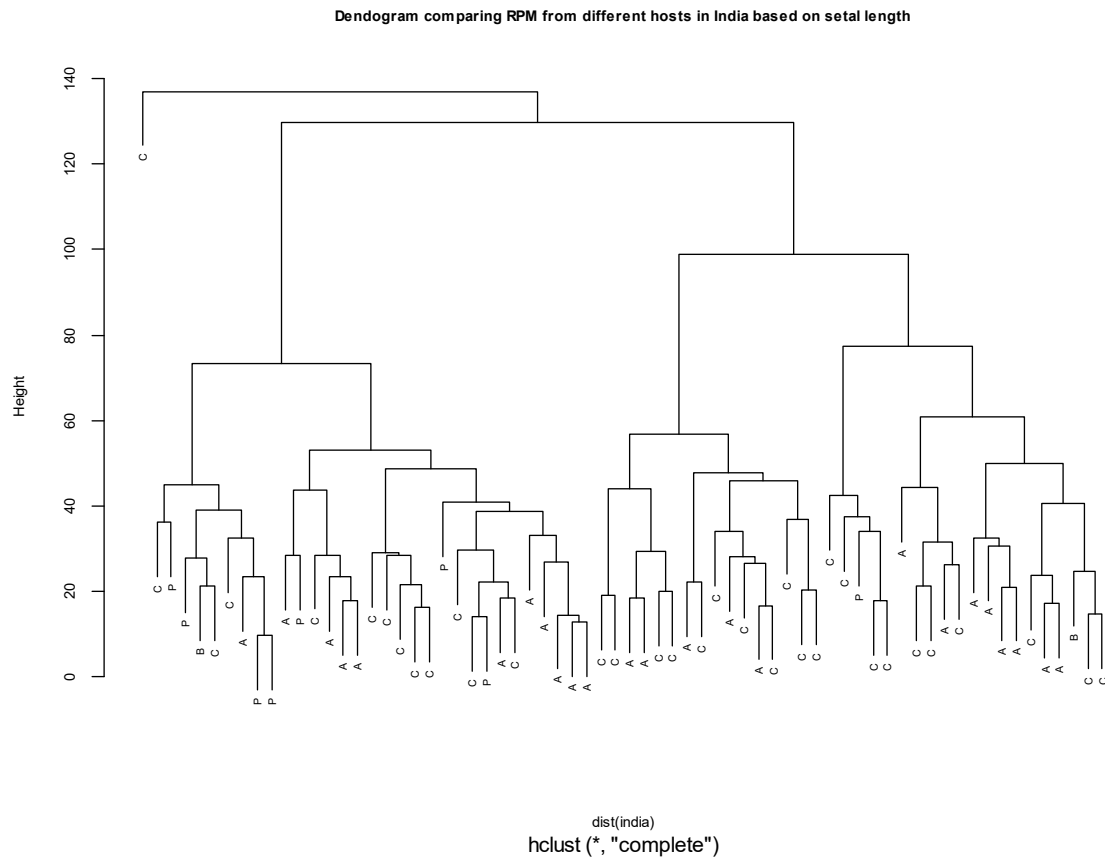


Figure 3.9 Hierarchical cluster analysis of specimens collected from four different host species in India.
(A = *Areca catechu*, B= *Musa spp.*, C= *Cocos nucifera*, P= *Phoenix roebelenii*).

Samples of RPM used for molecular work did not yield enough DNA for downstream sequencing work, therefore a molecular comparison was not possible between specimens and sites in India.

The next analysis compared the morphology of *R. indica* within the Caribbean on both a host plant and site level. A two-way MANOVA showed there was no significant interaction between host and site for all measurements, apart from setae d1 and f2 ($F(1,122)= 13.86$, $p<0.001$; $F(1,122)=7.01$ $p<0.01$ respectively), therefore this term was removed for all setae apart from d1 and f2 in subsequent analysis. The minimal adequate model showed there to be a significant difference in lengths of c2 and f2 between hosts ($F(1,123)= 5.16$ $p=0.02$ and $F(1,123)= 4.58$ $p=0.03$ respectively; Figure 3.10; Appendix 9) and significant differences in body length and width and lengths of d1, d2, e2, c1, c2, f2 and v2 between sites ($F(3,123)= 4.17$, $p<0.01$; $F(3,123)= 9.64$, $p<0.001$; $F(3,123)= 3.78$, $p=0.01$; $F(3,123)= 3.14$, $p=0.03$, $F(3,123)= 4.60$, $p=0.004$, $F(3,123)= 3.79$, $p=0.01$; $F(3,123)=3.70$, $p=0.014$; $F(3,123)= 5.77$, $p=0.001$; $F(3,123)= 2.81$, $p=0.04$ respectively; Figure 3.11; Appendix 8).

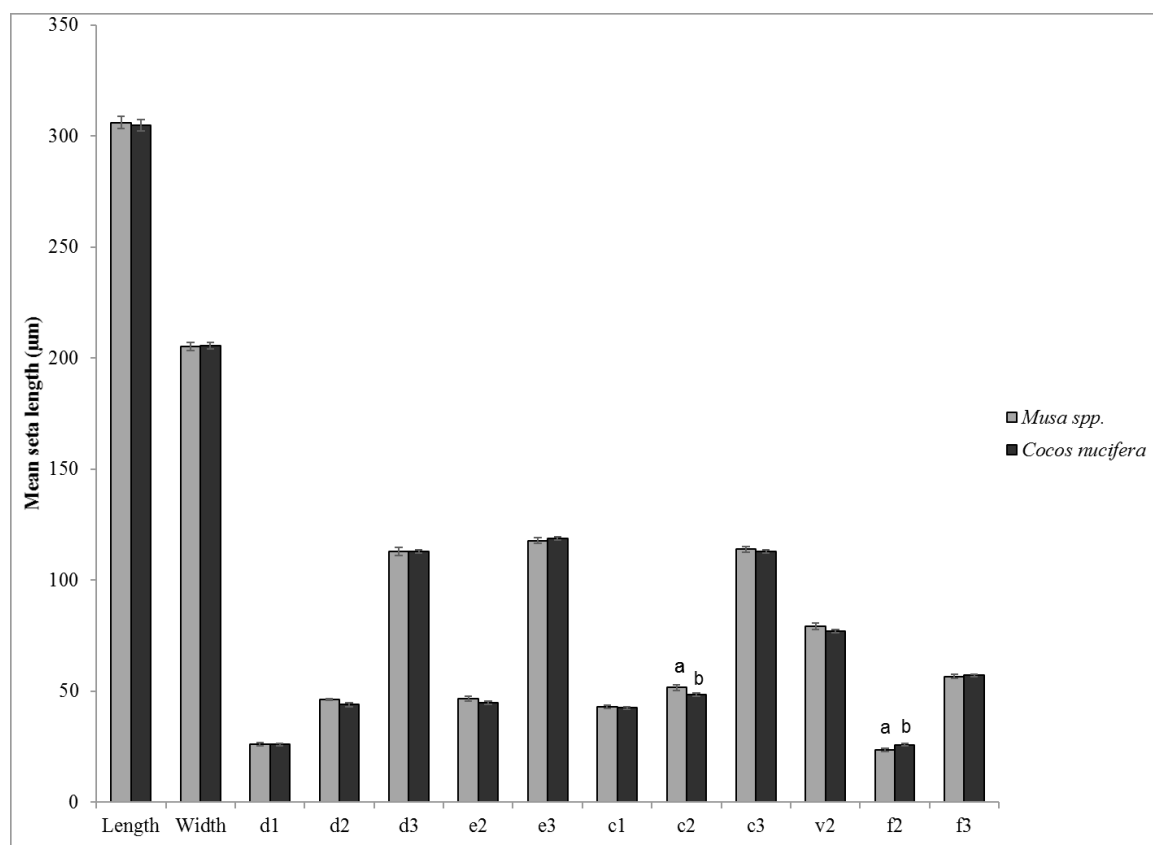


Figure 3.10 Lengths of dorsal setae, width and length (µm) of RPM collected from different host plants in the Caribbean. n=94 for RPM ex. *Cocos nucifera*, and=34 for RPM ex. *Musa spp.*. Significant differences in length are shown using different letters (p<0.05).

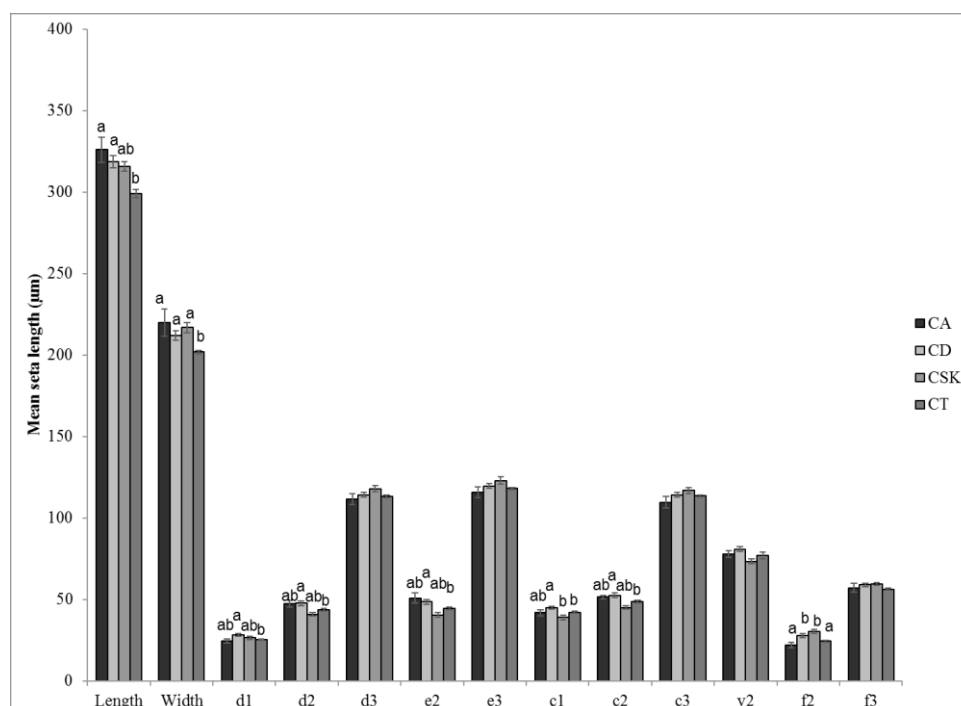


Figure 3.11 Lengths of setae and width and length (μm) of RPM collected from different islands in the Caribbean. $n=5$ for CA (Antigua), $n=27$ for CD (Dominica), $n=7$ for CSK (St. Kitts), $n=89$ for CT (Trinidad).

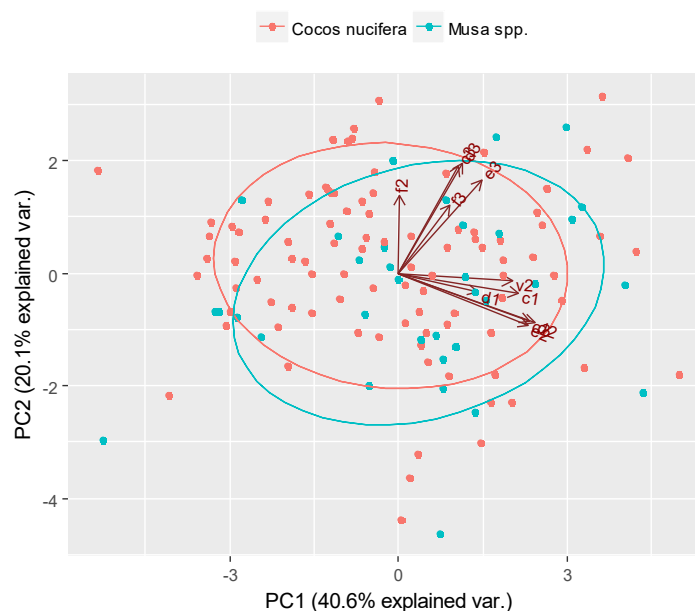


Figure 3.12 Principle components analysis bi-plot from RPM in the Caribbean categorised using host plants. Ellipses are normal contour lines drawn using ellipse.prob where the probability is set to a default of 68% for each group (Friendly, 2006).

A principle components analysis (Figure 3.12) showed similar results to the India specimens between hosts. There was a strong positive loading for PC1 for v2, c1, c2, d2 and e2. Setae f2, f3, c3 d3, e3, (all lateral setae apart from f2) had a strong positive loading for PC2. Principle Component 1 accounted for 40.6% of the variation in specimens whereas PC2 account for 20.1% of the variation in specimens. The most influential setae for PC1 were c2, d2 and e2, whereas for PC2 they were c3, d3 and e3- again very similar to results shown for Indian specimens. Further PCA analysis was applied to these data according to island of origin and year of collection. There was no evidence that there was any partitioning of populations of RPM at an island level (Figure 3.13) or over time (Figure 3.14). A MANOVA comparing setae lengths over time showed there were no significant difference in lengths of setae between specimens collected in 2009, 2011 and 2012, apart from d1 and f2 (v1: $F(1,125)=2.15$, $p=0.15$; c1: $F(1,125)=0.42$, $p=0.52$; d1: $F(1,125)=5.67$, $p=0.02$; c2: $F(1,125)=0.20$, $p=0.66$; d2: $F(1,125)=0.14$, $p=0.71$; e2: $F(1,125)=0.003$, $p=0.95$; f2: $F(1,125)=17.3$, $p<0.001$; c3: $F(1,125)=2.17$, $p=0.14$; d3: $F(1,125)=0.29$, $p=0.59$; e3: $F(1,125)=0.11$, $p=0.75$; f3: $F(1,125)=2.71$, $p=0.10$).

A linear discriminate analysis between hosts showed that there was no real distinction between specimens collected from separate islands (Figure 3.15) or host plants (Figure 3.16). The trait with the most influence according to LD1 between hosts was c2 (-0.18) however other traits were negligible. The LDA between islands showed that LD1 accounted for 43.7% of the variation and the most influential trait for LD1 was f2 (0.17). A hierarchical cluster analysis was not able to distinguish between populations from different hosts based on the setae length measurements (Figure 3.17) or by island of origin (Figure 3.18).

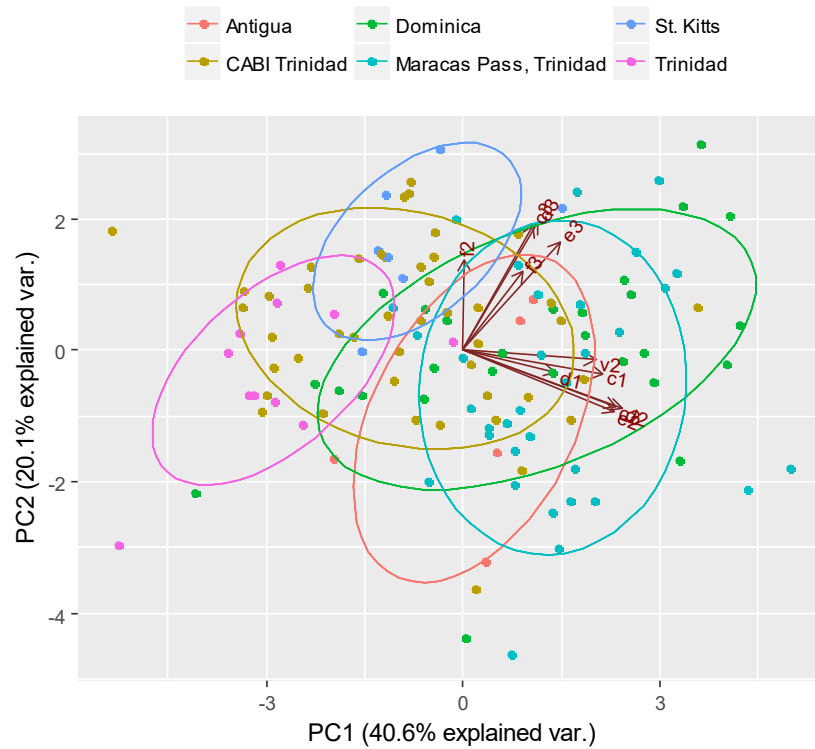


Figure 3.13 Results of PCA comparing RPM specimens collected from different islands in the Caribbean. Ellipses are Normal contour lines drawn using `ellipse.prob` where the probability is set to a default of 68% for each group (Friendly, 2006)

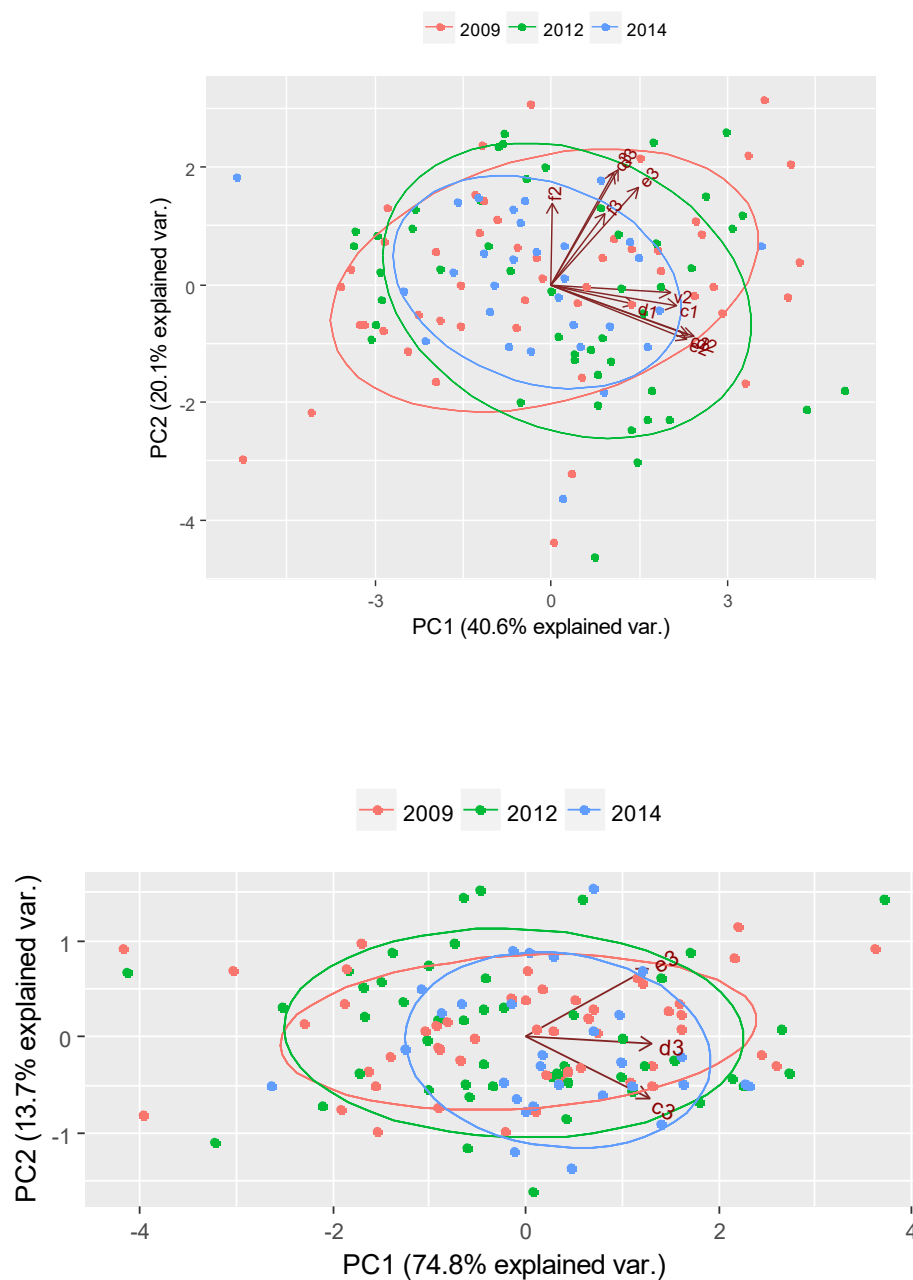


Figure 3.14 Results of PCA comparing RPM specimens collected over different years in the Caribbean using all setae measure (top) and just lateral setae (bottom). Ellipses are Normal contour lines drawn using ellipse.prob where the probability is set to a default of 68% for each group

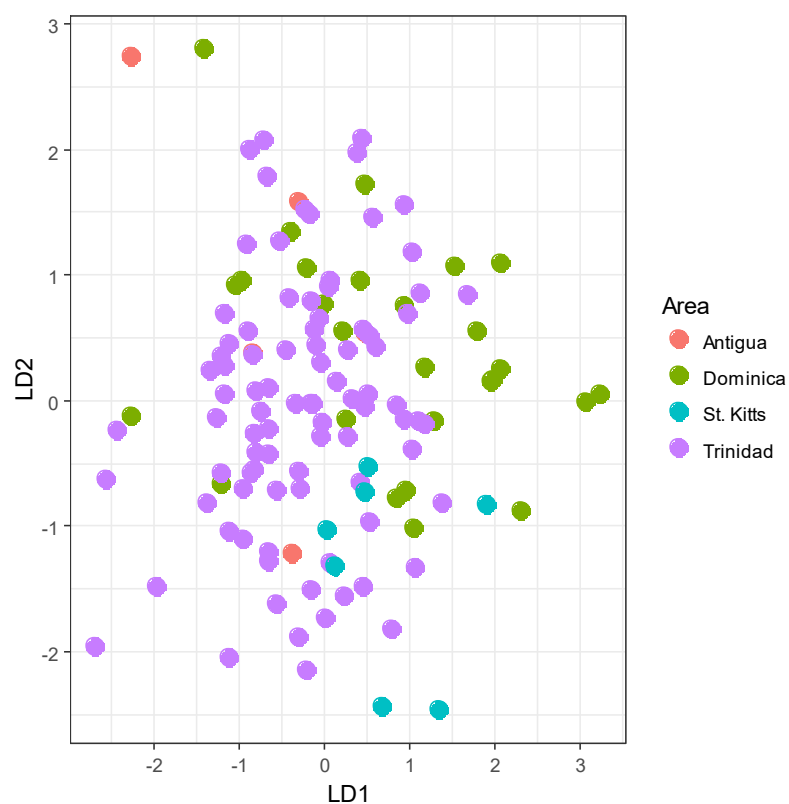


Figure 3.15 Results of LDA comparing RPM specimens collected from different islands in the Caribbean.

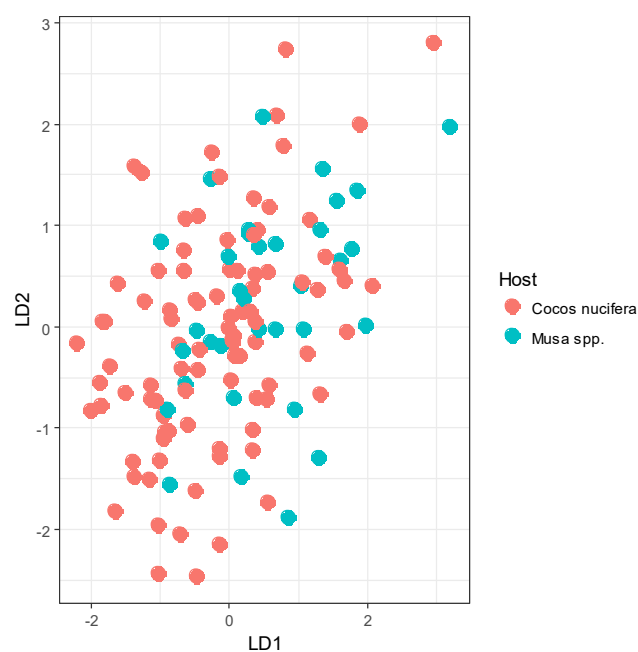


Figure 3.16 Results of LDA comparing RPM specimens collected from different host plants in the Caribbean.

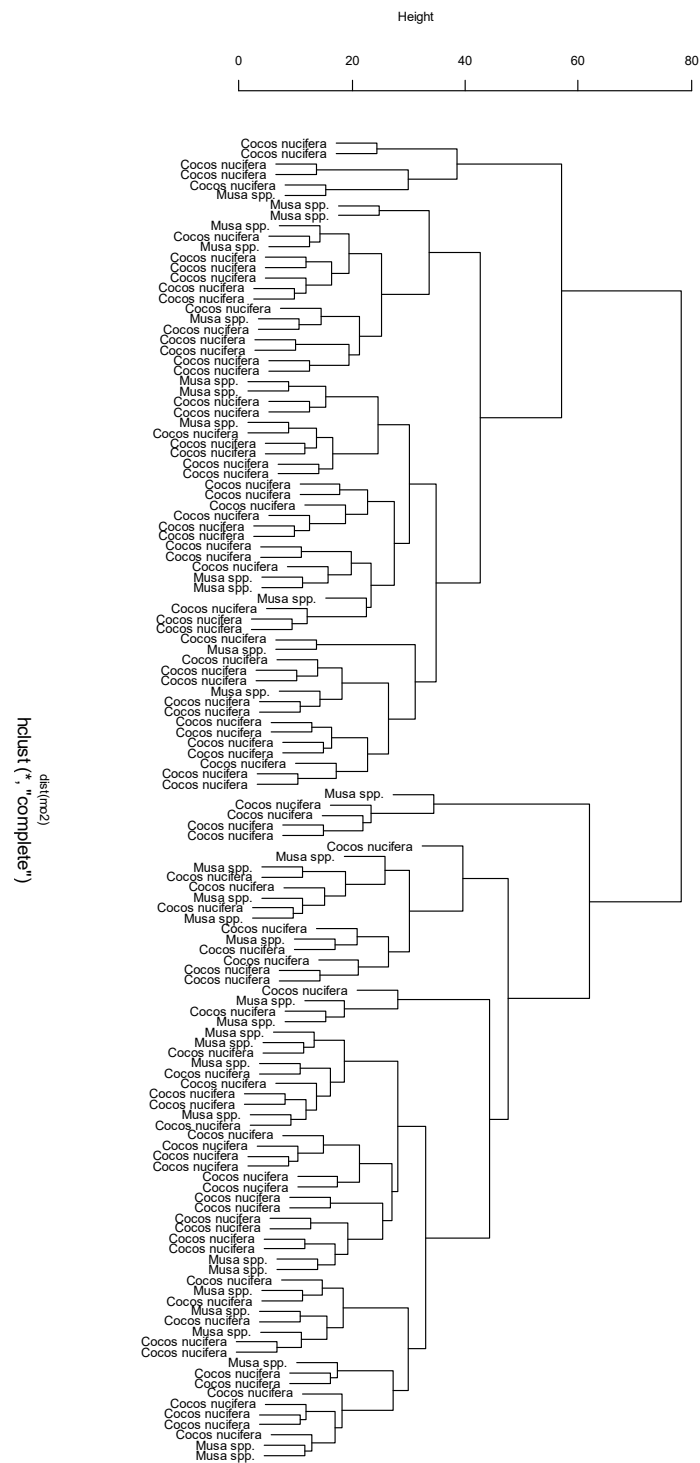


Figure 3.17 Dendrogram comparing RPM from *Cocos nucifera* and *Musa* spp. collected from four islands in the Caribbean based on dorsal setae measurements. Specimens are separated by host origin.

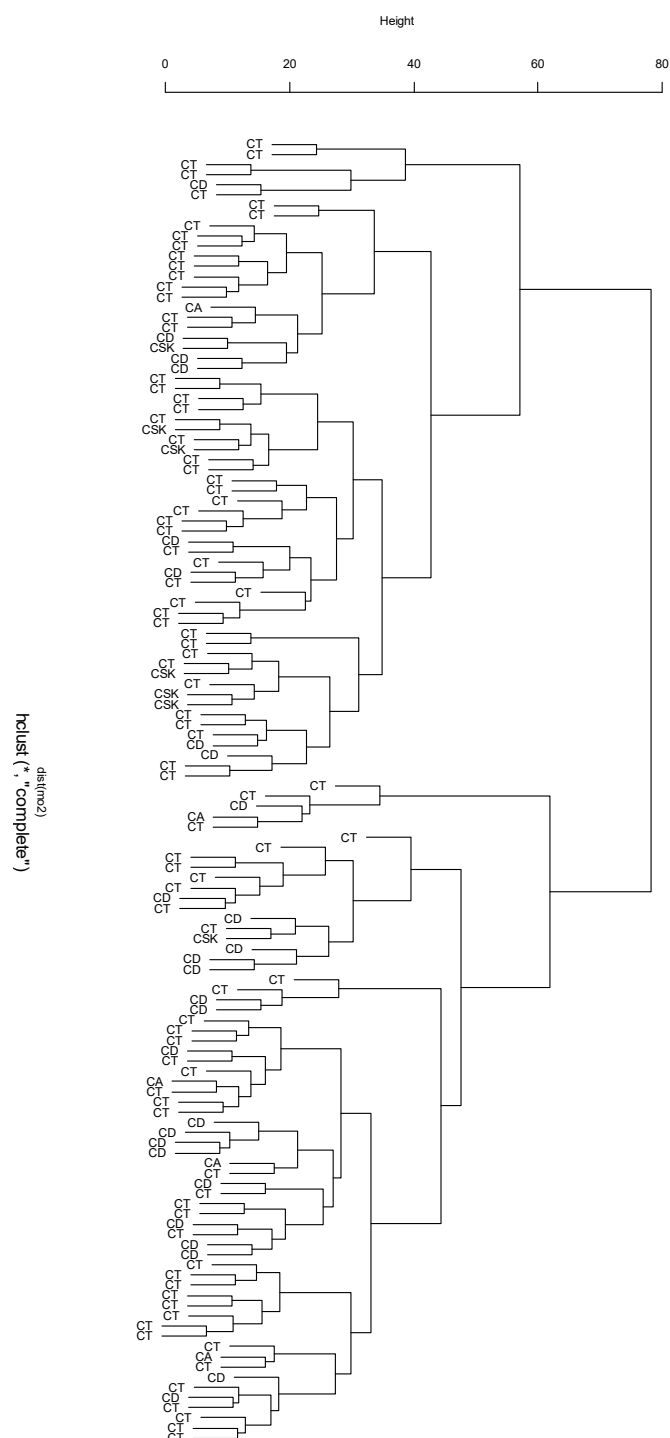


Figure 3.18 Dendrogram built using setae lengths of RPM from *Cocos nucifera* and *Musa* spp. collected from four islands in the Caribbean based on dorsal seta measurements, separated by island of origin (CT= Caribbean, Trinidad, CD= Caribbean, Dominica, CA= Caribbean, Antigua, CSK= Caribbean, St. Kitts).

As there was no observable difference between *R. indica* on *A. catechu* and *C. nucifera* within India, the samples from India from all host plants combined and the Caribbean were compared. Although in general the body size (length/width) of RPM was significantly smaller for specimens collected in the Caribbean ($F(1,195)=22.4$, $p<0.0001$; $F(1,195)=15.8$, $p<0.0001$ respectively), the setae were significantly longer in all cases apart from f2 ($F(1,195)=1.4$, $p=0.24$) (Figure 3.19; Appendix 10). In particular, lateral setae c3, d3 and e3 were significantly longer in specimens from the Caribbean compared to those from India (22.4, 19.9 and 19.0 μm longer respectively; $F(1,195)=493.2$, $p<0.0001$; $F(1,195)=285.5$, $p<0.0001$; $F(1,195)=328.4$, $p<0.0001$). A linear discriminate analysis (Figure 3.20) showed overlapping of the India and Caribbean populations but to a much lower extent to that seen within country. Figure 3.21 is a regression tree model built using setae lengths and it shows that Caribbean and Indian populations can be partitioned based on the length of c3 then d3 lateral seta lengths. A sub-set of the Caribbean population resides within the Indian population and can be differentiated using f2 length. Figure 3.21 also shows that populations of RPM can be almost perfectly separated into populations derived from India and those derived from Caribbean based on seta length alone with some minor crossover.

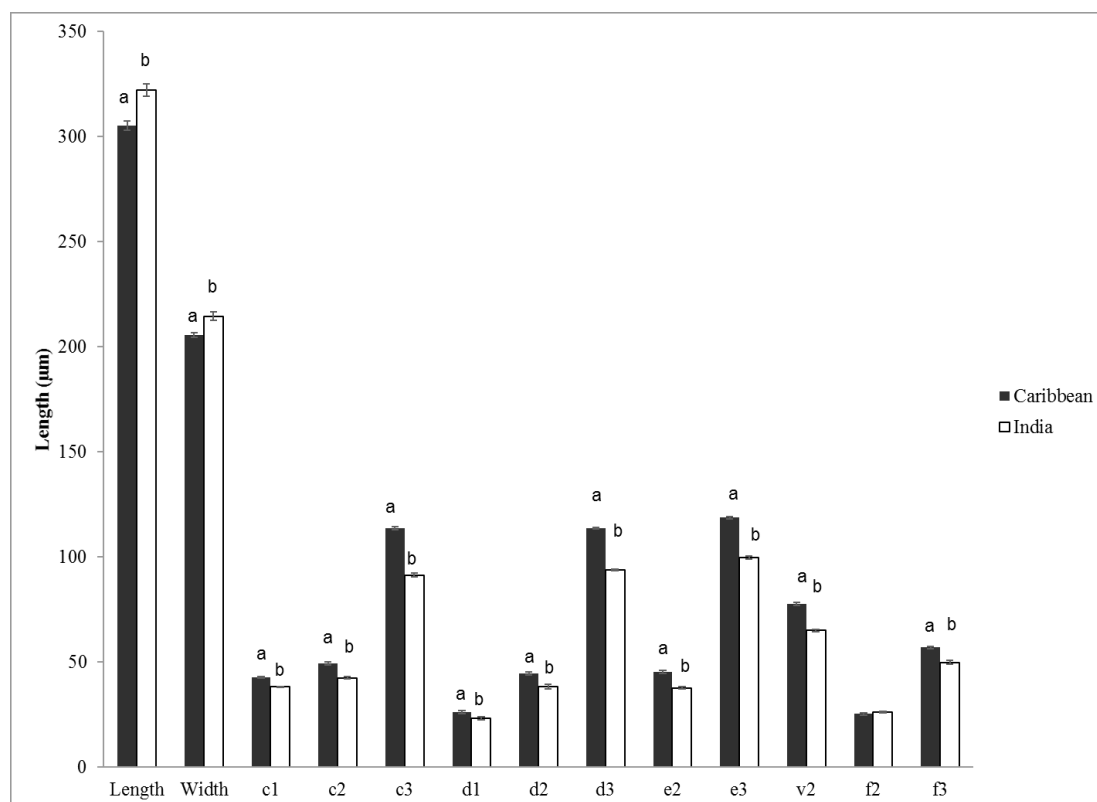


Figure 3.19 Lengths of setae, and width and length (μm) of RPM collected from the Caribbean and India (from all host plants). (n= 128 for Caribbean RPM, n= 69 for Indian RPM).

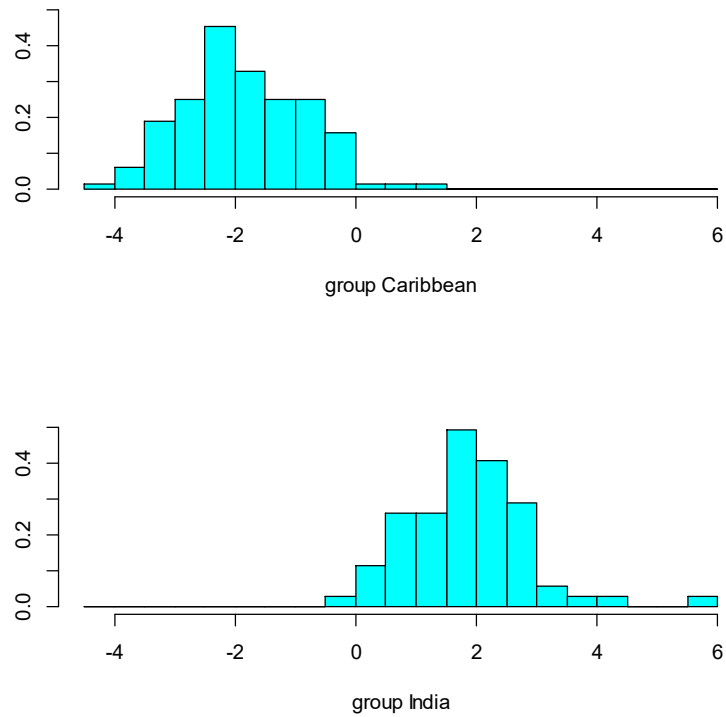


Figure 3.20 The result of an LDA on all traits measured on RPM between India and the Caribbean.

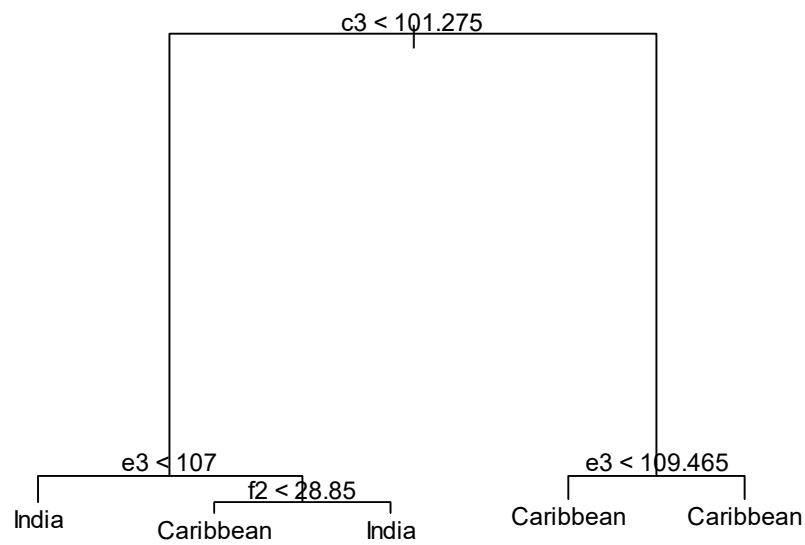


Figure 3.21 A regression tree model partitioning populations of RPM from Caribbean and India.

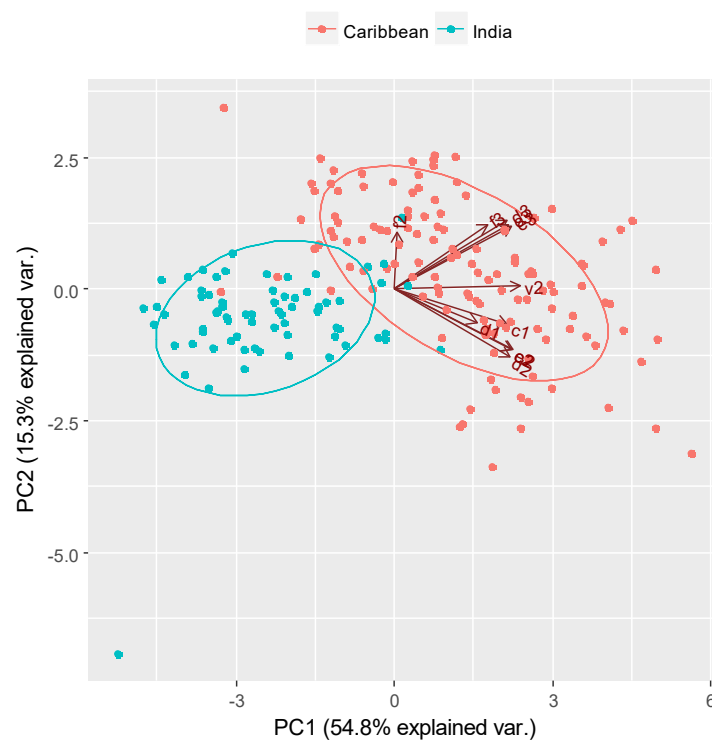


Figure 3.22 Principle components analysis of RPM populations derived from India and Caribbean based on all setae measurements and body length/width. Ellipses are normal contour lines drawn using ellipse.prob where the probability is set to a default of 68% for each group

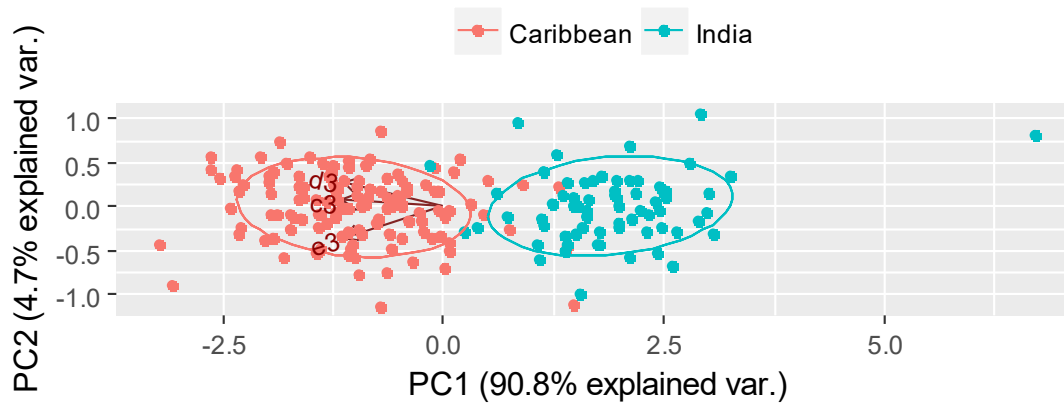


Figure 3.23 Principle components analysis using lengths of c3, d3 and e3 only. Ellipses are normal contour lines drawn using ellipse.prob where the probability is set to a default of 68% for each group.

A PCA on all setae lengths divided the specimens from the Caribbean and India into two groups, with minor overlapping. Principle Component 1 explained 54.8% of the variation and PC2 accounted for 15.4% of variation (Figure 3.22). When only lateral setae c3, d3 and e3 were used, PC1 increased to 90.8% of the variation between specimens explained and PC2 accounting for only 4.7% of the variations (Figure 3.23). The ellipses did not overlap in this analysis. The dendograms constructed using also almost wholly partitioned the two populations from India and Caribbean using all setae lengths (Figure 3.24) and to a greater extent setae c3, d3 and e3 (Figure 3.25).

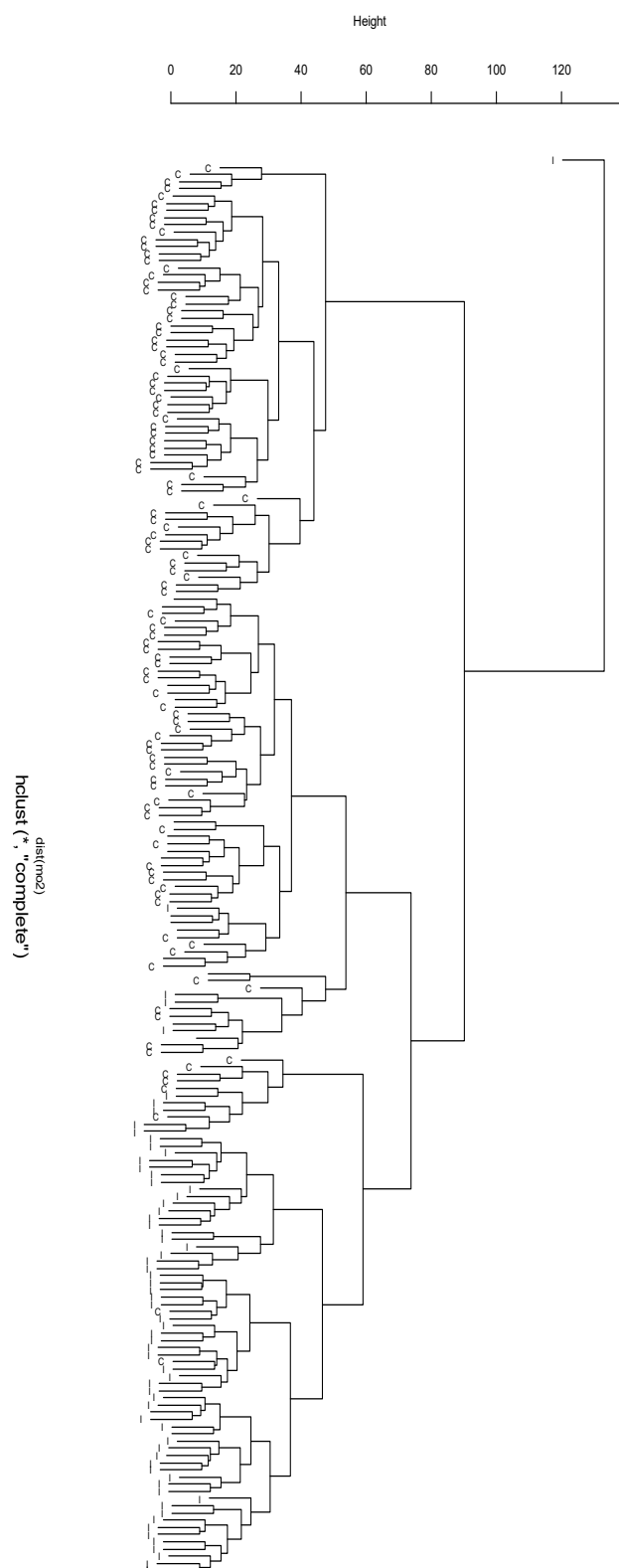


Figure 3.24 Dendrogram built using setae lengths of RPM from the Caribbean and India (C= origin Caribbean, I = origin India).

The ecology of *Raoiella indica* (Hirst) (Acari:Tenuipalpidae) in India and Trinidad

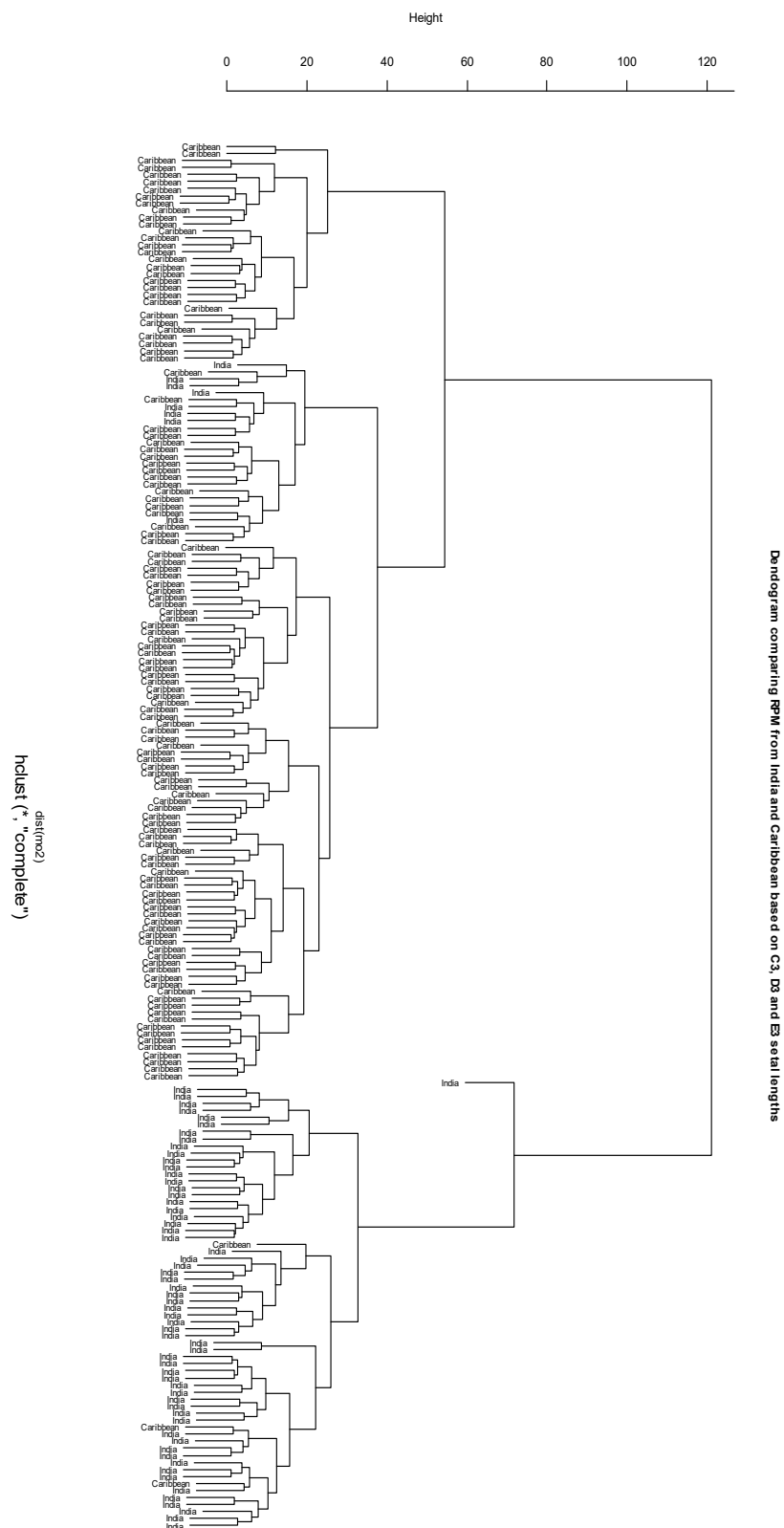


Figure 3.25 Dendrogram built using c3, d3 and e3 setae lengths of RPM from the Caribbean and India

Following the comparison of *R. indica* females between region, a follow up analysis was conducted to see if these differences were observable in male specimens also (Figure 3.26). A MANOVA showed that the specimens from the Caribbean were significantly narrower than the specimens from India ($F(1,22)=16.29$, $p<0.001$), however the length of the c3 seta was significantly longer ($F(1,22)=4.43$, $p<0.05$) with the mean length 82.0 μm in the Caribbean compared to 74.6 μm in India.

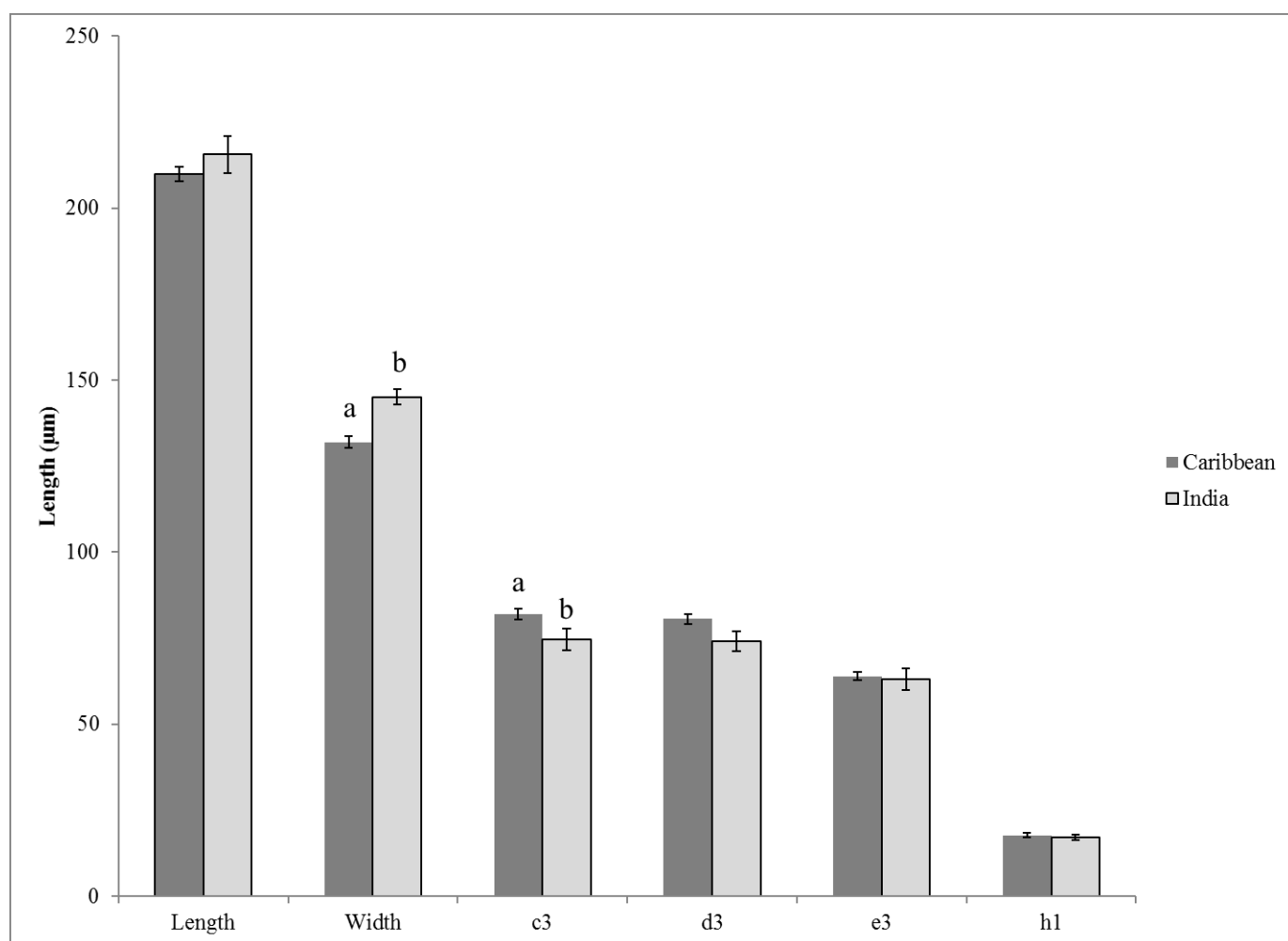


Figure 3.26 Comparison of males specimens from Caribbean and India (n=23 for Caribbean and n=11 for India)

With regards to the molecular analysis, DNA extractions were undertaken on several occasions, however, only one band of DNA was returned from all the DNA extractions/PCR's (from a RPM specimen collected in Trinidad; Figure 3.27; Appendix 5). The RPM sequence results were compared to those obtained in Dowling et al. (2012) from specimens collected from both *C. nucifera* and *A. catechu* in India, and to those collected from *C. nucifera* in the Philippines. The sequence was

identical to those collected from India ex *A. catechu* and the Philippines ex *C. nucifera*. From later DNA extraction undertaken it was shown that DNA was being extracted from samples, however it was not in sufficient quantities for PCR and sequencing. Appendix 11 give the quantities recovered from the final set of extractions.

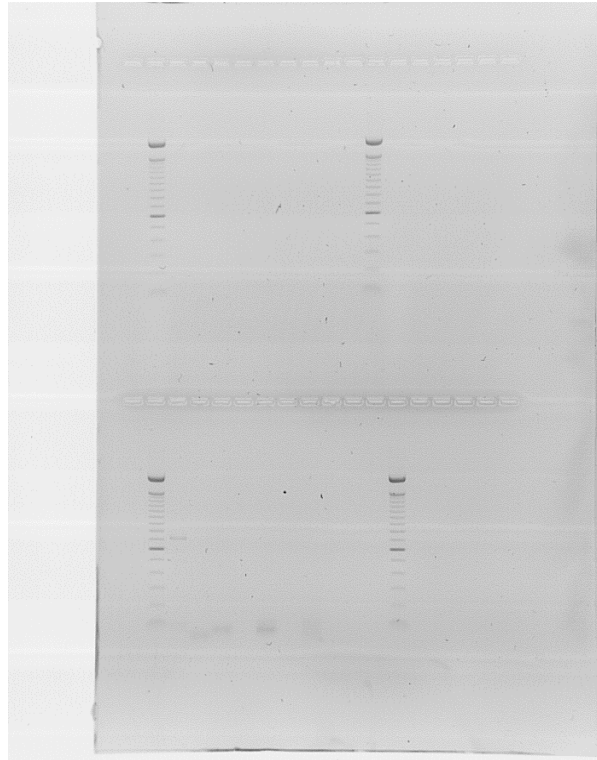


Figure 3.27 DNA band recovered from Trinidadian RPM sample.

3.5 Discussion

Hypothesis 1 predicted that there would be a significant difference in morphology between specimens collected from *A. catechu* and *C. nucifera* in India, as previous molecular studies have shown that there were genetic differences between *R. indica* specimens collected from these two host species in India (Dowling et al., 2012). It was not clear whether molecular differences indicated the presence of RPM host races or whether there may be geographical races. The results here showed that morphologically, there were differences observed between the lengths of setae c1, v2 and f3 on specimens collected from *C. nucifera* and *A. catechu* in India, however these setae were also shown to differ significantly between specimens collected from different sites- even when host plant was

controlled for; therefore, it is unlikely that these differences are indicative of host associated morphological differences between specimens collected from *A. catechu* and *C. nucifera* within this region in Kerala. Lengths of e2, c1, c2, v2 and f3 setae were also significantly longer on specimens collected from *P. roebelenii*, compared to those collected from *C. nucifera* and *A. catechu* in the same area, and differences between sites were likely due to the same influential specimens. The results showed that setae on individuals sampled from *P. roebelenii* were significantly longer than specimens from *C. nucifera* sampled from the same site on the same occasion indicating that there is either a host induced difference or perhaps a nutrient induced difference. The *P. roebelenii* plants were grown in pots on this site and not in the same earth as the coconut palms. These setae are found dorso-centrally (c1 and v2), dorso-sublaterally (e2 and c2) and laterally (f3). The PCA showed that these setae had a strong positive loading for PC1 which accounted for 41.4% of the variation. When a LDA was applied to populations from different hosts, there was no evidence to suggest that RPM could be separated using host or site and this was supported using the hierarchical cluster analysis and a tree diagram. Conclusions from the morphological work show that there is some variability between RPM collected from different sites and hosts in India, however there is no evidence that these differences were consistent with host therefore it is unlikely that there are different morphological host races. Thus, there was no strong supporting evidence to indicate that within Kerala there were two morphologically distinct biotypes of RPM from *A. catechu* and *C. nucifera* as could be hypothesised from the molecular data presented by Dowling et al. (2012). It is likely that differences observed between samples were due to extrinsic factors such as nutrition, especially given the differences observed between specimens collected from the same site on *C. nucifera* and *P. roebelenii*. It is possible that the differences observed by Dowling et al. (2012) may not manifest in the form of differences in setae length, thus it was disappointing that no useable amounts of DNA were extracted from the specimens to confirm the molecular status of the samples (reasons for this are discussed later). Setae length measurements have been used successfully to find differences in populations of mites between hosts previously (Navia et al., 2009; Skoracka et al., 2002) and differentiation between *Raoiella* species has traditionally been based largely on number, length and shape of setae (Beard et al., 2012). Further study of setae on RPM legs may reveal further differences.

Prior to this study, it was hypothesised that there would be no morphological or molecular differences between RPM found within the Caribbean collected from different host plants/sites. Again, the length of seta c2 was significantly different between hosts *Musa* spp. and *C. nucifera* along with seta f2. There were significant differences between sites also for setae d1, d2, e2, c1, c2, f2 and v2. As with India, there seemed to be significant variability in the lengths of both dorso-central and dorso-sublateral setae both between hosts and locations. It may be hypothesised that the length of dorso-

central and dorso-sublateral setae may be influenced by the nutritional conditions of the plant or other extrinsic factors. This may some way to explain the differences between populations collected from different sites and hosts within the region. Vásquez et al. (2014) observed some significant differences in the lengths of dorsal setae from RPM collected from different populations on *C. nucifera* in the New World. Their results showed that the lengths of sc2, c1, c2, d2 were significantly longer on RPM from some sites compared to others, but they did not measure lengths of lateral setae (c3, d3 and e3). In the present study, results from the PCA for within region analysis showed that 60.3% and 60.7% of variation was explained by PC1 and 2 for India and Caribbean results respectively when all measurements were considered. These were largely similar to those reported by Vásquez et al. (2014) who reported 65.8% variation between RPM collected from different host plants/sites.

Setae which were never significantly different between sites or hosts within country were the lateral setae c3, d3 and e3. These setae differed significantly between the specimens collected from the Caribbean and India but did not differ significantly between sites or between hosts within each region, therefore disproving the hypothesis that RPM specimens from populations in India ex *Areca catechu* and the Caribbean would be morphologically identical. Male specimens also had significantly longer c3 setae in the Caribbean compared to India.

It is unlikely that these differences were due to density of colonies between India and the Caribbean, as the baseline study which investigated the effect that crowding may have on the length of setae showed that there was no relationship between the length of setae and the degree of crowding of the colony from which the RPM specimen was collected. There was evidence however that the body length of RPM may differ depending on how crowded a colony was, even when statistical outliers were removed from the analysis.

If the molecular results from Dowling et al. (2012) are compared to these results, it may be that the population sampled in Kerala is consistent with those collected from *C. nucifera* in their study as they differ to those collected in the Caribbean. The results here indicate that rather than differences being host related, there may be evidence geographical subsets of RPM populations as the observed differences were consistent across all specimens from Kerala regardless of host plant. Further molecular work would be required to confirm this and the collection and study of RPM from a broader geographical range would confirm how widespread these differences are. The analysis in the Caribbean between 2009, 2012 and 2014 samples showed no evidence of change in setae length over time, therefore this trait may be considered heritable and is not likely to be affected by time of year, nutrition or other extrinsic factors.

The significant difference in length of the lateral setae enabled an almost complete partitioning of populations from India and the Caribbean using a hierarchical cluster analysis based on setae c3, d3 and e3 with only three samples from the Caribbean categorised with samples from India and eight samples from India categorised with those from the Caribbean. The PCA also showed that populations could be separated on setae length alone with some minor cross over. This low level of cross over showed that there is plasticity within each population to express shorter or longer lateral setae, however overwhelmingly, populations from India display the phenotype which has shorter lateral setae and populations in the adventive range have longer lateral setae. It could be hypothesised that the population which invaded the Caribbean was a small subset of a larger population which had more diverse lateral setae length. The population introduced to the Caribbean may have been a small population with long lateral setae and as it appears that this is a stable heritable trait, longer lateral setae have remained a consistent attribute of the invasive population. This would suggest that the RPM in the Caribbean were derived from a single introduction. Examples of genetic bottlenecks are common in invasion ecology (Tsutsui et al., 2000) and often lead to a reduction in diversity of particular traits. When the standard deviations for the lateral setae length in both India and the Caribbean were compared, there was little difference. If India were the area of origin for RPM, it may be hypothesised that the standard deviations and variability of setae lengths would be greater than those found in the Caribbean. The lack of diversity of lateral seta lengths in India suggest that populations in India may also be a subset population derived from a more diverse population, indicating that Kerala may not be the area of origin of RPM. This would be consistent with views of Dowling et al. (2012) who hypothesised the area of origin could be in the Middle East. Only wider geographical analysis of RPM populations would confirm this.

An alternative hypothesis for difference in setae lengths between regions may be as a result of adaptation post invasion caused by a change in temperature, photoperiod, exposure to competitors or predators (Lee, 2002). Release from predation pressure from one or more predators from a complex of co-evolved natural enemies found in the naturalised range may lead to different selection pressures faced by RPM in populations in the adventive range and thus differences in observed morphologies. The setae protrude laterally from the mite and could be hypothesised to be associated with defence- either for feeding area of an individual or defence against approach of predators, or may have an association with dispersal. It is likely that the setae which differ between regions on both males and females, are mechanoreceptors based on their shape and position (Evans, 1992) therefore they may act to either cue RPM to move when touched either by a predator or another RPM individual. The biological implication for mites with longer setae could be that it increases the fitness of the mite in some way i.e. better defence against predators, dispersal etc. Evolution of increased competitive

ability is a hypothesis that states that an invasive species in an environment with lack of predation pressure reallocates resources away from defence against predation to increase its competitive ability making it a better competitor in the adventive range. Using invasive plants as an example, the plant shifts resource allocation to increase biomass (Blossey & Notzold, 1995). Evolution of Increased Competitive Ability for a mite for example may include an increase in fecundity, ability to exploit food resource or defence against predation. Red Palm Mite has been reported to exploit a vastly greater number of hosts in the New World than the Old World and there is evidence that population densities are higher in the invasive range, therefore there is evidence to suggest there may be some increase in competitive ability, or RPM may be responding positively to a 'niche opportunity' created by an abundance of host resources or lack of predation pressure (Shea & Chesson, 2002). Whether these are related to advantages conferred by seta length remains to be confirmed. Comparative densities and parallel surveys are lacking, so further studies are required to confirm the density increase between the Caribbean and India and to investigate whether enemy release is a possibility in the invasive range and to what extent the role of these lateral setae may play in defence against predators.

Molecular evidence to date suggests that there are possibly two different haplotypes of RPM found in India. The population in the Caribbean was shown to be identical to those from *Areca catechu* in India, however there is a possibility that the population studied in Kerala could differ from the mites analysed by Dowling et al. (2012), as Bangalore where the specimens were collected is several hundred kilometres north-east of the collection sites in this study. Unfortunately, during this study, no DNA was extracted from the samples from India, and in the case where DNA was extracted, it was not in sufficient quantities to allow amplification and follow on sequencing. This may have been due to the time lag between collection and processing as samples were sent to the UK from India after arranging a material transfer agreement. Additionally, initial assays only used one RPM per extraction, and subsequent literature published for RPM DNA extraction highlighted the requirement for multiple RPM specimens and adjusted reaction conditions. Further work should pursue the molecular aspects of this study to see whether the morphological variations observed in this study have a molecular basis. Unfortunately, this was not possible during this PhD due to an expired MTA and Memorandum Of Understanding (MOU) with KFRI which meant further work in India was not possible following from 2012 onwards. In addition, studies should be undertaken to investigate the host plant relations of the population studied in Kerala. The morphological differences observed between the populations in India and Trinidad could be indicative of different races of RPM which may have different host plant associations.

4.0 Host plant relations in the Old World

4.1 Introduction

Since its introduction into the Caribbean, RPM has been reported on many different plant species, bringing the total number of reported host plants from both regions to 91, with the majority comprising of members of the Arecaceae family (72), and the remaining comprising of members of the Heliconiaceae (5), Musaceae (6), Strelitziaceae (2), Zingiberaceae (4) and Pandanceae (2) (Carrillo et al., 2012a). The extent to which these hosts are suitable for RPM to feed and reproduce has not been fully established for all species, however the number of host reports from the adventive range differ substantially from those reported in the Old World and it is therefore important to understand whether these differences in host reports are due to a lack of reporting from the Old World or whether other factors may be contributing to observed differences.

Since the first record of RPM in Coimbatore, India, the mite has been widely reported as a minor seasonal pest of *Areca catechu* (arecanut) and *Cocos nucifera* (coconut) throughout the southern states of India including Karnataka, Tamil Nadu and Kerala (Loganathan et al., 2000; Sathiamma, 1996; Senapati & Biswas, 1990; Yadavbabu & Manjunatha, 2007). Red Palm Mite populations build up in the hot dry months between December and April, peaking in April, and return to a low level with the onset of monsoon rains (Taylor et al., 2012); prior to 2009, there were no known published reports of RPM on *Musa* spp. or alternative host plants in the local area. Surveys carried out in 2009 found little evidence of breeding colonies of RPM on *Musa* spp. in Kerala, India (Taylor et al., 2011): only one colony (consisting of four cast skins, four eggs and three post embryonic stages) was found on *Musa* spp. out of 56 plants from 17 plots surveyed in an area spanning 10-20 kilometres. Since the reports on *Musa* spp. in the New World in 2005, RPM have reported colonies on *Musa* spp. in Oman (Hountondji et al., 2010), and in India (Shailesh et al., 2014) but these remain the only published reports in the Old World. A summary of *Musa* spp. and RPM incidence in the Old World and in the adventive range are given in Table 4.1.

In contrast, Kane et al. (2005) first reported the spread of RPM onto *Musa* spp. in the New World, naming *Musa acuminata* (Colla), *M. balbisiana* (Colla), *Musa uranoscopus* and *Musa x paradisiaca* as hosts, and reported for the first time high population levels of RPM on commercial banana plantations in Dominica. Subsequently, *Musa* spp. infested with multigenerational colonies were reported in the eastern Caribbean on commercially grown varieties Dwarf Cavendish, Giant Cavendish, Robusta, and Williams and of plantains Apem, Cents Livre, Ordinary, Dwarf French, and

Horn (Cocco & Hoy, 2009). Ramos-Lima et al. (2011) studied populations of RPM on coconut and *Musa* spp. in Cuba and found that the period of egg to adult on coconut and *Musa* spp. was 31.4 ± 3.31 and 33.4 ± 4.76 days respectively, indicating that RPM was breeding successfully on *Musa* spp., although the authors found that female oviposition period, longevity and total oviposition was longer/higher on coconut when compared to *Musa* spp.. Further studies have demonstrated the ability of RPM to establish and reproduce on *Musa* spp. throughout the adventive range (Balza et al., 2015; Otero-Colina et al., 2016; Rodrigues & Irish, 2012; Rodrigues & Peña, 2012). In contrast to field observations, Cocco & Hoy (2009) conducted survival analyses of the introduced pest on various banana cultivars using detached leaf disc arenas in Florida, USA and found that RPM females could not establish on Dwarf Cavendish, Dwarf Nino, Gran Nain, Dwarf Zan Moreno, Dwarf Green, Truly Tiny, *Musa sumatrana* x Gran Nain, Dwarf Puerto Rican, Rose, Nang Phaya, Misi Luki, Manzano, Lady Finger, Glui Kai, and Eburn Musak indicating there may be an effect of cultivar on the ability of RPM to survive and reproduce on some of the reported hosts.

Table 4.1 Cultivars of *Musa* spp. which have had populations of RPM reported on them (New World) or have been surveyed for RPM (India).

<i>Musa</i> spp. cultivar	Ploidy	Country	RPM infestation?
Dwarf Cavendish	AAA	East Caribbean	Successive generations (Cocco & Hoy, 2009)
Giant Cavendish	AAA	East Caribbean	Successive generations (Cocco & Hoy, 2009)
Robusta	AAA	East Caribbean	Successive generations (Cocco & Hoy, 2009)
Williams	AAA	East Caribbean	Successive generations (Cocco & Hoy, 2009)
Apem	AAB	East Caribbean	Successive generations (Cocco & Hoy, 2009)
Cents livre	AAB	East Caribbean	Successive generations (Cocco & Hoy, 2009)
Horn	AAB	East Caribbean	Successive generations (Cocco & Hoy, 2009)
Ordinary	AAB	East Caribbean	Successive generations (Cocco & Hoy, 2009)
Dwarf French	AAB	East Caribbean	Successive generations (Cocco & Hoy, 2009)
Cavendish subgroup	AAA	Cuba	Successive generations (Ramos-Lima et al., 2011)
Bluggoe	ABB	Mexico	Moderate infestation (Otero-Colina et al., 2016)
Silk or Manzano	AAB	Mexico	Moderate infestation (Otero-Colina et al., 2016)
Poovan	AAB	Kerala, India	No generations found (Taylor et al., 2011)
Palayam kodan	AAB	Kerala, India	1 colony found after extensive survey (Taylor et al., 2011)
Nendran	AAB	Kerala, India	No generations found (Taylor et al., 2011)
<i>Musa</i> spp. no cultivar given	?	Salalah, Oman	No details given. No major damage observed (Hountondji et al., 2010)
Grand Naine	AAA	South Gujarat, India	Present on 1-9% of plants between October 2007-April 2008 (Shailesh et al., 2014) (Recorded as <i>Raoilia indica</i>). No information on numbers/density

In total 72 species of Arecaeae have now been reported as hosts of RPM (Carrillo et al., 2012a; Kane et al., 2005; Welbourn, 2006; Étienne & Flechtmann, 2006) compared to the five species reported prior to the introduction of RPM to the adventive range. A study by Carrillo et al. (2012a) in Florida, found established colonies of RPM on 31/33 palm species inspected in a botanical garden, of which only one species (*C. nucifera*) had been reported previously as a host in the Old World prior to the invasion. The study highlighted that around 70% of host plants reported associated with RPM were native to the Old World however, around 30% were native to the neo-tropics suggesting they were new associations. The field survey confirmed there has been at least a 30% increase in reported host associations since the introduction of RPM to the adventive range, however the high number of new associations reported which are native to the Old World suggests that these should also be reported as host plants in this region also thus warranting further study.

With erroneous hosts excluded, there are still large differences in the observed host range of RPM in the Old World and New World and this raises several questions. Is the relatively narrow host range in the Old World, a reflection of a lack of field survey data on alternative hosts and thus these hosts are under-reported? Or, is there a biological reason for this apparent difference in host use such as a difference in susceptibility of cultivars grown? Or are RPM in India to some degree host specific, favouring only plants from the order Arecales (palms) in India as opposed to both Arecales and Zingiberales (bananas and heliconias) in the adventive range?

Under the scenario where the restricted host range in the Old World is due to a lack of published observations on alternative hosts, surveys in the naturalised range would in theory reveal unreported host associations similar to those in the adventive range from both Arecales and Zingiberales, given similar species and cultivars. This is a possibility given recent reports of RPM on *Musa* spp. in other parts of the Old World. It is possible that colonies of RPM exist on alternative hosts however, they may be found at lower population densities due to exogenous factors such as cultivar differences in plants such as *Musa* spp. grown between the two regions (thus leading to lower survival / establishment), the action of natural enemies or it may be that RPM reside on uneconomically important plants. Cocco & Hoy (2009) demonstrated differential survival of RPM on different cultivars of *Musa* spp. and palm species using leaf disc survival assays in the laboratory; thus, field surveys should be tested alongside the performance of RPM on different hosts in the laboratory to establish any host level effects on RPM establishment. With regards to natural enemies, Bernays & Graham (1998) argued that in addition to traditional chemical co-evolution of the host-arthropod relationships, predation by generalist natural enemies may exert a significant selection pressure on an arthropod, thus restricting its realised host range. The concepts of the enemy release hypothesis put

forward by Keane & Crawley (2002) for invasive plants, could explain a widening of host range if generalist predator fauna was less diverse or effective in the adventive range whereby the increase in RPM populations on primary hosts could lead to an overspill of RPM onto less favourable secondary hosts. To separate out the effect of host plant and exogenous factors such as the effects of natural enemies, the study approach firstly needs to establish presence/absence of RPM colonies in the field on alternative hosts (observed field range), then establish to what extent the plant host-RPM relationship is responsible for this i.e. are hosts unsuitable for RPM establishment? The first part of this chapter will assess both the observed field host range of RPM and biological host range of RPM in laboratory studies. The effects of exogenous factors such as natural enemies are explored in more detail later in this thesis (chapter 5).

Under the scenario where RPM are not found on alternative hosts during surveys, the host-RPM relationship within India requires further investigation. Magalhães et al. (2007) proposed that phytophagous mites may be more prone to host race formation due to a low propensity to disperse from the natal site, a low dispersal ability in general, a strong association with the host, host associated mating and a high reproductive capability- all of which apply to RPM given current knowledge and would thus support the hypothesis that host races of RPM may be present in the field. Drès & Mallet (2002) outlined the empirically testable criteria for existence of host races within a species. They stated that if host races were in existence there would be use of different host taxa in the wild; individuals would exhibit 'host fidelity'; host races would co-exist in the same environment i.e. on Arecales and Zingiberales; they would be genetically differentiated at multiple loci and this would be replicable on a spatial and temporal scale; there would a correlation between host choice and mate choice; evidence of restricted gene flow between races; host races would have a higher fitness on their natal host compared to an alternative host and hybrids would less fit than parents. Magalhães et al. (2007) outlined approaches for testing host race existence such as reciprocal transplants from original host to alternative host and testing fitness parameters. Local adaptation was reviewed by Kawecki & Ebert (2004) and they highlighted different methods to test for this including a range of indicators such as juvenile survival, fecundity, age at first reproduction.

To test the concepts discussed above, two hypotheses are suggested:

1. The host range is wider than reported for RPM in the Old World. Colonies of RPM will be found on other palms/host plants; however, their associations have not been reported to date in the literature (e.g. because they are not economically important i.e. indigenous palm

species, RPM are found in low densities, numbers are kept in check by natural enemies).

2. RPM have host specific races. The race found in Kerala is specific to the order Arecales (palms) and will not feed and reproduce plants from the order Zingiberales (*Musa* spp. and *Heliconia* spp.) or Pandanales (as previously reported in literature).

Through testing these hypotheses, outcomes of studies and experiments will provide more information about why there is a low level of alternative host plant species reported for RPM in the Old World and give insights into the possible mechanisms responsible for field observations. Hypotheses were tested through a combination of field and laboratory work undertaken in Kerala, India where RPM has been long established.

4.2 Methods

4.2.1 Hypothesis 1.

Field surveys were undertaken on a broad range of Arecales (palms) and Zingiberales (*Heliconia* spp. and *Musa* spp.) species in the study area. Surveys included a general survey of plants from the orders Arecales and Zingiberales in the study region in Kerala, followed by more targeted surveys assessing the presence/absence of RPM on *Musa* spp.. Each survey was complemented with a laboratory assay assessing RPM survival and fecundity analyses using RPM collected from *C. nucifera* palms at field sites. Data from the survival analyses were inputted into a model to demonstrate the implications of results from the survival analyses.

Host plant survey in wider area

To assess the presence or absence of RPM on Arecales and Zingiberales, surveys were carried out on field plots at smallholder premises around the towns of Vadakkenchery, Vandazhi and Mudapullur where there are many home gardens and plantations and the Banana Research Station, Kannara, Kerala (12th March 2010). This area was chosen as Taylor et al. (2012) demonstrated that RPM populations were most abundant and dense in this area compared to three other areas studied in Kerala. Ten plots were selected that had alternative host plant species present. *Cocos nucifera*, *A. catechu* and any potential alternative hosts were examined for the presence/absence of RPM individuals / colonies. If individuals were found, they were collected in 80% alcohol along with individuals collected from nearby *C. nucifera* or *A. catechu* palms.

Laboratory survival and fecundity assays on alternative host species

Survival analyses and an assessment of numbers of eggs laid were conducted in line with methodologies published by Cocco & Hoy (2009) which assessed survival over a seven day period on leaf discs. This experiment commenced on 6th April 2011. The leaf disc sections were taken from palms and bananas found in the survey area. Seven host plant species were chosen as they represented species which were frequently grown in the study area and represent potential hosts for RPM given the host range reported in the adventive range. Species chosen were *Areca catechu*, *Borassus flabellifer*, *Cocos nucifera*, *Heliconia* spp., *Musa* sp. (cv Palayam kodan), *Pandanus* sp., and *Cyrtostachys renda* (Red Palm). *Cocos nucifera* and *A. catechu* were used as control plants as these were already reported hosts in the region. *Borassus flabellifer* is a native palm grown frequently throughout the study area and *C. renda* was chosen as this was grown as an ornamental on several different study sites. Other palm species were not grown in sufficient numbers in the area to provide replication required for the experiment. *Musa* sp. cv Palayam kodan and *Heliconia* spp. were chosen to represent members of the Zingiberales. *Pandanus* sp. was chosen as this was frequently grown throughout the study area but was suspected to be an erroneous host given that it does not belong to Arecales or Zingiberales. All plants were found in the Vadakkenchery area of Kerala. In total one leaf was collected from five separate plants of each species grown on geographically separate plots (apart from *Heliconia* spp.) giving an n of five for each host plant. Seven *Heliconia* plants, comprised of three cultivars/species which were: *Heliconia rostrata* (labelled *Heliconia* sp. 1 in text) (one plant) *Heliconia psittacorum* (labelled *Heliconia* sp. 2 in text) (four plants) and *Heliconia stricta* (labelled *Heliconia* sp. 3 in text) (two plants). Species selection was guided by availability of replicated plants from different plots in the area surveyed. From each of the plants the end section of a frond/ or whole leaf (*Heliconia/Musa* spp.) was removed from the plant. Removed sections were wrapped in newspaper and kept in the back of an air-conditioned vehicle and returned to the laboratory.

In the laboratory, 90mm glass Petri dishes were prepared by lining with cotton wool pads (Boots, UK) and replacing lids (to keep cotton moist prior to experiment). Approximately 20ml of tap water was added to the cotton wool to dampen it and plates were labelled clearly on the underside with species ID and replicate number. Dishes were numbered, and a randomisation was prepared using a random number sequence prepared using R (R, 2016). Leaf sections collected from the field were prepared firstly by wiping with tap water to clean them and ensure no other insects or mites were present; then using a scalpel, sections were taken from each leaf. For palms, sections were 4 x 4cm used (where the width of the leaflets allowed), a small incision was made along the mid rib to keep leaf sections flat. *Musa* sp. and *Heliconia* spp. sections were 4 x 4 cm and taken approximately 1-2 cm from the mid rib unless there was damage to the leaf section, whereby the section was taken as close to this area as

possible. The leaf disc was put straight on to the soaked cotton wool, abaxial side facing upwards and the lid replaced on the Petri dish. Edges were sealed with thin strips of tissue paper to ensure the leaf section remained hydrated and to contain RPM. In total, five separate leaf sections were prepared per species of plant (from separate leaves/plants). Petri dishes were kept on the laboratory bench (due to lack of a controlled temperature room facility).

Red Palm Mite for the survival analyses were collected from two heavily infested *C. nucifera* palms (local variety 'red coconut') (GPS: N 10° 35' 32.3" E 76° 31' 02.3"). Adult females were collected from approximately 10-15 coconut leaflets and transferred into two petri dishes prepared with coconut leaf discs to act as a seed colony for experiments. Petri dishes for the experiment were laid out in a grid pattern in a fully randomised order and females were added one by one to each dish in order of randomisation. After one RPM had been added to each dish, a further two were added in order of randomisation (between dishes), and finally a further two were added in the same manner to give five RPM per dish. This method was used to remove selection bias from RPM allocation. Plates were checked underneath a stereo microscope to ensure all plates had adult females only on them and that none had died/been damaged during transfer.

Survival of the mites was assessed daily using a stereo microscope. Dead mites were removed from the experiment; mites on the tissue paper or at extremities were moved back onto the leaf section to avoid drowning when tissue paper was moistened daily. If mites were missing these were noted and a note was also made of the number that had 'run off' and found on the tissue not the leaf section. The number of RPM individuals with droplets on setae were also noted on day 2-6 as it is possible that these may act as an indicator of fitness (this is discussed and analysed in chapter 6). The temperature and humidity range throughout the experiment was monitored and conditions were between 29.8-30.6°C 63-67% RH. In total, 37 petri dishes were prepared.

Field survey of bananas of different ploidies

Through the host plant survey in the wider area, it was noted that the majority of cultivars of *Musa* spp. grown by smallholders in the study area were varieties such as Palayam kodan, Poovan and Nendran, thus cultivars were not comparable with those reported in the adventive range. To find cultivars which were comparable to those in the adventive range, surveys were carried out at the Banana Research Station, Kannara, Kerala which grows 212 different cultivars of *Musa* spp. (12th March 2010).

Many of the varieties grown on this site had been treated recently with chemical pesticides therefore were unsuitable for survey, however, four abandoned or non-chemically treated plots were chosen with the aim to compare locally grown cultivars with those reported as hosts in the Caribbean. Two AAB varieties widely grown in Kerala; Nendran (equivalent to plantain), and Poovan (local equivalent of 'Mysore' variety in Caribbean), and two AAA varieties 'Red' [Red subgroup, local name Chenkadali; (Simmonds & Stover, 1987)] and 'Robusta' [Giant Cavendish group; (Simmonds & Stover, 1987)] were selected (Figure 4.1). *Musa* sp. cultivars Nendran and Poovan are grown widely throughout Kerala in homesteads and small plantations, and Robusta is reported to have multigenerational host in the Caribbean (see Table 4.1). Red was chosen as this is an AAA cultivar, not grown widely in this region. Very few Robusta or Red cultivars are grown in this part of India, although, there is an area in South Kerala known to grow the Red Cultivar and a region about 400km north east of Mumbai which grows Dwarf Cavendish (pers. comm., Dr Suma, Banana Research Institute, Kannara). Bananas grown at the banana research station generally follow the same growing regime. Suckers from mother plants are used to stock the gene bank grown at the banana research station. Suckers of each plant are dipped in Chlorpyrifos before planting. On planting, soil was treated with Carbofuran as granules which are active as a systemic for 50 days. Plants were allowed to grow with no additional chemical pesticide treatments added until they are 3 months old. Plants chosen for this study had no additional pesticide treatment since planting. After two months, fertilizer is added to each plant plot (100g N, 75g P₂O₅, 150g K₂O). The plots are generally irrigated twice weekly using 40 litres of water per plant, unless otherwise stated. In the case of the Red banana plots and the Nendran plots, these had no pesticides applied to them and were irrigated twice a week. The Red banana plot was comprised of individuals approximately 6 months old and the Nendran plot was approximately 4-5months old. Because of the regular irrigation, these plots were paired. The Robusta and Poovan plots were categorised as abandoned plots. Robusta plots were 4-5 months old and had had not additional fertilizer or pesticides applied since planting and no regular irrigation. Poovan plots were also abandoned with no additional pesticides, fertilizer or irrigation. Robusta and Poovan plants were approximately 2m in height.



Figure 4.1 The four varieties of banana chosen for the leaf disc experiment and field surveys. a) Nendran (AAB) b) Red (AAA) c) Poovan (AAB) d) Robusta (AAA).

Two lower leaves from six plants in each plot were examined for the presence of RPM, along with at least three leaflets from two fronds from adjacent *C. nucifera* or *A. catechu* plants which often fringed the plots. Red Palm Mite colonies/individuals were noted and collected in 80% alcohol and identified by mounting on a slide using Heinze media. Leaf samples were also taken to conduct survival and fecundity analyses in a laboratory assay.

Local *Musa* spp. cultivars survival and fecundity analysis

Four cultivars of *Musa* spp. (Nendran (AAB), Poovan (AAB), Red (AAA) and Robusta (AAA)) grown on the plots described previously at the BRS were selected for a survival and fecundity analysis comparing RPM on local *Musa* spp. cultivars (experiment commenced 12th March 2010) *C. nucifera* plants from the BRS and one from Kerala Forest Research Station (KFRI), Peechi (due to low plant numbers on the BRS site) were used as controls. Leaf number was determined from the growing point at the top of the plant. Leaf 1 was defined as the newly formed leaf at the top of the plant, and then successive leaves were counted from the top of the plant. Red and Nendran cultivars were faster growing and slightly older so leaf 7 was selected for sampling. On Robusta and Poovan,

leaf 4 was selected as they were smaller (roughly 2m in height). A section roughly 25-35cm long was removed from the middle section of the leaf and the depth was determined by the length from the distal edge to the midrib which varied amongst cultivars. Leaf sections were kept cool and transported back to the laboratory for leaf disc preparation. Coconut leaflets were also collected from coconut palms surrounding the banana growing areas so control leaflets were grown in the same soil. Seventy-two, 90mm glass Petri dishes were prepared as described previously for survival analyses. Petri dishes were kept on the laboratory bench (due to lack of controlled temperature room facility). Leaf discs were taken from five different banana plants of the same cultivar and from each of the leaves; three sub-sections were cut using a scalpel (Figure 4.2). In total, 60 banana leaf sections were tested. For the coconut control, leaflets from four different trees were used and three subsections of these were tested to give n=12 leaves. Coconut controls were set up with the same dimensions as the banana leaf discs; this included the leaflet mid-rib along the centre, or where the leaflet could not be flattened the midrib was cut to flatten the leaflet. The temperature range throughout the experiment was maximum 31.3°C, minimum 29.5°C; and the humidity range was maximum 61.4%, minimum 55.0%. For this assay, a band of Vaseline was applied to the extremities of the disc to prevent the mites from walking off the disc and drowning however, this was not used for other survival assays as it was suspected this may have affected the results. Preliminary observations showed that mites turned around when they detected Vaseline.

RPM were collected from the Vadakkencherry area of Kerala from multiple coconut palms and leaflets. Leaflets were collected and stored separately in cotton bags and returned to the laboratory where they were stored in an air-conditioned environment overnight. Leaflets were inspected under a stereo microscope and adult females and deutonymphs were removed onto a separate dish with a coconut leaf disc. If females were part of a mating pair the male was also transferred over. Females were then transferred over to the experimental leaf discs in the order of the randomisation from left to right as described previously. Five females per leaf disc were transferred using this methodology, however, on inspection after day one, some males were misidentified as females although at least three females were transferred per leaf disc. To correct for this when assessing number of eggs laid; the number of males per dish were noted and an adjustment factor was applied to the final counted egg number.



Figure 4.2 a) Leaf disc set up on cotton wool with tissue paper around the edge and Vaseline border. b) Example of the leaf section taken from banana plants and returned to the lab. c) RPM counted from coconut leaflets onto Petri dish with coconut leaf disc.

Assessment of presence of RPM on *Musa* spp. in close proximity to *Cocos nucifera* (mixed plot survey)

To further investigate the occurrence of RPM on *Musa* spp., a study was conducted to assess the presence/absence of RPM when grown in close proximity to RPM infested *C. nucifera* plants. The aim of the study was to quantify accurately the presence/absence of RPM on both *Musa* sp. and two varieties of coconut when grown in close proximity to each other. The plot for this survey (GPS: N 10°35'37.50", E 76°30'13.10") was chosen because it consisted of nine *C. nucifera* (cv 'red') seedlings (up to 3-4 m in height), 13 *C. nucifera* (cv 'green') seedlings and 19 *Musa* sp. plants cv Palayam kodan (AAB). The plot had been surveyed previously and was known to harbour annual populations of RPM on *C. nucifera* plants (Taylor et al., 2012). The plants on the plot were mapped spatially to plot host plant distribution/proximity and assess RPM distribution in the plot. The numbers of RPM mobile life stages were counted on five leaflets, on two fronds of each palm, and on two whole leaves of the *Musa* sp. plants using a hand lens (x12.5). Leaflets were not removed as this plot was part of an on-going study area. The mean number of RPM per coconut leaflet or banana leaf was calculated and plotted on a spatial graph which plotted location of plants. Representative samples of RPM were stored in 80% alcohol for identification. The survey was conducted in March 2010.

Laboratory study on live plants

Preliminary experiments were set up using *Areca catechu* seedlings and *Musa* spp. cv Dwarf Cavendish and Robusta plants grown from tissue culture (all purchased from the BRS) (commenced 17th March 2010). Other cultivars were considered however they were unavailable at the time of running the experiment (no *C. nucifera* seedling were available). Plants were selected which were at the 4-6 leaf stage of development and six plants from each cultivar/ species were used. No pesticides were applied to the seedlings. *Areca catechu* seedlings were selected at the four-leaf stage. Female RPM were removed from heavily infested *A. catechu* leaves collected from BRS (16th March 2010)

and were temporarily stored on a leaf disc culture until they were transferred onto the host plants for the experiment. Plants were randomised and placed in two rows of nine and leaves were cleaned with distilled water and checked for the presence of other organisms (i.e. predators) (Figure 4.3). Care was taken to ensure leaves between plants did not touch. At the base of the second youngest leaf from each of the plants, Vaseline applied in a band to prevent mites escaping from the leaves or from predators moving onto the leaf. Ten female RPM were transferred to one leaf per plant and observations were made to assess mite number over time.



Figure 4.3 Set up of lab study on live plants. Plants used were *Areca catechu*, *Musa* sp. cv Robusta and cv Dwarf Cavendish.

A simple demonstration of the implications of survival and fecundity results

To illustrate the implications of results from the survival and fecundity analyses in terms of how they would relate to observations in the field in Kerala, a simplistic model was constructed using the mean data gathered from the palms survival assay. This illustration aimed to demonstrate how RPM would perform on different host plants given the results from the survival and fecundity analyses. The model was run to cover population growth over six months as this reflected the length of the RPM season in Kerala (December-May).

The model used data from the laboratory survival and fecundity assays on alternative host species, and combined them with data taken from the literature to calculate the net reproductive rate (R_0) given no extrinsic mortality due to predation. The model did not account for nymphal mortality during development (which was not measured during experiments) and assumed a constant temperature. Net reproduction rate (R_0) was calculated as $l_x m_x$, where l_x was the probability at birth of being alive at age x (here we used the mean survivorship taken from the palms leaf disc experiment) and m_x was the mean number of female offspring produced per unit time. To calculate l_x , the mean survivorship of the cohort over 7 days was used for each of the plant species. To calculate m_x , mean fecundity per female per day data was multiplied by the mean sex ratio of RPM taken from a field study conducted in 2010

for which the multiplier was 0.74 (data unpublished, not shown in this thesis; mean of sex ratios taken between December 2009 and May 2010). The net reproductive rate (R_0) for the generation was calculated by multiplying by the mean number of egg laying days given by Moutia (1958) for RPM in Mauritius which was 27 days. The generation time T (time from egg-adult) was fixed at 22 days for all plants (Moutia, 1958) in the absence of data from experiments. All eggs were presumed to hatch and develop.

4.2.2 Hypothesis 2

To test whether there was evidence of Arecales and Zingiberales specific races in Kerala, the survival and reproduction of RPM on a natal host was compared to survival and reproduction on an alternative host (i.e. *Musa* sp.) using the technique described by Cocco & Hoy (2009). This experiment commenced 5th March 2012. Leaflets and leaves of *Cocos nucifera*, *Areca catechu* and *Musa* sp. cv Palayam kodan were collected from the Vadakkenchery area of Kerala, India from five separate plants on geographically separated sites, and transported back to the laboratory. Red Palm Mite were collected from infested *C. nucifera* and *A. catechu* leaflets found at N 10°35'08.5" E 76° 31' 10.1" (5th March 2012). No breeding populations of RPM were found on *Musa* spp. in the field therefore this cross could not be made. For *Musa* sp., two subsets of leaves were collected from the same plant- old (lower leaves) and new (upper leaves) from the same plant to control for leaf age.

Survival assay dishes were prepared as described previously, however 10 sections were prepared for each species (from five separate fronds/leaflets) to allow for crosses to be made. Onto five petri dishes for each plant species, five RPM females were placed which had originated from *Areca catechu* palms. Additionally, onto five separate dishes for each plant species, RPM which had originated on *Cocos nucifera* were placed. The final lists of crosses are shown in Table 4.2. Red Palm Mite were added in the order of the randomisation from left to right, top to bottom (of the grid of petri dishes). Initially one female was transferred per plate, then two females on the next round, and two in the round after that, to ensure there was no bias in selecting RPM for each plate. Five females per leaf disc were transferred using this methodology. Leaf discs were inspected daily and scored for survival of the mites and the number of eggs laid. If the mite had left the leaf disc (i.e. was on the tissue) this was noted; if it was still alive it was moved back onto the leaf disc. On occasion, not all mites could be found, in these instances they were marked as missing, if not seen again it was censored during the survivorship analysis according to the day it went missing. The experiment was run for seven days.

Table 4.2 Crosses of RPM made for hypothesis 2.

Origin of RPM	Plant species
<i>Areca catechu</i>	<i>Areca catechu</i>
<i>Areca catechu</i>	<i>Cocos nucifera</i>
<i>Areca catechu</i>	<i>Musa</i> sp. cv <i>Playam kodan</i> “older leaves”
<i>Areca catechu</i>	<i>Musa</i> sp. cv <i>Palayam kodan</i> “newer leaves”
<i>Cocos nucifera</i>	<i>Areca catechu</i>
<i>Cocos nucifera</i>	<i>Cocos nucifera</i>
<i>Cocos nucifera</i>	<i>Musa</i> sp. cv <i>Playam kodan</i> “older leaves”
<i>Cocos nucifera</i>	<i>Musa</i> sp. cv <i>Palayam kodan</i> “newer leaves”

4.2.3 Analyses

Survival analyses

Dead mites were allocated the status 1 and missing mites/live mite at the end of the experiment were allocated the status 0. Each leaf section was the lowest unit of replication for the plant, thus the mean time of death/survival for mites on each leaf subsection was calculated and used in the analysis (individual mite death on leaf sections could not be used as the five mites on a leaf section were not independent from each other). Where there were leaf subsections, the mean time at death for the three subsections was then calculated to give a mean time at death for mites on each of the banana plants. This mean was then used in statistical analysis to form the raw data unit used in the survival analysis, thus each leaf section had $n=5$. Survival analysis was then carried out using the *survreg* survival analysis in R statistical computing (R, 2016). A comparison between the model assuming a constant hazard and the model with Weibull errors showed that the model using Weibull errors was significantly better in all assays ($p < 0.001$ when tested using ANOVA). Differences in survival were considered significant where $p < 0.05$.

Mean number of eggs

For the local *Musa* spp. cultivars fecundity analysis, a linear mixed effects model was fitted to the average number of eggs laid per female per day, with the error structure defined as “Average eggs per female per day $\sim$ Cultivar + (1 | DAY) + (1 | rep)” to account for the temporal pseudo-replication and the pseudo-replication within cultivars (3 sub sections per leaf tested). The mean number of eggs laid per female per day was calculated by taking the sum of the eggs from the leaf sections or three sub-leaf sections (where subsections were used) and dividing by the number of mites on the dish. In this assay, as there were some males introduced at the beginning by mistake an adjustment factor was calculated to take into account the number of males per dish. For all other fecundity analyses, after the mean number of eggs laid per leaf was calculated, the mean of number of mites from the five different

plants from each of the different cultivars tested was calculated. Standard errors were calculated using these five mean values. The numbers of eggs were $\log(x+1)$ converted to account for the nature of the count data (strictly bound above zero). It was not possible to fit a GLM using poisson errors as the numbers of eggs for each leaf section had to be presented as a mean number of eggs per live female; therefore, the data was not in integers. Differences between numbers of eggs laid per day were calculated using an ANOVA for each day and a Tukey post hoc test was applied to show differences between treatments. Similar analyses were applied to data from all other survival assays. Differences in numbers of eggs laid were considered significant where $p < 0.05$.

Proportion of mites leaving leaf discs

The proportion of mites found on the tissue (either dead or alive) were noted during the palm and natal/non-natal survival assays, and converted to a proportion of the total dead/live that day. A vector comprised of the number of RPM on the tissue and the total number dead/live that day was formed and a generalised linear model using binomial errors was applied to these data on each day separately. Quasibinomial errors were applied to models which showed evidence of over dispersion.

Differences between leaf number/watering regime

In the local *Musa* spp. cultivars survival and fecundity analysis, plots from which leaves were collected had been managed in different ways. Two plots had been watered regularly, and two plots had not been managed to the same extent. Additionally, due to the difference in age of plants, different leaf numbers were collected. Therefore new factor levels 'Leaf 4 Abandoned' for Robusta and Poovan; and 'Leaf 7 Managed' for Red and Nendran were set up to test to see if management of the plot or leaf number selected had any significant effect on the survival of RPM. Survival analysis was then carried out on these new factor levels as stated in the 'survival analysis' section above.

Assessment of presence of RPM on *Musa* spp. in close proximity to *Cocos nucifera*

A generalized linear mixed model using maximum likelihood ['glmerMod'] was fitted to these data using Poisson errors to account for the nature of the count data. Voucher specimens of RPM were taken from coconuts and all suspected RPM specimens found on banana were stored in 80% alcohol for identification in the laboratory. These specimens were also used in chapter 3 of this thesis.

4.3 Results

4.3.1 Hypothesis 1

Host plant survey in wider area

The only additional host found with multi-generational colonies of RPM on leaflets was *Phoenix roebelenii* (Arecales:Arecaceae), a relative of the date palm *Phoenix dactylifera*. The plants surveyed were found underneath a heavily infested coconut palm (GPS: 10°35'32.30"N 76°31'2.30"E) (Table 4.3). Other palm species were found to have solitary female RPM on them and were often underneath an infested coconut palm (see Table 4.3). Observations from all surveys indicated that RPM may be found on alternative species found in close association with coconuts infested with RPM, however subsequent reproduction and colony formation was only observed on *C. nucifera*, *A. catechu* and *P. roebelenii*. From investigating the presence of RPM on alternative host plants it was noted that in the area surveyed, there were low numbers of alternative species to survey.

Table 4.3 Host plants surveyed and the presence or absence of individual RPM (f= female RPM)

Order	Family	Species	Common name	RPM	Comments
Arecales	Arecaceae	<i>Borassus flabellifer</i>	Talipot palm	0	No
	Arecaceae	<i>Borassus flabellifer</i>	Talipot palm	0	4 seedlings inspected, no RPM on nearby <i>Cocos nucifera</i>
	Arecaceae	<i>Caryota urens</i>	Fish tail palm	0	Growing wild on roadside, no <i>Cocos nucifera</i> in vicinity
	Arecaceae	<i>Cyrtostachys renda</i>	Red palm	2 f	Directly under infested <i>Cocos nucifera</i> palm
	Arecaceae	<i>Cyrtostachys renda</i>	Red palm	0	No
	Arecaceae	<i>Dypsis lutescens</i>	Areca palm	0	No
	Arecaceae	<i>Licuala grandis</i>	Fan Palm	2 f	Under heavily infested <i>Cocos nucifera</i> palm
	Arecaceae	<i>Livistona rotundifolia</i>	Footstool palm	0	No
	Arecaceae	<i>Phoenix roebelenii</i>	Dwarf date palm	Multiple colonies of RPM	Under a heavily infested <i>Cocos nucifera</i> palm
	Arecaceae	<i>Roystonea regia</i>	Royal palm	0	Infested <i>Areca catechu</i> seedling approx. 100m away
Pandanales	Pandanaceae	<i>Pandanus</i> sp.	Pandan	0	5 trees sampled in same stand. <i>Areca catechu</i> and <i>Cocos nucifera</i> within 100m were infested with RPM
Zingiberales	Heliconiaceae	<i>Heliconia</i> sp.	Unknown	0	100s RPM per leaflet found on coconut 5-10m away
	Heliconiaceae	<i>Heliconia</i> sp.	Unknown	0	Approx 100-200m away from heavily infested <i>Areca catechu</i> seedlings
	Heliconiaceae	<i>Heliconia</i> sp.	Unknown	0	Approx 100-200m away from heavily infested <i>Areca catechu</i> seedlings
	Zingiberaceae	<i>Elettaria cardamomum</i>	Cardamom	1 f	Under <i>Cocos nucifera</i> palm

Laboratory survival and fecundity assays on alternative host species

The mean survival time was highest on *C. nucifera* (5.94 days), then *Musa* sp. cv Palayam kodan and *A. catechu* (5.83 days and 5.23 days respectively) (Figure 4.4). Lowest survival time was recorded on *Heliconia* sp. 1 (3.13 days), then *Pandanus* sp. (3.75 days), *B. flabellifer* (4.16 days) and *C. renda* (4.30 days). *Heliconia* sp. 2 and 3 mean survival was 4.54 and 4.70 days. A survival analysis using Weibull errors showed survival on *Pandanus* sp. was significantly lower than on *C. nucifera* ($z = -2.31$, $p = 0.021$) however, the overall model was non-significant ($\chi^2 = 10.89$ on 8 d.f, $p = 0.21$). Survival on *B. flabellifer* and *C. renda* was marginally significantly lower than *C. nucifera* ($z = -1.73$, $p = 0.08$, $z = -1.94$, $p = 0.052$ respectively). Survival on *A. catechu*, *Heliconia* sp.1-3 and *Musa* sp. cv Palayam kodan was not significantly lower than on *C. nucifera*. Given the indications from the non-significant model, factor levels were collapsed to form two groups comprising *A. catechu*, *Heliconia* sp. 2-3, *Musa* sp. cv Palayam kodan and *C. nucifera* (group 1- higher survival) and *B. flabellifer*, *Heliconia* sp. 1, *Pandanus* sp. and *C. renda* (group 2 – lower survival); there was a significant difference in survivorship between the two groups ($\chi^2 = 8.08$, 1 d.f., $p = 0.005$; Figure 4.5).

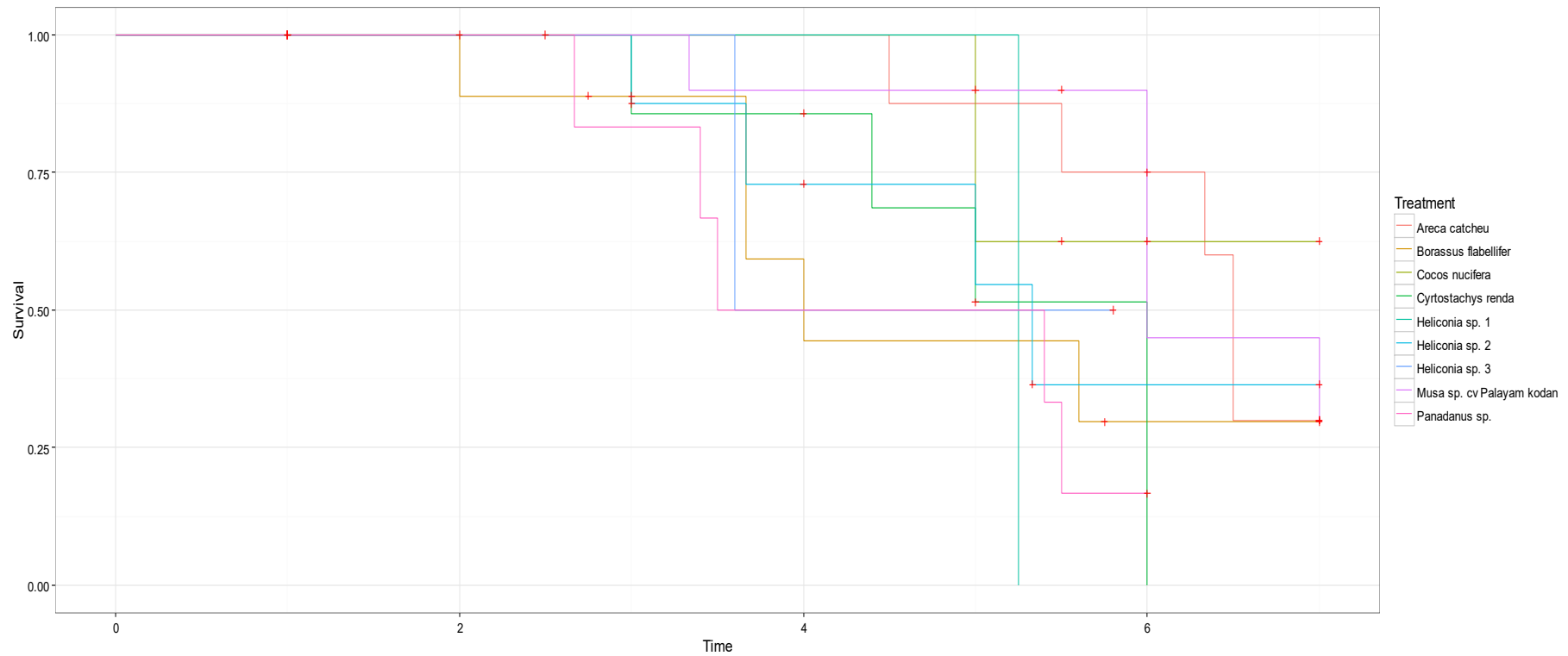


Figure 4.4 Survival analysis showing differential mean survival of RPM on different plants (*Areca catechu*, *Borassus flabellifer*, *Cocos nucifera*, *Cyrtostachys renda*, *Heliconia* spp., *Musa* sp. cv Palayam kodan and *Pandanus* sp.) (Time is in days).

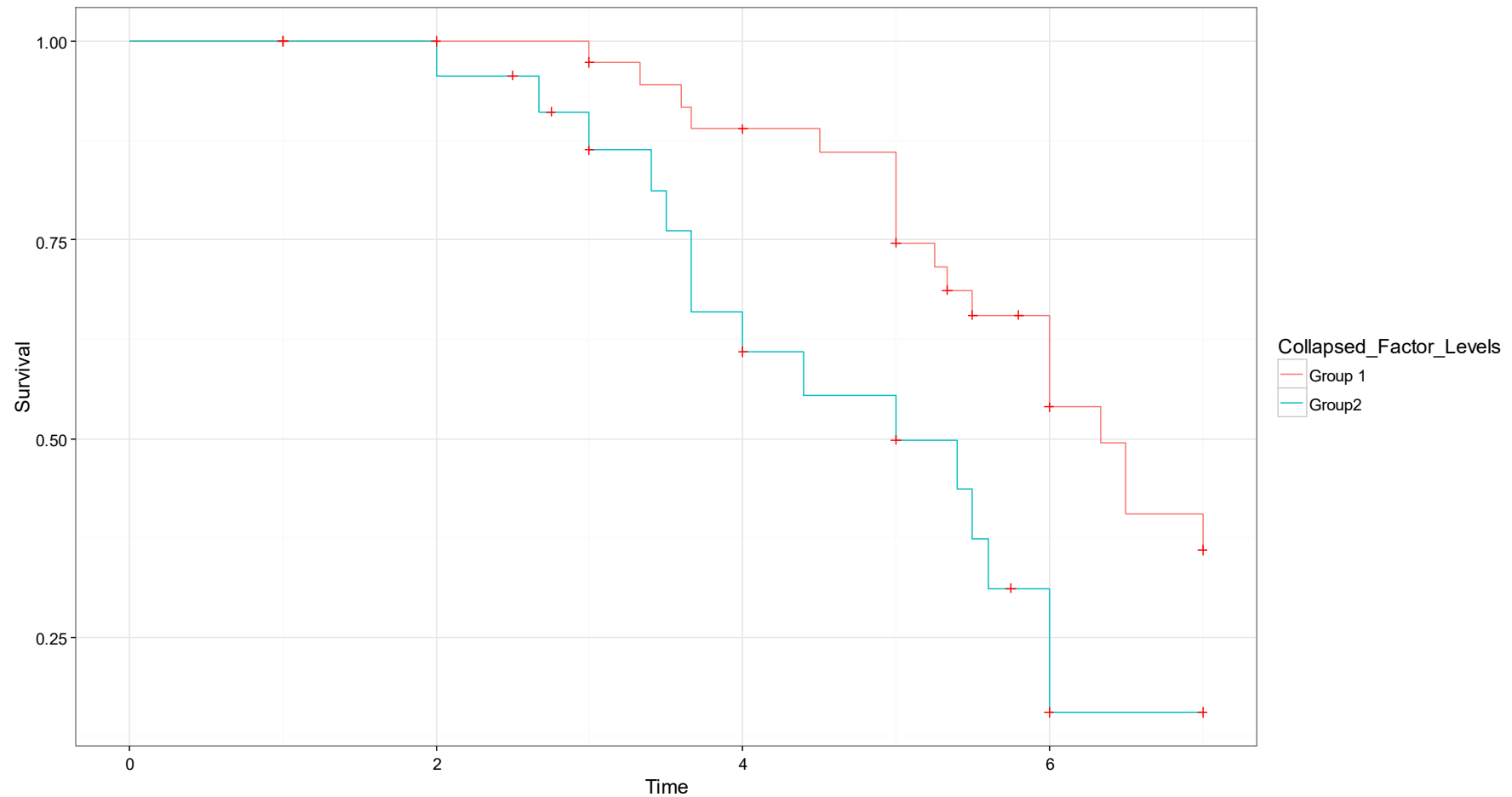


Figure 4.5 Survival analysis showing differential mean survival of RPM on Group 1 and Group 2 plant species. Group 1= *Areca catechu*, *Cocos nucifera*, *Heliconia* spp. and *Musa* sp. cv Palayam kodan. Group 2= *Borassus flabellifer*, *Cyrtostachys renda* and *Pandanus* sp.. (Time is in days).

For the number of eggs laid, a linear mixed effects (LME) model showed that the majority of variation was accounted for between treatments (95.5%) rather than over time (4.54%). When compared to the null model, the LME showed there was a significant effect of host on the numbers of eggs laid (AIC difference= 104.5, $\chi^2 = 120.5$, chi d.f.=8, $p < 0.001$). A post hoc test (lsmeans using Tukey Honest Significant Difference) showed that there were significantly more eggs laid per female on coconut and when compared to all other plants (d.f.=244, $p < 0.0001$ in all cases, *Areca catechu* z-ratio = 6.06, *Borassus flabellifer* z-ratio= 9.47, *Cyrtostachys renda* z-ratio = 9.36, *Heliconia* sp. 1 z-ratio = 5.88 *Heliconia* sp. 2 z-ratio = 6.95, *Heliconia* sp. 3 z-ratio = 4.83, *Musa* sp. cv Palayam kodan z-ratio = 8.17, *Pandanus* sp. z-ratio= 10.18). Additionally, significantly more eggs were laid on *A. catechu* when compared to *Borassus flabellifer* (z-ratio=, 3.41, $p = 0.02$), *Cyrtostachys renda* (z-ratio = 3.30, $p = 0.03$) and *Pandanus* sp. (z-ratio= 4.12, $p = 0.001$).

After 24h on the leaf sections (day 1) mites laid eggs on all species of host plant apart from *Pandanus* sp., *Heliconia* sp. 1 and *B. flabellifer*. No eggs were laid on sections from *Pandanus* sp. or *Heliconia* sp. 1 throughout the whole experiment. There were no significant differences between the numbers of eggs laid on all plants on day 1 ($F(8,28)=1.90$, $p=0.10$) or day 2 ($F(8,28)=1.03$, $p=0.44$). From day 3 onwards however, there was a significant difference in eggs laid between treatments (day 3: $F(8,28)=5.36$ $p < 0.001$, day 4: $F(8,28)=3.31$, $p=0.009$, day 5: $F(8,28)=15.58$ $p < 0.001$, day 6: $F(8,28)=9.73$, $p < 0.001$, day 7: $F(8,28)=5.60$, $p < 0.001$). Figure 4.6 shows the mean number of eggs laid per day ($\pm 1SE$). On all days from day 3 onwards, there were significantly more eggs laid on coconut compared to *Borassus flabellifer*, *Cyrtostachys renda* *Heliconia* sp. 1 and *Pandanus* sp. ($p < 0.01$) and after day 5 the numbers of eggs laid on *A. catechu* were significantly lower compared to those on *C. nucifera*. On days 3, 4, and 7 there were no significant differences in numbers of eggs laid on coconut than *Heliconia* sp. 2 and *Heliconia* sp. 3

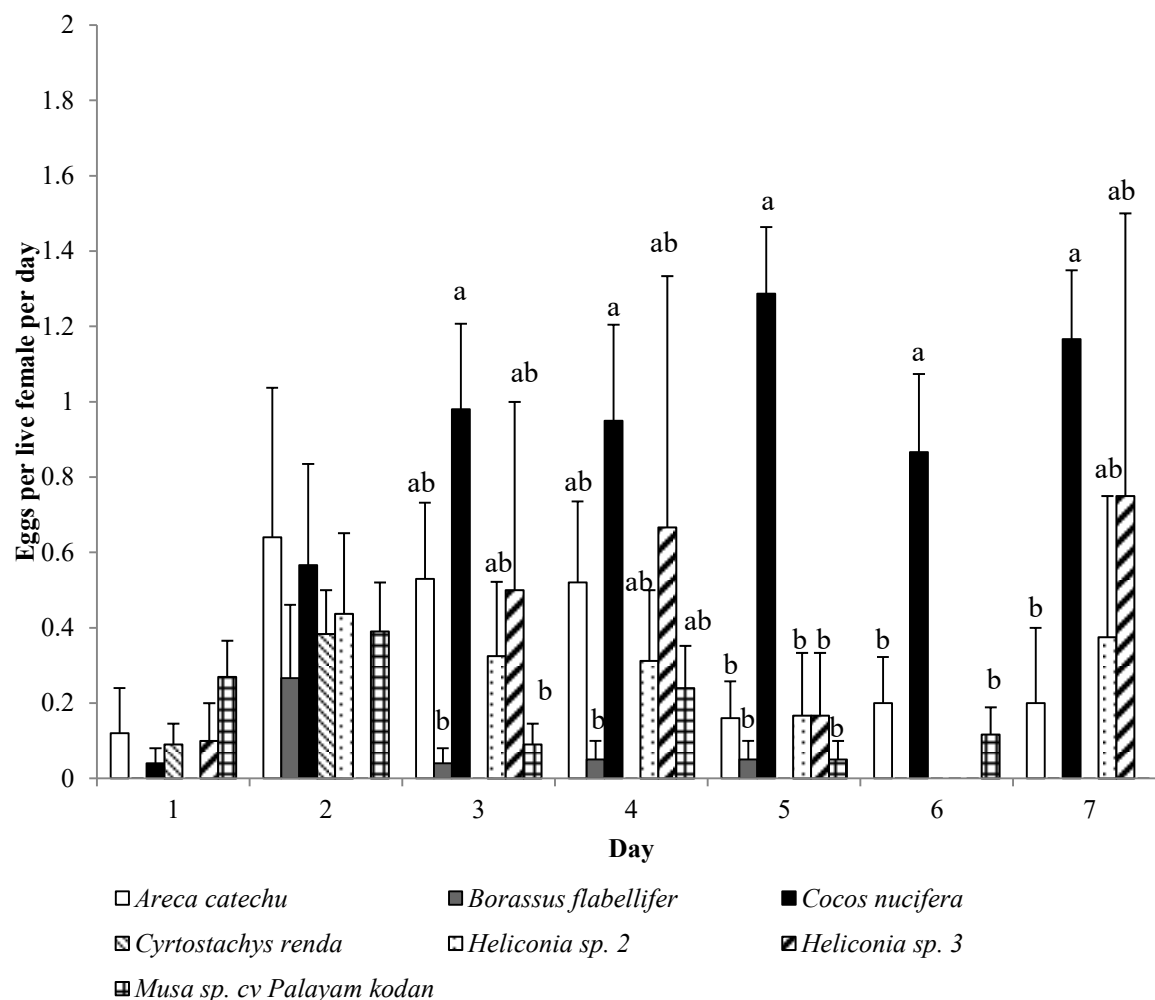


Figure 4.6 Mean numbers of eggs laid per live female per day on each host plant species (± 1 SE). No significant differences were observed between numbers of eggs on day 1 and day 2. For day 3 onwards, statistically significant differences ($p < 0.05$) are indicated within day using letters. *Heliconia* sp. 1 and *Pandanus* sp. were not included in this figure as no eggs were laid for the duration of the experiment

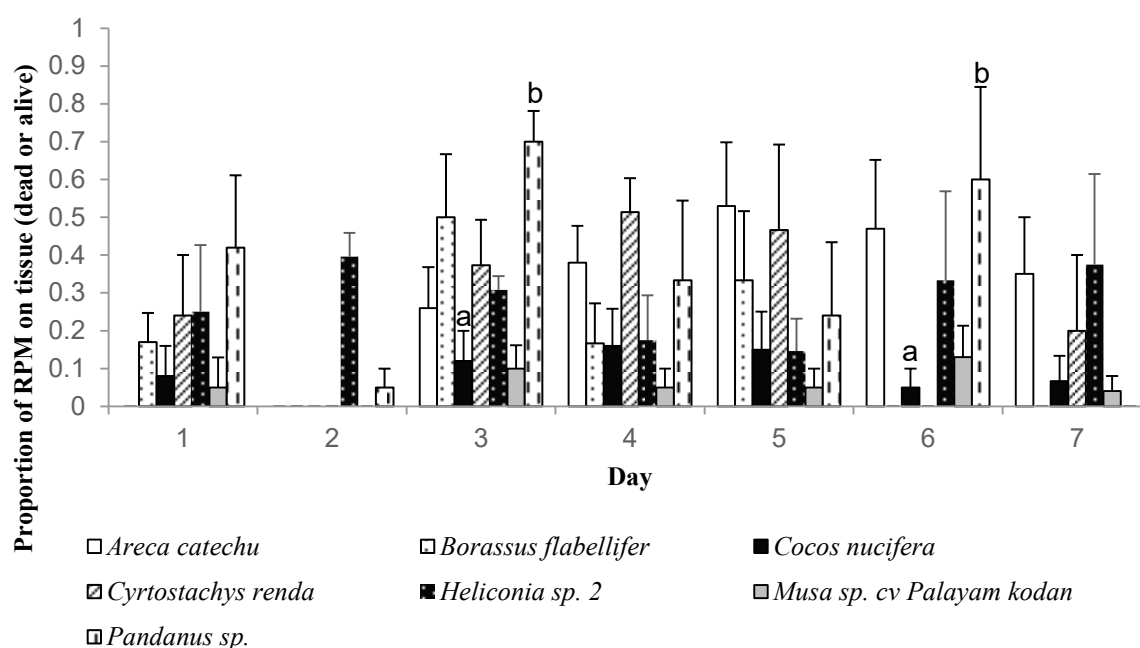


Figure 4.7 Proportion of RPM found on the tissue (exiting leaf disc) on each day of the survival assay ($\pm 1SE$). Significant differences ($p < 0.05$) are shown using different letters.

The level of run off from the leaf discs was consistently low for RPM on *C. nucifera* and *Musa sp. cv Palayam kodan*, never exceeding 20% of all RPM/host species/day (Figure 4.7). The highest run-off proportion was 0.16 for *C. nucifera* on day 4 and 0.13 on *Musa sp. cv Palayam kodan* on day 6. A linear mixed effect model showed there was a significant effect of host on the level of RPM run off when compared to the null model ($\chi^2 = 37.7$, chi d.f.=8, AIC difference = 21.7, $p < 0.001$). A post-hoc test (lsmeans using Tukey HSD) showed that significantly more RPM exited leaf discs on *Heliconia sp. 1*, ($p = 0.002$) and *Pandanus sp.* ($p = 0.01$) when compared to *C. nucifera*. On days 3 and 6 the proportion of RPM found on the tissue surrounding the leaf disc were significantly higher on *Pandanus sp.* compared to *C. nucifera* ($z = 2.34$, $p = 0.02$; $z = 2.44$, $p = 0.01$ respectively). The proportions of RPM on the tissue were high on *A. catechu* from day 3 onwards with a mean proportion of 0.26, 0.38 and 0.53 found on the tissue on days 3-5 respectively. Over 20% of RPM exited the leaf disc for *Pandanus sp.*, *C. renda*, *Heliconia sp. 2* and *A. catechu* on five out of the seven days observed.

A simple demonstration of the implications of survival and fecundity results

Results from the simple demonstration of the implications of the survival and fecundity data (Figure 4.8; Table 4.4) showed that population increase varied depending on the host plant on which RPM was feeding/laying eggs. Assuming a founder population of $n = 1$ RPM, after nine generations which is

roughly equivalent to the time period through which populations increase in abundance and density in the Kerala area (December-May) (Taylor et al., 2012), there was predicted to be 1.17×10^8 RPM in the general environment if colonies were feeding on *C. nucifera*, 1.77×10^5 if feeding on *A. catcehu*, 55 on *Heliconia* sp. 3, 35 on *Musa* sp. cv Palayam kodan and 1 on *Heliconia* sp. 2. No establishment occurred on *C. renda*, *B. flabellifer*, *Heliconia* sp.1 or *Pandanus* sp..

Table 4.4 Population model of RPM survival on different palm and plant species based on experimental results, plus information from literature. I_x is the mean survivorship over 7 days; m_x is the mean eggs per female per day multiplied by the mean proportion female per generation * R_0 based on mean eggs per female per day multiplied by the average adult female lifespan taken from Moutia 1958.

Species	Mean survivorship over 7 days (I_x)	Mean eggs per female per day ($\pm 1SE$)	Mean eggs per female per day x mean proportion female per generation	m_x	Net Reproductive Rate (R_0)*
<i>Cocos nucifera</i>	0.81	0.69 $\pm$ 0.17	0.51	13.90	11.20
<i>Areca catechu</i>	0.63	0.43 $\pm$ 0.10	0.32	8.54	5.42
<i>Borassus flabellifer</i>	0.49	0.08 $\pm$ 0.04	0.06	1.55	0.75
<i>Heliconia</i> sp. 1	0.37	0	0	0	0
<i>Heliconia</i> sp. 2	0.54	0.17 $\pm$ 0.07	0.13	3.43	1.84
<i>Heliconia</i> sp. 3	0.56	0.23 $\pm$ 0.05	0.17	4.58	2.55
<i>Musa</i> sp. cv Palayam kodan	0.74	0.17 $\pm$ 0.09	0.12	3.31	2.46
<i>Pandanus</i> sp.	0.34	0	0	0	0
<i>Cyrtostachys renda</i>	0.51	0.05 $\pm$ 0.05	0.05	1.35	0.69

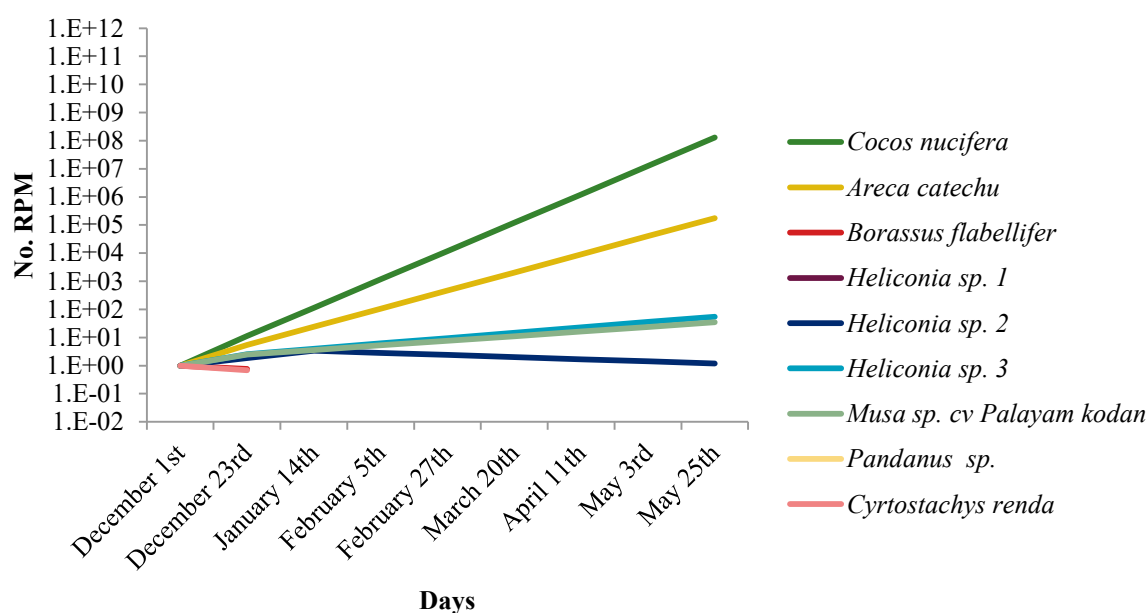


Figure 4.8 Graph showing exponential population growth given R_0 (calculated using survivorship of RPM on each plant species, and relative fecundity/sex ratio/generation time). Egg to adult time is 22 days.

Field survey of bananas of different ploidies

The survey conducted on different *Musa* spp. cultivars at the banana research station, observed no RPM individuals on any of the *Musa* spp. inspected; however, five heavily infested *A. catechu* seedlings were observed in the vicinity of the Red and Nendran cultivars (approximately 100m away) indicating the presence of RPM on the site, although these seedlings were not directly adjacent to the plots inspected. Population levels were extremely dense on all *A. catechu* leaflets and migration of RPM females was observed going down the stem. On the banana cultivars surveyed there was evidence of other groups present such as spider mites and natural enemies such as thrips, Coccinellids, spiders and Phytoseiids, although these were not collected and identified.

Local *Musa* spp. cultivars survival and fecundity analysis

The survival of RPM was highest on the coconut leaflets over time ($4.5 \text{ days} \pm 0.30$), followed by Robusta ($4.2 \text{ days} \pm 0.27$) and then Red ($4.1 \text{ days} \pm 0.47$) (Figure 4.9). Survival was shown to be lower on both the Nendran ($3.5 \text{ days} \pm 0.19$) and Poovan ($3.5 \text{ days} \pm 0.25$) with the poorest survival shown on Nendran. A survreg model using Weibull errors showed that there was a significant difference in survival between cultivars ($\chi^2 = 10.54$ on 4 degrees of freedom, $p = 0.03$). The results of Cox's proportional hazard showed that survival of mites on the cultivar Nendran was significantly different to those reared on coconut leaflets ($z=3.23$, $p=0.001$) whereas those reared on Red, Robusta and

Poovan were not; however, those reared on Poovan were marginal ($z=1.73$, $p=0.08$).

Because of the marginal significance of Poovan, two new factors were created; AAA and AAB ploidy and RPM survival was compared with the coconut control (Figure 4.10). The mean survival of the mites on the AAA ploidy leaflets, was 4.2 days (± 0.26 SE) compared to 3.48 (± 0.15 SE) days on AAB ploidy. The mean survival on the coconut control was 4.45 (± 0.30 SE). A significant difference between survival of mites on leaf discs of different ploidies was shown using a survreg model ($\chi^2=7.41$ on 2 degrees of freedom, $p=0.03$); and a Cox's proportional hazard test showed that survival was significantly lower on the AAB ploidy leaflets when compared to coconut control ($z=2.53$, $p=0.012$). Mites reared on AAA ploidy leaves were not found to have significantly different survival to those on coconut ($z=0.39$, $p=0.70$). All leaf sections in this experiment were banded with Vaseline, however RPM entered the Vaseline band (when attempting to exit the leaf sections); thus it is thought this may have affected survival. As all sections were treated the same results are still indicative of differential survival on these plants.

No significant difference in RPM survival was observed when new factor levels were created for leaf number and banana management (watering regime) ($\chi^2 = 1.31$ with 2 degrees of freedom, $p = 0.52$).

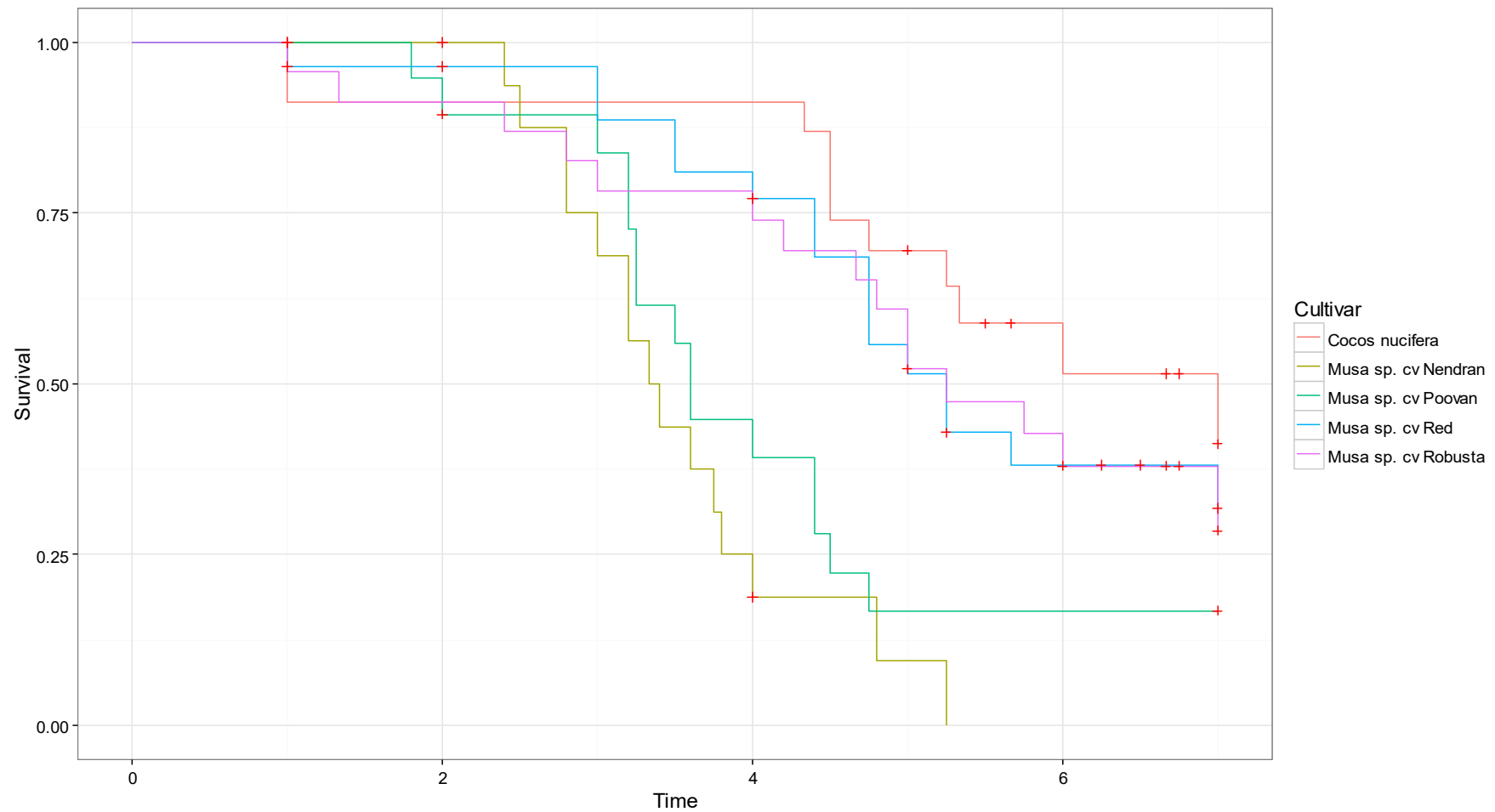


Figure 4.9 Mean survivorship of RPM on four different *Musa* spp. cultivars (Red, Robusta, Nendran and Poovan) and a *Cocos nucifera* control. (Time is in days).

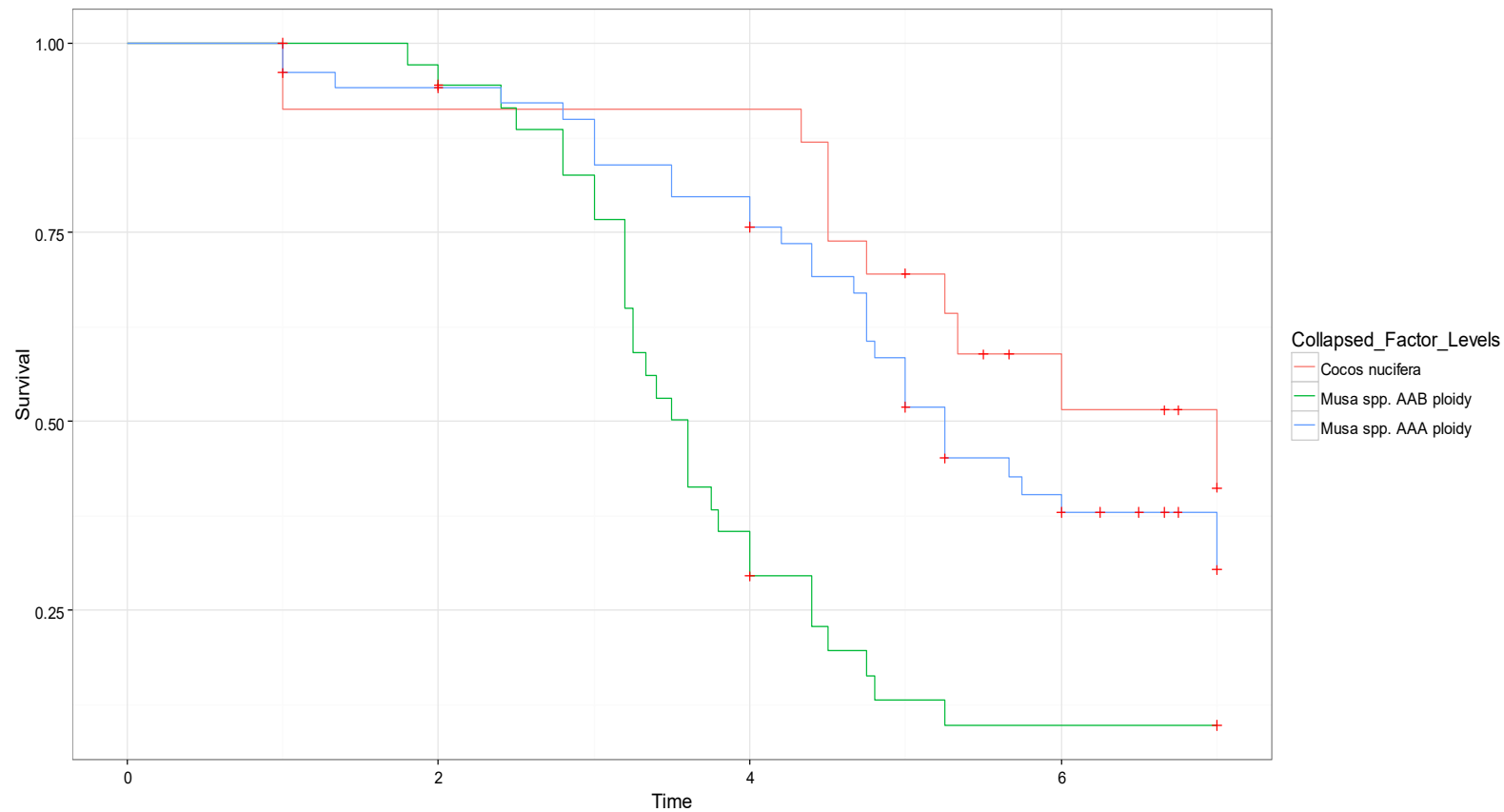


Figure 4.10 Mean survival of RPM on *Cocos nucifera* leaf discs and discs from two different ploidy levels of *Musa* spp. (AAA-cv Red and cv Robusta; AAB cv Nendran and cv Poovan). Crosses on chart show censorship where an individual went missing or individuals which remain alive at the end of the experiment. (Time is in days).

The analysis of the numbers of eggs laid using the linear mixed effects model showed that the majority of variation was accounted for between cultivars (83.7%), with variation over time lower at 3.3% and 12.9% between reps. When compared to the null model, there was a significant effect of cultivar on the numbers of eggs laid per female per day (Difference in AIC = 150.6, $\chi^2 = 86.15$, chi d.f.=4, $p < 0.001$). A post hoc test showed that the number of eggs laid on coconut leaf sections were significantly higher than those laid on all four cultivars of *Musa* spp. ($p < 0.0001$ for all cultivars, Nendran z-ratio = -9.10, Poovan z-ratio = 9.10, Red z-ratio = 7.72, Robusta z-ratio = 8.17). There were no significant differences between the numbers of eggs laid per female between the different cultivars of *Musa* spp. ($p > 0.05$ in all cases).

Mean eggs counts per day were converted using $\log(x+1)$ to account for the nature of the count data (Figure 4.11). A one-way ANOVA were applied to each time point. Results showed that there was no significant difference between the numbers of eggs laid per live female on day 2 ($F(4,19) = 1.82$, $p = 0.17$; 0 eggs were laid on day 1). There was however a significant difference in the number of eggs laid between treatments on day 3 ($F(4,19) = 8.40$ $p < 0.001$) with significantly fewer eggs laid on Nendran, Poovan and Robusta cultivars ($p < 0.01$ for each). There was not a significant difference in eggs numbers laid between Red and *C. nucifera* treatments ($p = 0.48$). On all following days, there were significantly more eggs laid on *C. nucifera* than on each of the *Musa* spp. cultivars (day 4, $F(4,19) = 8.69$, $p < 0.001$; day 5 $F(4,19) = 7.12$, $p = 0.001$, day 6 $F(4,19) = 11.85$, $p < 0.001$, day 7 $F(4,19) = 16.44$, $p < 0.001$), with no significant difference between the numbers of eggs laid between each of the *Musa* spp. cultivars ($p > 0.05$). There was evidence that more eggs were laid per day on cultivars Red and Robusta, however these were not significantly higher than those laid on cultivars Poovan and Nendran. Overall mean numbers of eggs per female per day over the 7-day period were 0.45 ± 0.30 on *C. nucifera*, compared to 0.02 ± 0.02 on *Musa* sp. cv Nendran, 0.02 ± 0.02 on *Musa* sp. cv Poovan, 0.09 ± 0.02 on *Musa* sp. cv Red and 0.07 ± 0.04 on *Musa* sp. cv Robusta.

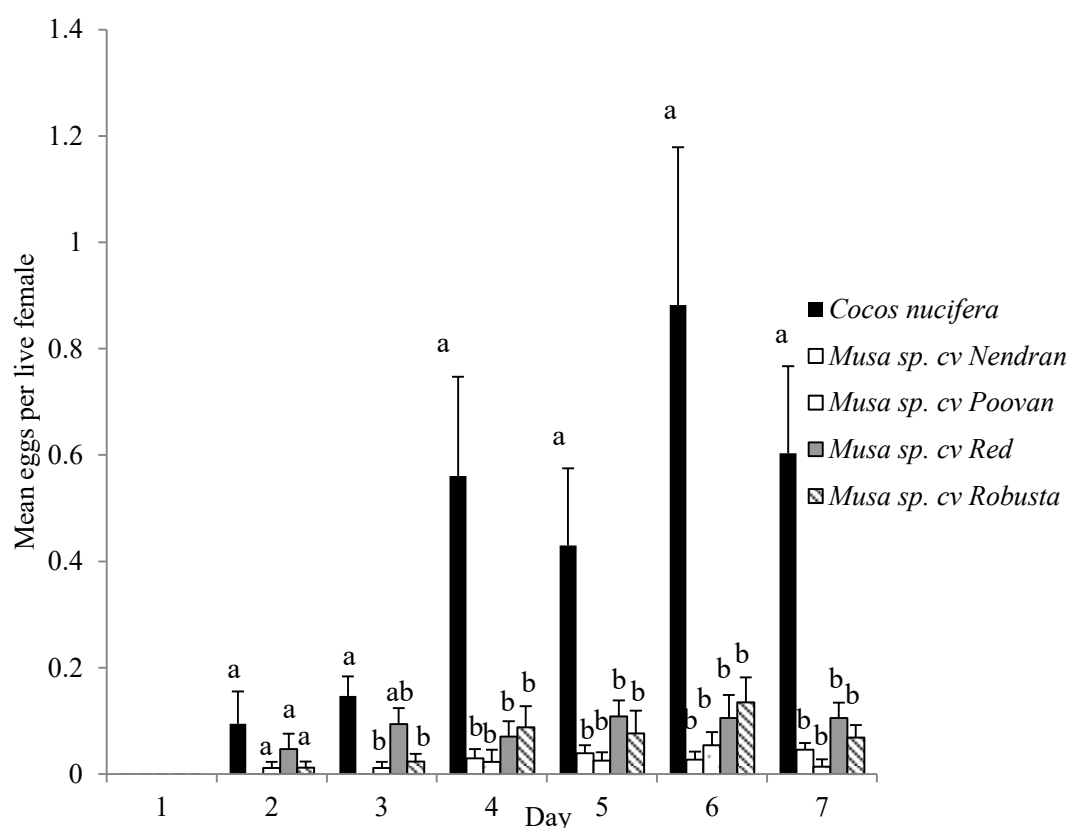


Figure 4.11 Mean number of eggs laid per female on each day on *Cocos nucifera* and *Musa* spp. cv Nendran, Poovan, Red and Robusta. (n=5 per time point apart from *C. nucifera* which had 4 replicates) different letters indicate significant differences in egg number between *C. nucifera* and *Musa* sp. cultivars.

Assessment of presence of RPM on *Musa* spp. in close proximity to *Cocos nucifera* (mixed plot survey)

Figure 4.12 shows the mean density of RPM per leaflet on *C. nucifera* cv Red, *C. nucifera* cv Green and per leaf of *Musa* sp. cv Palayam kodan on a spatial scale. Mean RPM densities on *C. nucifera* cv “Red” were 22.5 ± 6.77 , 11.5 ± 3.01 on *C. nucifera* cv Green and 0.14 ± 0.10 on *Musa* sp. cv Palayam kodan. A generalised linear mixed effects model showed there was a significant difference in the numbers of RPM per leaflet between the host plants surveyed when compared to a null model (χ^2 (d.f.=2)= 12.6, difference in AIC = 8, $p = 0.002$). There were significantly more RPM on *C. nucifera* cv Red and cv Green than on *Musa* sp. cv Palayam kodan (z- ratio = 7.6, $P < 0.0001$ and $z = 6.7$, $p < 0.0001$ respectively). There was no significant difference between densities of RPM on *C. nucifera* cv Red or cv Green varieties (z-ratio= -1.1 , $p=0.52$). Results showed that RPM was found in very low abundance on *Musa* spp. even though the plants were in close association with infested coconuts. Two banana plants out of 21 had RPM individuals found on the leaves, however these plants were found directly underneath a mature coconut palm at the edge of the plot and there was no evidence of

colony formation (i.e. eggs or cast skins). Seven out of nine ‘red’ coconut palms and nine out of eleven ‘green’ coconuts were found to have RPM colonies on. In total, in the plot, there were 2029 RPM sampled from *C. nucifera* cv. Red, 1375 from *C. nucifera* cv. Green and five sampled from *Musa* sp. cv Palayam kodan.

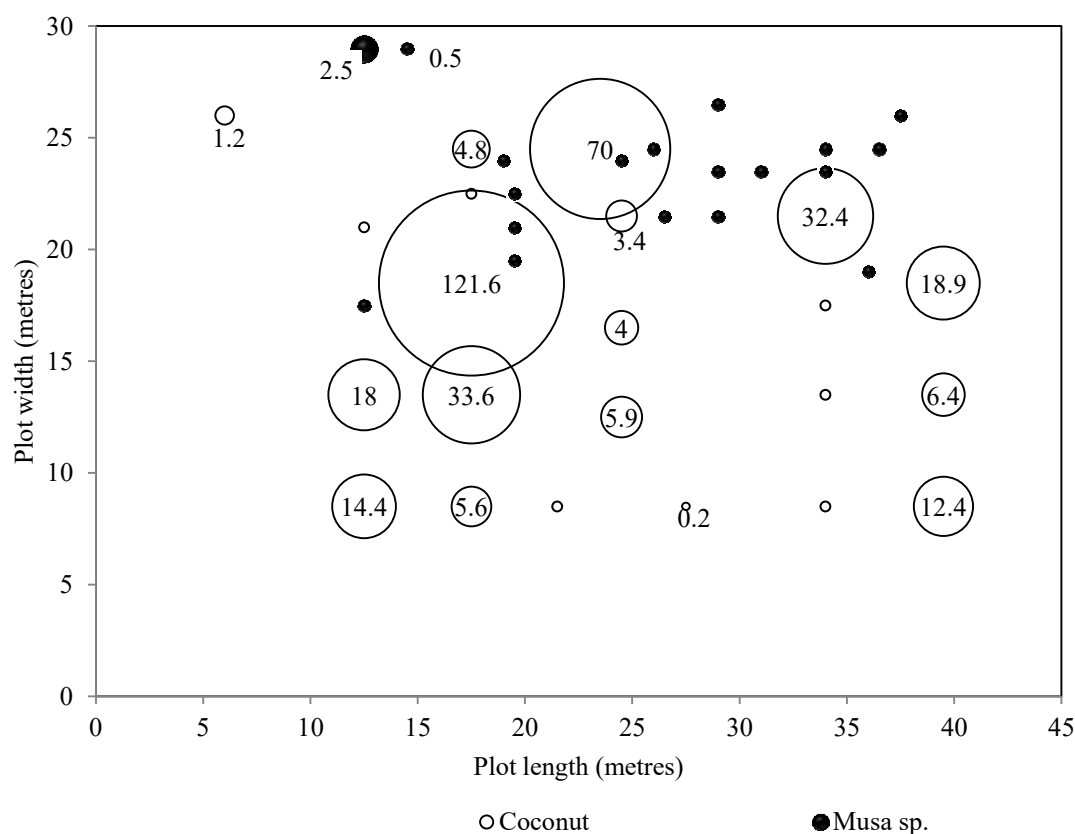


Figure 4.12 Spatial spread of RPM through a mixed *Cocos nucifera* (coconut) and *Musa* sp. plot. X and Y axis represent the length and width of the plot in metres. Numbers in circles indicate the mean number of RPM found per leaflet on the host plant, which is proportional to the size of the circle. Circles with no numbers indicate a host plant that had no RPM present.

Laboratory study on live plants

When live RPM were placed onto *Areca catechu*, *Musa* sp. cv Dwarf Cavendish and *Musa* sp. cv Robusta, there was a drop in the number of RPM recovered from all plants over the course of 8 days. A linear mixed effects model showed that there was a significant effect of host plant species on the numbers of RPM counted on each plant during the observation period compared to the null model ($\chi^2 = 8.3$, chi d.f.=2, difference in AIC = 4.29, $p = 0.02$), and a post hoc test showed that there were significantly more RPM observed on *Musa* sp. cv Robusta than on *A. catechu* (z ratio= -2.81, $p = 0.01$).

There were significantly more RPM were recovered from *Musa* sp. cv Robusta on days 6 and 8 than on *Areca catechu* ($z=-2.39$, $p=0.017$, $z= -2.067$, $p= 0.039$ respectively; Figure 4.13)

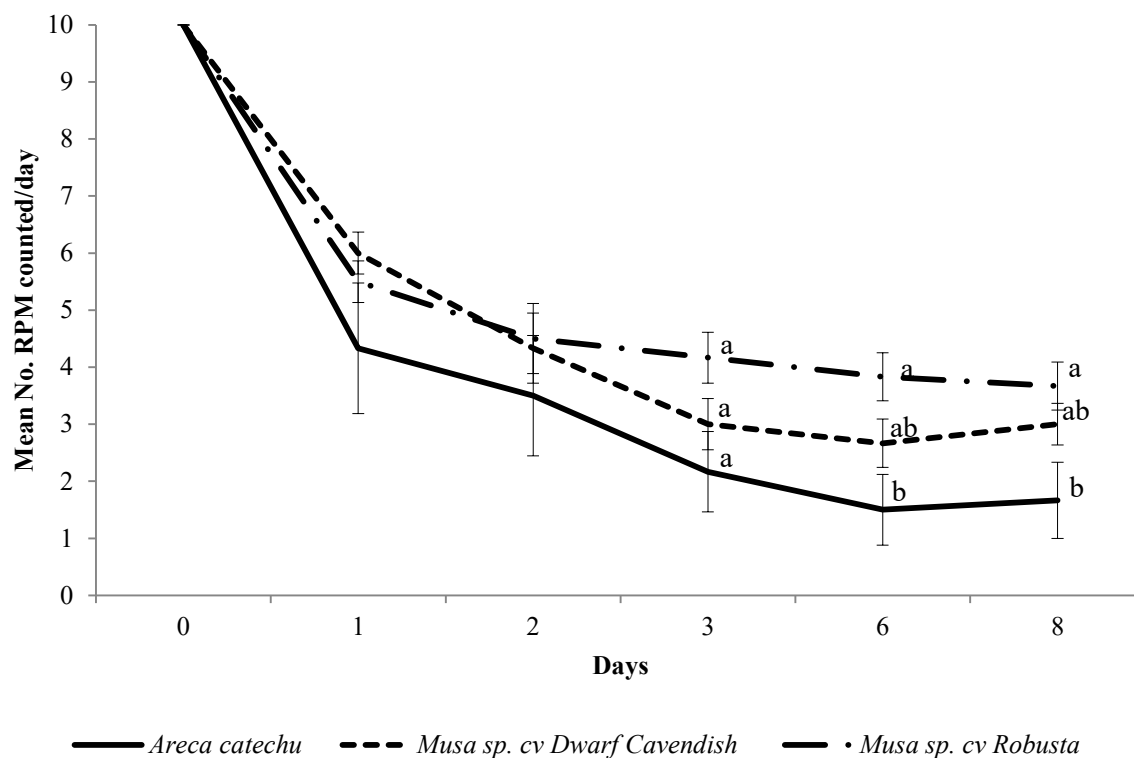


Figure 4.13 Numbers of live RPM counted per day on infested plants held in the laboratory (*Areca catechu*, *Musa* sp. cv Dwarf Cavendish and *Musa* sp. cv Robusta). ($\pm 1SE$)

4.3.2 Hypothesis 2

Survival and reproduction of RPM on natal host compared to alternative host.

Results from survival analysis showed that there was no significant difference in survival of RPM between any of the treatments ($\chi^2=2.25$, 7 d.f., $p= 0.94$; Figure 4.14). The scale parameter for the model with Weibull errors was 0.35. Data were separated into RPM originating from *A. catechu* or *C. nucifera*, and there was no significant difference in survival relating to RPM origin ($\chi^2=0.7$, 1 d.f. , $p= 0.4$, Figure 4.15). There was also no significant difference between survival of RPM originating on palms and transferred onto palms, and RPM originating on palms and transferred onto *Musa* sp. ($\chi^2=0.13$, 1 d.f. , $p= 0.72$; Figure 4.16). Table 4.5 shows the mean time to death $\pm 1SE$. The lowest survival was seen on *Musa* sp. (older leaves) with mites originating from *C. nucifera*, however the survival analysis showed this to not be significantly different from the other mean survival times.

Table 4.5 Mean time to death of RPM originating on *Areca catechu* and *Cocos nucifera* on different leaf types.

Origin of RPM	Plant species	Mean time of death	Mean eggs per female per day ($\pm 1SE$)
<i>Areca catechu</i>	<i>Areca catechu</i>	4.9 ± 0.47	0.58 ± 0.26
<i>Areca catechu</i>	<i>Cocos nucifera</i>	4.4 ± 0.88	0.26 ± 0.20
<i>Areca catechu</i>	<i>Musa</i> sp. cv Playam kodan "older leaves"	4.9 ± 1.00	0.18 ± 0.09
<i>Areca catechu</i>	<i>Musa</i> sp. cv Playam kodan "newer leaves"	5.5 ± 0.48	0.15 ± 0.08
<i>Cocos nucifera</i>	<i>Areca catechu</i>	5.5 ± 0.59	0.21 ± 0.10
<i>Cocos nucifera</i>	<i>Cocos nucifera</i>	3.3 ± 0.33	0.47 ± 0.21
<i>Cocos nucifera</i>	<i>Musa</i> sp. cv Playam kodan "older leaves"	3.0 ± 0.71	0.43 ± 0.18
<i>Cocos nucifera</i>	<i>Musa</i> sp. cv Playam kodan "newer leaves"	5.9 ± 0.39	0.27 ± 0.10

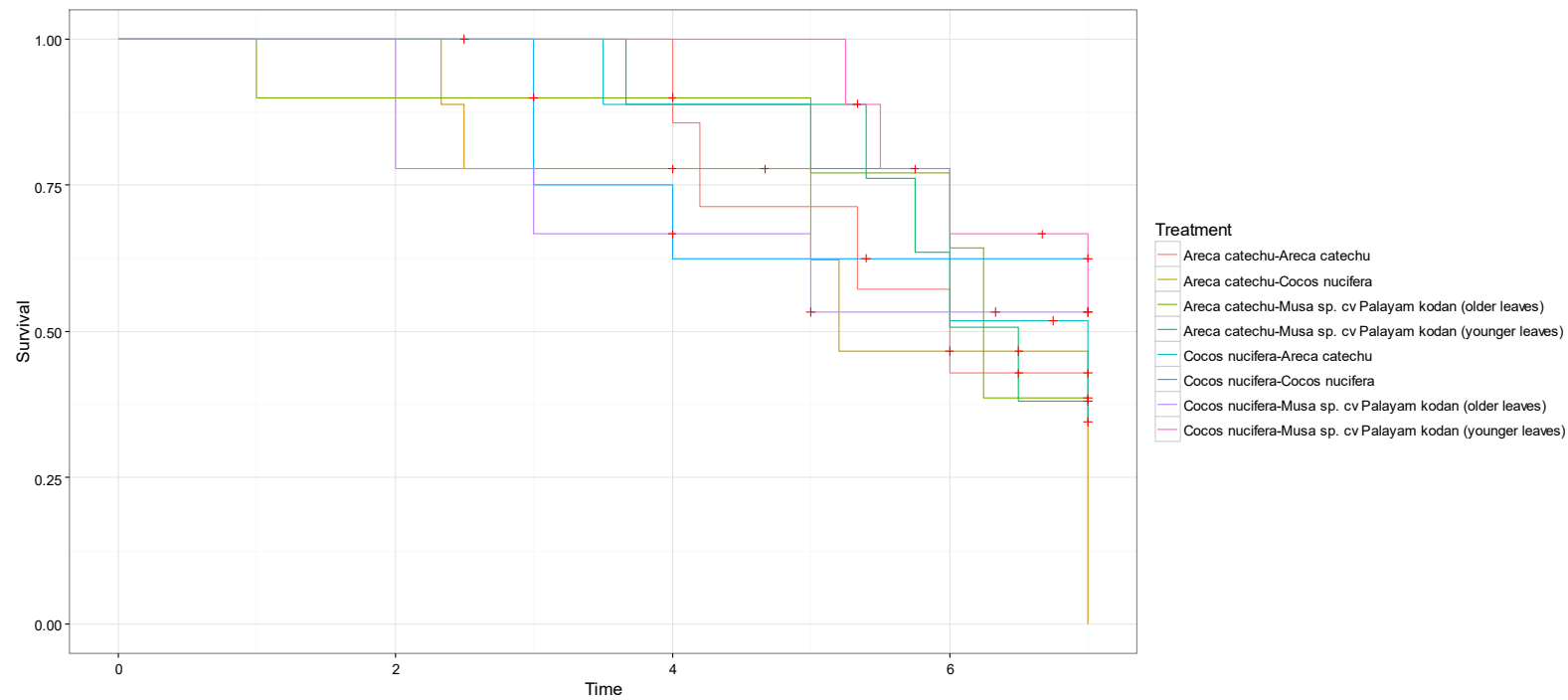


Figure 4.14 Survival plot of RPM (including censorship) originating from *Areca catechu* and *Cocos nucifera*, placed on leaf sections originating from *Areca catechu*, *Cocos nucifera*, *Musa* sp. cv Palayam kodan (young and old leaves). (Time is in days)

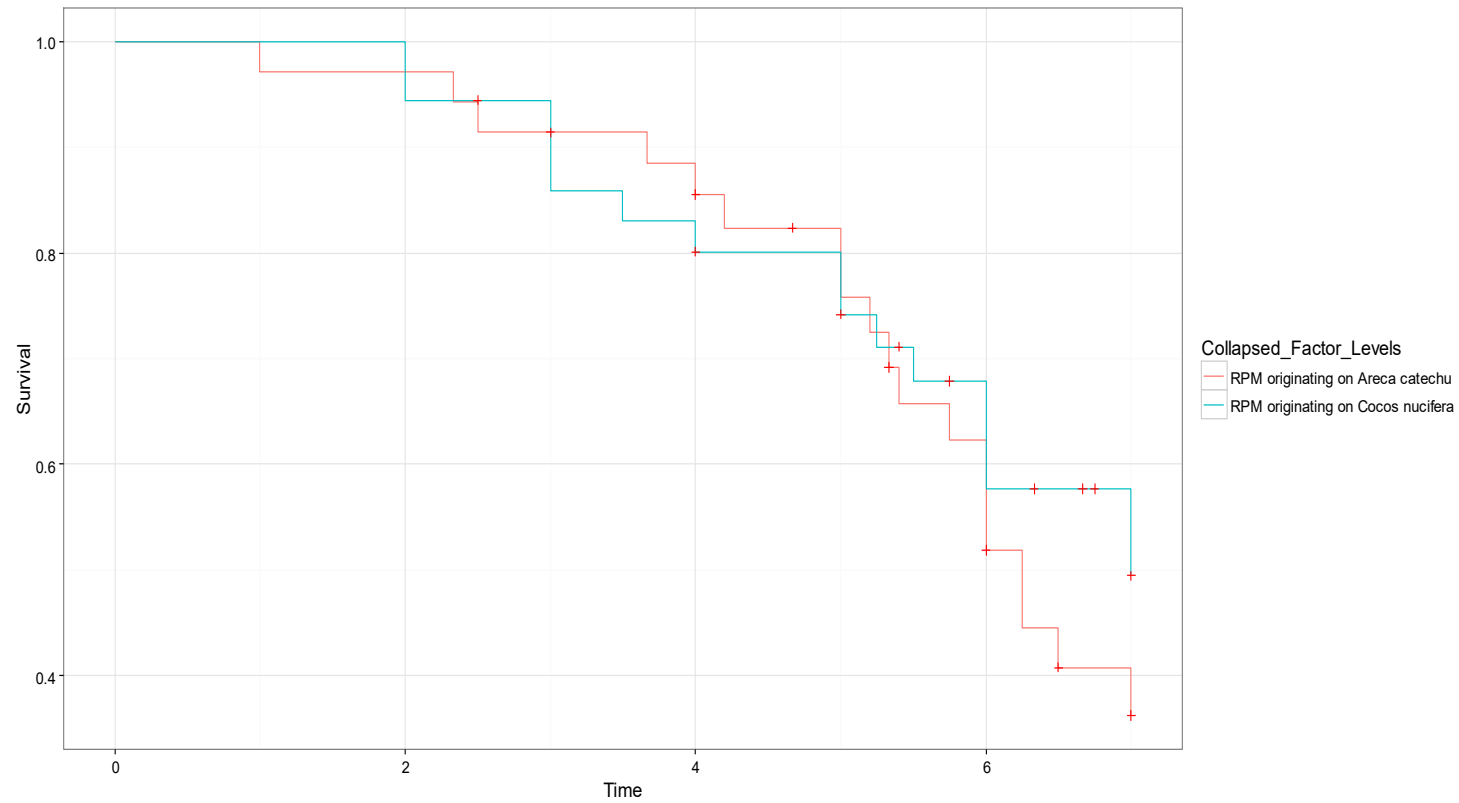


Figure 4.15 Survival of RPM originating on *Areca catechu* (Areca) versus survival of RPM originating on *Cocos nucifera* (coconut) across all treatments (*Areca catechu*, *Cocos nucifera*, *Musa* sp. cv Palayam kodan (young and old leaves). (Time is in days)

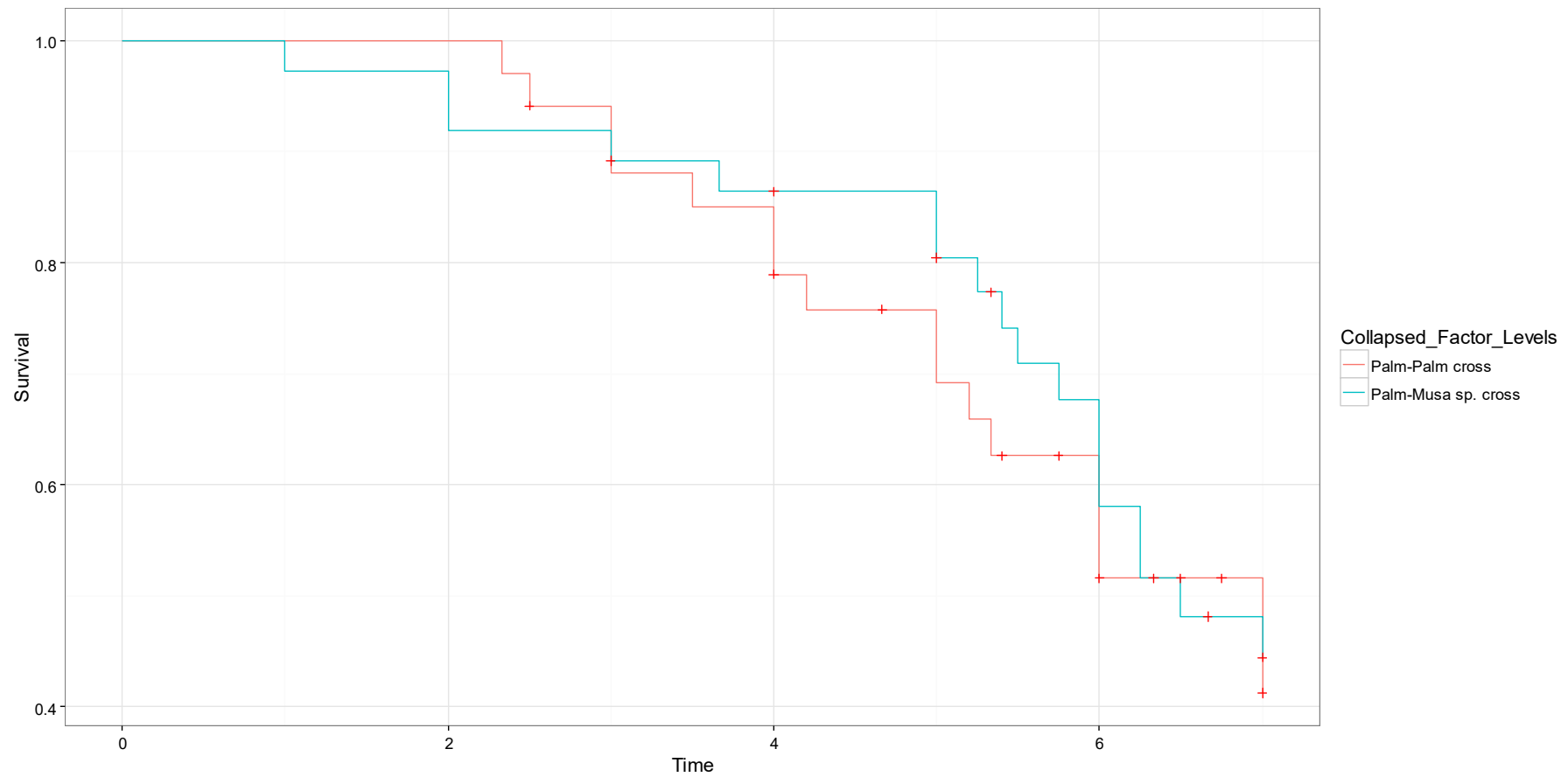


Figure 4.16 Survival of RPM originating on a palm host and placed onto a palm host, compared to RPM originating on a palm host and placed on *Musa* sp. host (Time is in days).

Results showing the number of eggs laid per live female per day are shown in Figure 4.17. There was no significant differences in the numbers of eggs laid on any of the days tested (day 1: $F(7,31)=1.41$, $p=0.24$; day 2: $F(7,31)=1.90$, $p=0.10$; day 3: $F(7,31)=1.91$, $p=0.10$; day 4: $F(7,31)=2.01$, $p=0.09$; day 5: $F(7,31)=1.49$, $p=0.21$; day 6: $F(7,31)=2.25$, $p=0.06$; day 7: $F(7,31)=0.78$, $p=0.61$), although slightly lower numbers of eggs were laid on Palayam kodan leaves compared to *A. catechu* and *C. nucifera*. A linear mixed effects model showed that overall there was a significant difference in the numbers of eggs laid between treatments when compared to the null model ($\chi^2 = 31.0$, chi d.f.=7, difference in AIC= 17.0, $p<0.001$). A post hoc showed that there were significantly more eggs laid on leaves of *A. catechu*, with RPM originating from *A. catechu*, than those transferred on *Musa* sp. cv Palayam kodan (old or new leaves) regardless of whether they were collected from *C. nucifera* (z ratio = 3.73, $p=0.005$ for old leaves; z ratio = 3.32, $p=0.02$ for young leaves) or *A. catechu* (z ratio= 3.63, $p=0.007$ for old leaves, z ratio= 3.87, $p=0.003$ for young leaves).

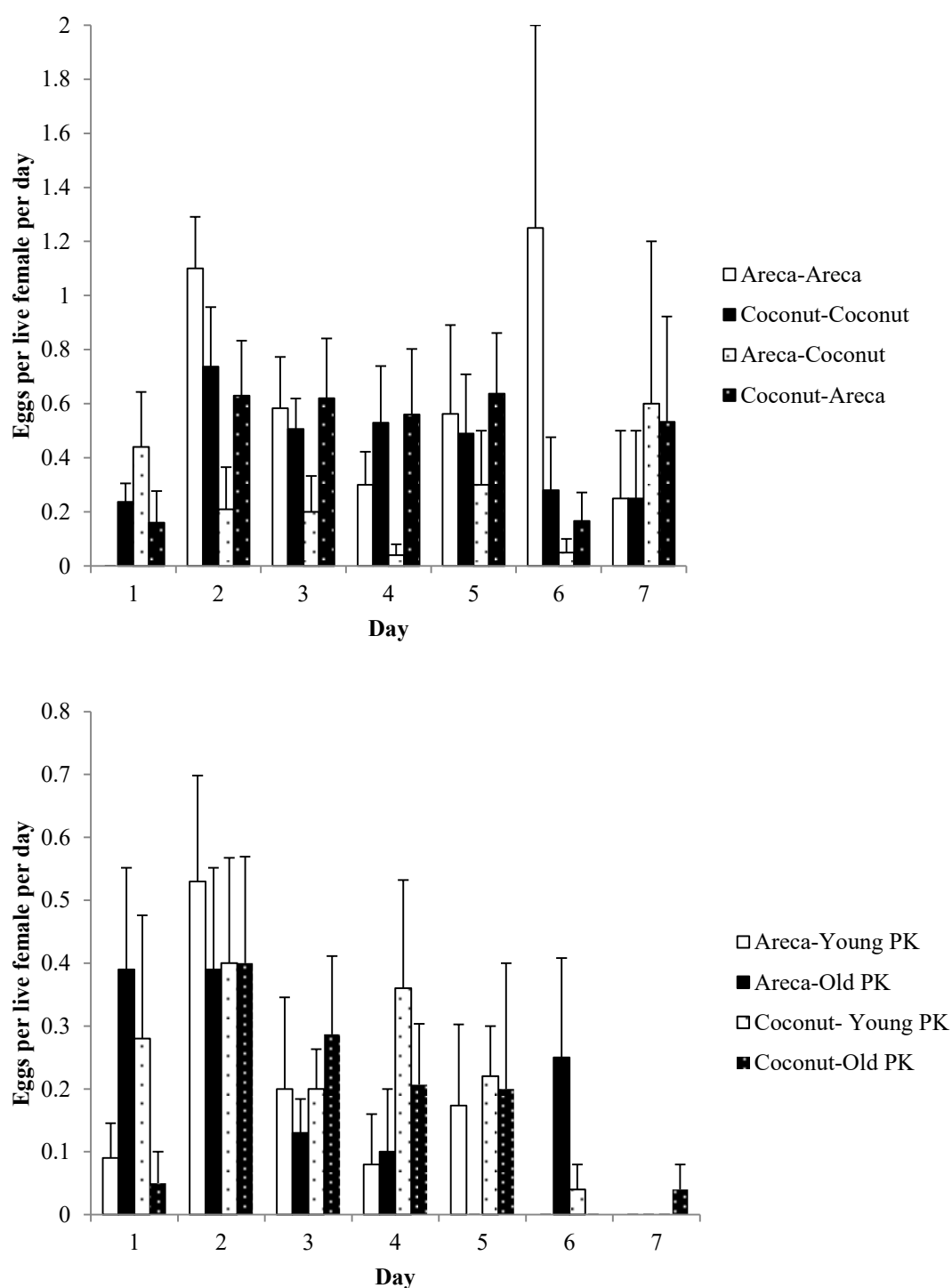


Figure 4.17 Mean number of eggs per live female laid per day over 7 days ($\pm 1SE$) by RPM originating from *Areca catechu* and *Cocos nucifera* on leaf sections of *Areca catechu*, *Cocos nucifera* and *Musa* sp. cv Palayam kodan old and new leaves.

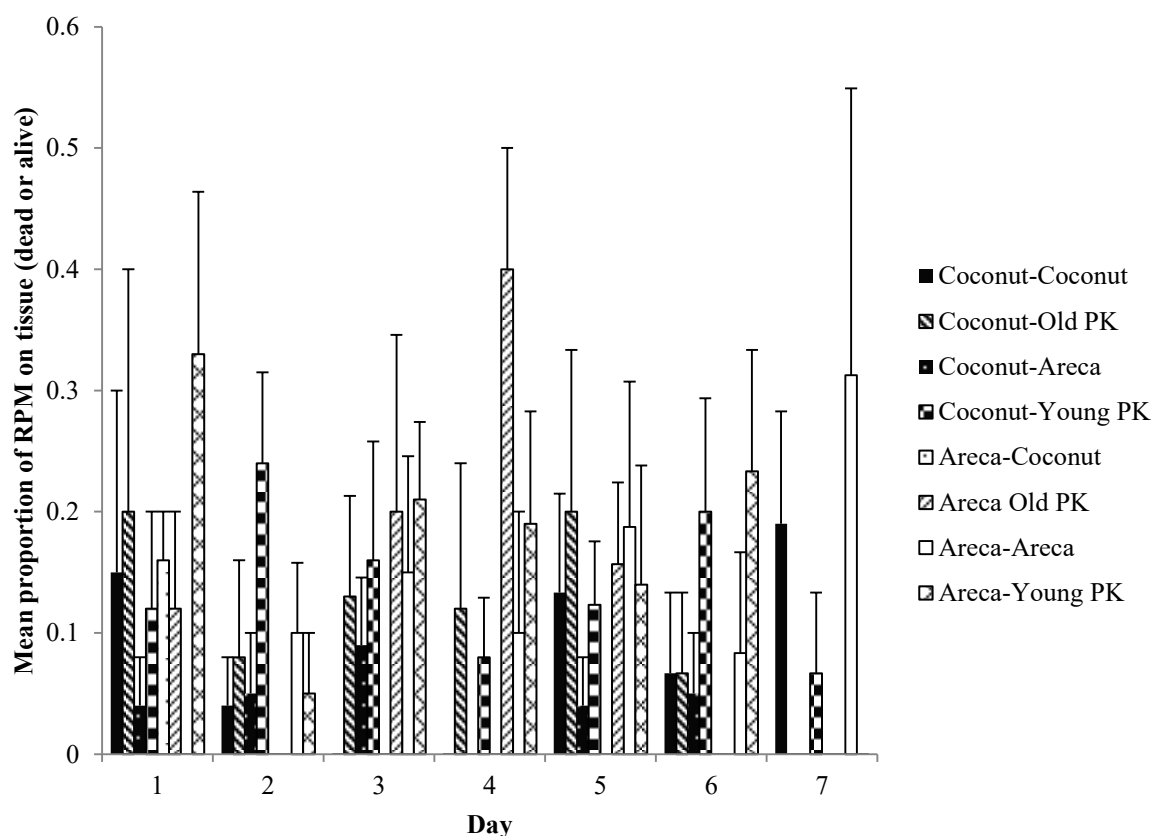


Figure 4.18 Mean proportion of RPM found on the tissue (exiting leaf disc) on each day of the survival assay ($\pm 1SE$). Coconut= *Cocos nucifera*; Areca= *Areca catechu*; Old PK= lower leaves taken from *Musa* sp. cv Palayam kodan; Young PK= upper leaves taken from *Musa* sp. cv Palayam kodan.

No significant difference was observed in the numbers of RPM found on the tissue surrounding leaf discs on any of the day ($p > 0.05$ for all tests; Figure 4.18).

4.4 Discussion

The first hypothesis stated that the host range in the Old World will be found to be wider than reported in the literature, and it was speculated that the narrow host range in the literature was due to under-reporting of RPM occurrence on other hosts. The field surveys, although limited in size in this chapter, showed evidence that RPM numbers were very low on alternative palm hosts and no breeding colonies were found on any of the alternative hosts apart from those found on *P. roebelenii*. Taylor et al. (2012) showed that *C. nucifera* and *A. catechu* supported high populations of RPM in the study area, and these observations were reconfirmed. In the area surveyed, there were few alternative palm species available to survey apart from those surveyed in this chapter on smallholder premises,

thus more survey data is required to confirm this conclusion. The study area was chosen as it supported high populations of RPM (Taylor et al. 2012) thus it was expected that alternative palm utilisation would be evident in this area if it was occurring. Kulkarni & Mulani (2004) stated that there are 20 genera of palm represented by 96 species in India, however the majority of these aside from widely cultivated species such as *C. nucifera*, *A. catechu*, *P. dactylifera* and widespread indigenous species *Phoenix sylvestris*, *Caryota urens* and *B. flabellifer* are found in restricted ecological niches meaning extensive geographic surveys would need to be undertaken to assess the presence/absence of RPM on these species. *Borassus flabellifer* was more common in the survey area, however the palms tended to be found in inaccessible stands or were too tall to collect samples from. Specimens of *B. flabellifer* which were examined showed no evidence of RPM populations or individuals. Further work should focus on more extensive surveys on indigenous and ornamental palms in India.

The survival analysis data on palms gave a good insight into which alternative species RPM can survive and reproduce on. Host selection was limited to the availability of at least five separate palms from the field to ensure replication, therefore only *C. renda* and *B. flabellifer* were tested as alternative hosts. *Musa* sp. cv Palayam kodan and 3 varieties of *Heliconia* spp. were also tested as Zingiberales comprised an important new host report in the adventive range. *Pandanus* sp. was tested as this species was reported as a host plant in the literature, although it's placement outside of the Arecales and Zingiberales highlights it as an anomalous host plant. No significant difference was found in survival on *C. nucifera*, *A. catechu*, *Musa* sp. cv Palayam kodan and *Heliconia* spp. (sp. 2 and sp. 3), however significantly poorer survival was observed on *B. flabellifer*, *C. renda*, *Heliconia* sp. 1 and *Pandanus* sp. The latter four species were excluded as potential hosts given RPM performance *in vitro*, with low survival rates, low numbers of eggs laid (if any) and a high rate of run off from the leaf discs (>20% on 5 days out of 7), which link in with the observations from the field. The poor performance of RPM on *Pandanus* sp. indicated that previous records of *R. indica* (originally named *R. pandanae*) found on *Pandanus* sp. were likely to have been wind-blown specimens that were unable to form colonies on this host, therefore it should be removed from reported host lists. Specimens found during surveys on *C. renda* were likely to have dropped from *C. nucifera* palms overhead and did not have the potential to form colonies given the results from the survival assay.

The fecundity assessment supported the survival results. Red Palm Mite on *C. nucifera* laid consistently higher numbers of eggs than those on alternative host plants, significantly so when compared to *B. flabellifer*, *C. renda*, *Heliconia* sp. 1, *Pandanus* sp., *Musa* spp. cv's Robusta, Red,

Poovan and Nendran but not significantly more than on *A. catechu*, *Heliconia* sp. 2 and 3 and *Musa* sp. cv Palayam kodan, although numbers of eggs laid in general were lower than on *C. nucifera*. Numbers of eggs laid on *A. catechu* dropped off significantly after day five compared to *C. nucifera* in the palm assay, however in the assay conducted for hypothesis 2, there was no evidence of this. On *B. flabellifer*, *C. renda*, *Heliconia* sp. 1, *Pandanus* sp., *Musa* spp. cv's Robusta, Red, Poovan and Nendran the significant drop in egg production occurred from day three onwards, which may relate to the quality of nutrition RPM are receiving for oogenesis. A pre-oviposition period of 3.3 days on average was recorded by (Zaher et al., 1969) for RPM on *P. dactylifera*. Although the pre-oviposition period relates to the time for RPM to lay first eggs upon emergence as an adult, this may also be linked to the time for eggs to develop upon commencement of feeding, thus a decline in oviposition after three days on the leaf discs as observed in these experiments may be indicative in the drop in nutritional quality the mite is receiving; thus, lowering the numbers of eggs laid. When these data were combined with the survival data in the demonstrative model, it was shown that high populations of RPM would only be able to develop on *C. nucifera* and *A. catechu*. There was evidence that populations could establish on *Musa* sp. cv Palayam kodan and *Heliconia* sp. 2 and 3, however the numbers of RPM would be much lower. In summary, laboratory evidence suggested that there was the potential for RPM to be found on *A. catechu*, *Heliconia* sp 2 and 3 and *Musa* sp. cv Palayam kodan in the field, however when surveys were conducted in the field no evidence of breeding colonies were found on any species other than *A. catechu* and *P. roebelenii*.

A more focused study was undertaken on *Musa* spp. specifically, given the increase in numbers of occasions where *Musa* spp. were reported as host plants in the adventive range. The centre of diversity for AAB ploidy *Musa* spp. is thought to be India as there is a wide variety of clones and somatic mutations of these triploids grown there (Purseglove, 1972), thus making India an ideal study location. The field survey found no evidence of colonisation of RPM on the *Musa* spp. plants surveyed in the study areas in Kerala, even when grown in close proximity to RPM infested hosts (i.e. *A. catechu* and *C. nucifera*). Laboratory studies showed that survival of RPM was possible on certain cultivars, and longevity differed according to the cultivar on which RPM were placed. Survival was significantly lower on the Nendran (AAB) cultivar and marginally different on Poovan (AAB) when compared to *C. nucifera*. Survival did not significantly differ on cultivars Red (AAA), Robusta (AAA) or Palayam kodan (AAB). Only Nendra, Poovan and Palayam kodan are grown commonly in the area surveyed (pers. comms Dr Suma, Banana Research Station), thus it may be possible that colonies of RPM maybe found outside the survey region in the Old World where these different cultivars are grown.. A paper recently published has recorded RPM on *Musa* sp. cv Grand Nain (AAA) in the field in South Gujarat, India however there was no information given on densities,

life stages or how mite species identification was undertaken (Shailesh et al., 2014). Although it is hypothesised that ploidy may influence the resistance of a *Musa* sp. cultivar to pests (the ‘balbisiana’ genome is thought to confer higher levels of disease resistance, hardiness and resistance to drought (Purseglove, 1972), there is no evidence from this study or other studies to suggest that this could be the mode of action for resistance. Evidence suggests that RPM performance is cultivar specific and some cultivars may be more resistant to RPM than others. This is in line with findings by Rodrigues & Irish (2012) who found differences in susceptibility to RPM depended on the cultivar of *Musa* sp. when tested in Puerto Rico.

The absence of field observations of colonies on *Musa* spp. may be linked to the significantly lower level of egg laying observed on all *Musa* spp. tested, meaning that establishment of colonies may be more difficult on these hosts. It may be hypothesised that cultivars conveying resistance to local pests such as RPM (which has been established in India for almost 100 years), may have been selected by farmers and smallholders in India, whereas in regions such as the New World, no such selection has occurred. Surveys carried out by Taylor et al. (2011) in the same study area in Kerala showed that cultivars grown by smallholders were of the varieties Palayam kodan, Nendran and Poovan. In the Caribbean, there is a mix of both AAA and AAB ploidy cultivars which have been shown to support successive generations of RPM. In the region of Kerala, India investigated however, there appears to be mainly AAB varieties grown. Synonymies between the AAB varieties grown between countries are difficult to confirm, as regional names are commonly given for bananas.

Red Palm Mite have been shown to feed through the stomata of leaves (Ochoa et al., 2011) and questions have been raised about the effectiveness of leaf disc assays using *Musa* spp. leaves, as they are highly hydrated, and stomata are prone to close quickly once removed from the plant (Rodrigues & Irish, 2012). Results here gave good indications of differences in RPM performance related to cultivar thus still contribute to the understanding of the relationship RPM has with these plants in India. Further assays on live plants would further the understanding of this relationship.

The study site assessing the presence of RPM on *Musa* spp. in close proximity to *Cocos nucifera* was selected as high populations of RPM were known to infest *C. nucifera* on this site and it was intercropped with many *Musa* sp. cv Palayam kodan plants. Red Palm Mite individuals were found on only two out of 22 of the *Musa* spp. plants surveyed in the spatial plot even though RPM densities were high on *C. nucifera* on this site. No evidence of breeding on the *Musa* sp. cv Palayam kodan was found i.e. cast skins and eggs, even though laboratory assays showed that RPM transferred from *C. nucifera* onto *Musa* sp. cv Palayam kodan showed no significant difference in survival or numbers of

eggs laid when compared (although numbers of eggs laid were lower). Further assays investigating more lifetable parameters would elucidate whether RPM is able to complete its lifecycle on this cultivar and assessments of egg-adult time and survival at each stage could add additional information to observations. At the Banana Research Station, no colonies or RPM individuals were found on the four cultivars of *Musa* spp. surveyed, even though extremely dense colonies of RPM were found in the vicinity of survey sites on *A. catechu*. These results suggest that there is a strong host fidelity to *C. nucifera* or *A. catechu* in the field, and although RPM can survive and lay eggs on *Musa* spp. it is unlikely that this will be a primary host. Interestingly, the level of run-off of RPM on *Musa* sp. cv Palayam kodan was low during survival assays indicating possible host acceptance by RPM which raises the question of why colonies were not seen on leaves during surveys. Similar evidence of remaining *in situ* on live plants was shown on the *Musa* sp. cv Robusta, although it is not clear why RPM did not remain *in situ* on *A. catechu* to the same extent. The absence of RPM colonies on *Musa* sp. cv Palayam kodan in the mixed plot survey did not relate to findings by Rodrigues & Irish (2012) in Puerto Rico. They found that susceptible *Musa* sp. cultivar PITA 16, when out numbering infested coconut palms 4:1, and when infested coconut palms surrounded a plant at a ratio of 4:1, sustained populations of RPM reaching around 120 per leaf, however the cultivar Palayam kodan, which appears to be susceptible in leaf disc assays, does not appear to sustain RPM populations in the field, even when surrounded by coconut hosts. These results therefore suggest that other mechanisms in addition to host related effects may be controlling populations on *Musa* sp. cv Palayam kodan in the field and this should be further investigated.

In conclusion, for hypothesis 1, further surveys on palms need to be conducted to ascertain the host range, as multiple species were not present in the survey area. Species which were grown and tested in the laboratory did not support RPM survival or egg laying thus can be ruled out as potential hosts. For *Musa* spp. and *Heliconia* sp. 2 and 3, it appears as RPM are able to survive and reproduce in the laboratory, however under field conditions, no evidence of RPM colonies were found, even when in close proximity to *C. nucifera* palms infested with RPM.

Under hypothesis 2, RPM was hypothesised to have host specific races; and the race found in Kerala would be specific to the order Arecales (palms) and will not attack plants from the order Zingiberales (*Musa* spp. and *Heliconia* spp.) or Pandanales. Drès & Mallet (2002) stated that host races will display a higher degree of fitness on natal hosts than on alternative hosts, use different taxa as hosts in the wild and exhibit 'host fidelity'. Laboratory assays were conducted as an initial assessment to evaluate the fitness of RPM on natal and alternative hosts. Given the restrictions of time in country it was not possible to breed RPM *in vitro* on different hosts to different host lines, therefore transfer

experiments were conducted from field populations. Results showed that there was no significant difference in survival on all hosts regardless of whether they originated on *A. catechu* or *C. nucifera*, although the numbers of eggs per live female were significantly lower on *Musa* sp. cv Palayam kodan when compared to RPM originating from *A. catechu* on both *A. catechu* and *C. nucifera*. It was not possible to test populations which originated from *Musa* sp. cv Palayam kodan as there were no field populations found in the survey area. Groot et al. (2005) conducted similar experiments with *B. phoenicis* and found that the species was comprised of a series of specialised clones based on results showing a low level of oviposition and reduced survival when transferred onto alternative hosts. This study showed no significant difference between survival on *Musa* sp. cv Palayam kodan compared to *A. catechu* and *C. nucifera* only evidence of lower numbers of eggs laid on *Musa* spp. compared to that on *A. catechu* and *C. nucifera*. These results combined with those found in chapter 3 suggest that the RPM studied in this thesis do not have host specific races. Differences in numbers of eggs laid per live female may have been due to nutritional suitability of each of the hosts or ability of RPM to feed on leaves. Recent research in Venezuela has shown that resistance of *Musa* sp. cultivars to RPM may be related to thickness of leaves, density of stomata and the concentration of phenolic compounds (high concentrations affect RPM colonisation) (Balza et al., 2015).

The results so far for *Musa* spp. contrast reports of multigenerational colonies of RPM found in the New World on various cultivars of *Musa* spp. (Cocco & Hoy, 2009; Kane et al., 2005). Even when *Musa* sp. were planted within metres of coconut palms supporting multiple colonies of RPM in the mixed plot survey, very little evidence of RPM transfer or indeed colony formation was found. Further field surveys would confirm if this is the case for multiple sites. The survival analysis aimed to further explore these results and investigated the hypothesis that the difference in cultivars grown between the Old World and New World may contribute to the observed differences. Results showed differing susceptibility of local cultivars, and the presence of colonies on Palayam kodan in the field could theoretically be expected, however it is likely that colonies would be in very low abundance. Only one very small colony has been found to date on *Musa* sp. cv Palayam kodan by Taylor et al. (2011) after extensive surveys. Drivers such as competition and predation are important factors to consider when examining apparent host range expansion or differences between regions. High population densities of up to 4,000 RPM per leaflet have been reported on host plants in the New World (Carrillo & Pena, 2010), possibly indicating that a lack of predation pressure or abiotic factors have led to lower mortality rates in RPM populations and to conditions of high competition. This increased competition may be hypothesised to be an underlying driver in ‘pushing’ mites from a preferred host such as coconut, onto a previously marginal host such as *Musa* spp., however to date there are no studies directly comparing RPM populations on *Musa* sp. and *C. nucifera* on the same

plot under field conditions to investigate this. Fry (1989) demonstrated that *Tetranychus urticae* (Koch) displayed the ability to adapt to hosts which were initially unfavourable in less than 10 generations, which supported and added to findings shown in a study by Gould (1979) who demonstrated that a single herbivore community (in this case *T. urticae*) contained enough genetic variation to adapt rapidly to a previously marginal host. The idea that host range is a fluid concept was put forward by Bernays & Graham (1988) who suggested that the switching between biotypes by phytophagous arthropods in a matter of generations as demonstrated by authors such as Gould (1979) and Fry (1989) may be seen as an 'ecologically dynamic' process rather than a shift in co-evolution between host plant and pest. This ability of an arthropod to broaden host preference may be an important trait in a niche where competition from conspecifics may be high, leading to high population densities and low resource availability.

In summary, the susceptibility of *Musa* spp. cultivars appears to be cultivar specific and therefore it is likely that more resistant *Musa* spp. cultivars are grown in the study area in Kerala. The laboratory assays suggest there is no evidence that RPM has host specific races between Arecales and Zingiberales, however field observations do indicate a strong fidelity to *C. nucifera* in the field. Results have shown the host range in Kerala has the potential to be wider given the results from the laboratory assays, however surveys in the field do not match the laboratory data, indicating that populations may be held in check on alternative host species by other factors.

5.0 A comparison of RPM and related predator complex between the adventive range (Trinidad) and naturalised range (India)

5.1 Introduction

Although there have been several studies on the population dynamics of RPM in the Old and New World which suggest populations are much denser in the latter region (Chandra & Channabasavanna, 1984; Pena et al., 2009; Taylor et al., 2012), there have been no parallel studies which directly quantify, compare and confirm these differences using common measures. Such a study is important to clarify the actual population densities in the two regions. Likewise, no studies have been made to compare the natural enemy communities in the two regions and to identify if the diversity and/or impact of natural enemies in the New World is lower than in the Old World. If population densities of RPM are higher and natural enemy communities poorer/have less impact on RPM populations than in the Old World then this lends support to the Enemy Release Hypothesis (Keane & Crawley, 2002). The literature relevant to these subjects is reviewed, followed by an outline of the objectives of the experimental work.

5.1.1 An overview of RPM population studies to date

To date, several studies have been carried out to estimate RPM populations in both the Old World and the New World (Table 5.1). A study by Moutia (1958) in Mauritius gave only general times of year when RPM would be abundant, however no information on RPM densities were given. Daniel (1983) was the first author to assess RPM density in Kerala, India, between the months of March-June over a three-year period. The assessments were made for a 1cm² area of *A. catechu* leaflet with reported densities ranging between 0-95/cm² over the course of the observations, however no information was given as to the numbers of trees inspected, the numbers of leaflets/their location in the canopy or how the unit area was derived. When similar surveys were carried out by Yadavbabu & Manjunatha (2007) on *A. catechu* the sampling unit used was number of RPM counted in 1cm² using a hand lens and results showed on average 44 RPM/cm² at the height of infestation. Both of these studies were carried out multiple plants at single site locations and when compared to Taylor et al. (2012) where mean densities when taken on an area wide level in Kerala, India (20 separate sites in one area) and used density per unit area (taken from a whole leaflet as a sampling unit), densities per cm² were much lower at 0.4-5 RPM/cm² at peak population levels. These examples show how differing sampling methodologies- even when conducted in similar localities/times of year (although not the

same year)- may lead to differing interpretations of population levels. Red Palm Mite population data from the New World has in general reported only RPM/leaflet values, apart from Ramos-Lima et al. (2011) who gave per 10 cm² densities on *Musa* sp. cv Cavendish. Studies to date have indicated that populations in the New World are much denser than those in India [a maximum of 4000 RPM/leaflet reported in USA (Pena et al., 2009)] compared to mean of 30.75 mobile stages/leaflet in Bangalore, India (Chandra & Channabasavanna, 1984), however the range of units reported for RPM population densities given and the differing sampling methodologies makes like for like comparisons extremely difficult. To compare accurately populations between the two regions, analogous sampling methods need to be undertaken to confirm the indicated differences in the historical literature. Among the list of sampling criteria outlined in Southwood (1966) was a specification that the sampling unit must lend itself to conversion in to unit areas. This is particularly important when comparing populations between host plants and regions. Other factors to consider include chance of selection from the sampling universe, stability of sample numbers i.e. leaflets per frond, the sampling unit is easily selected in the field and not be too small in comparison to the animal's size. Roda et al. (2012) examined RPM population sampling data in detail and determined that the optimal sampling unit would be to sample one leaflet from many different palms rather than multiple leaflets within a palm.

Employing standardised sampling methods between regions is important not only to estimate populations of RPM on *C. nucifera* to confirm severity of infestations in the New World, but also to compare populations on *Musa* spp. in both regions where RPM host use has been hypothesised to differ. By utilising standardised sampling methodologies which convert RPM density to unit areas, this would also allow for comparison of RPM population densities between regions and between host plants on the same site. In Chapter 4 it was hypothesised that increased competition could be an underlying driver in 'pushing' mites from a preferred host such as coconut, onto a previously marginal host such as *Musa* spp.; however, to date there are few studies directly comparing RPM populations on *Musa* spp. and *C. nucifera* on the same sites. Experiments have indicated that higher densities of mites may be found on *Musa* spp. when there is a higher occurrence of mites falling from the palms above the *Musa* spp. plants (Rodrigues & Irish, 2012) and recent work by Otero-Colina et al. (2016) has shown that infestation rates were slightly higher on *Musa* spp. growing close to infested *C. nucifera* palms, however there was no significant relationship found between infestation level and distance. Standardised sampling would also allow for other factors affecting population density to be estimated such as predator pressure within each region.

Table 5.1 Table of RPM population surveys up until 2012, including sampling methodologies, sampling units and results

Author	Host plant	Country	RPM density estimation method	Sampling methodology	Range
Daniel (1983)	<i>Areca catechu</i>	India (Kerala)	RPM/ 1 cm ²	Not given	0-95/cm ²
Chandra & Channabasavanna (1984)	<i>Cocos nucifera</i>	India (Bangalore)	RPM / leaflet	6 trees, 4 bottom fronds, 2-3 leaflets per frond. 10 leaflets per palm	Average of 30.75 mobile stages/leaflet (excluding no. eggs=20.83)
Somchoudhury & Sarkar (1987)	<i>Cocos nucifera</i>	India (West Bengal)	RPM / leaflet	5 plants, 3 fronds, 3 leaflets (45 leaflets in total)	maximum 5.75 per leaflet
Sarkar & Somchoudhury (1989)	<i>Cocos nucifera</i>	India (West Bengal)	RPM / leaflet	5 plants, 3 fronds, 3 leaflets (45 leaflets in total)	maximum 8 per leaflet (June 1982)
Yadavbabu & Manjunatha (2007)	<i>Areca catechu</i>	India (Karnataka)	RPM/ 1 cm ² (counted in 1 square centimetre using a hand lens)	10 plants, 5 leaflets (from top middle and base fronds)	max average 44.2 in a cm ² (15/04/03)
Peña et al. (2009)	<i>Cocos nucifera</i>	USA (Florida-West Palm Beach)	RPM / leaflet	2 sites, each with 8 plants, 1 leaflet from 2 fronds per plant, monthly n=16/site	ca. max 4000 RPM/leaflet (March 2008)
Peña et al. (2009)	<i>Cocos nucifera</i>	USA (Florida-Broward)	RPM / leaflet	2 sites, each with 8 plants, 1 leaflet from 2 fronds per plant, monthly n=16/site	ca. max between 1600 -1800 RPM/leaflet (April and June 2008)
Peña et al. (2009)	<i>Cocos nucifera</i>	Trinidad	mobile RPM per leaflet	5 plants, 3 fronds/plant, 3 leaflets/frond	Data not given
Peña et al. (2009)	<i>Cocos nucifera</i>	Puerto Rico	average number of mobile RPM per 10 leaflets cited	10 leaflets per site, washed with ethanol	max between 2000-2500 (mean of 10 leaflets, 3 sites) (March 2008)
Ramos-Lima et al. (2011)	<i>Musa accuminata</i> Cavendish subgroup	Cuba	average per 10cm ² leaf sample	20 plants, from each a 10cm ² leaf sample every 10 days	max between 2000-2500 per 10cm ² leaf sample (April 2009)

The ecology of *Raoiella indica* (Hirst) (Acari:Tenuipalpidae) in India and Trinidad

Author	Host plant	Country	RPM density estimation method	Sampling methodology	Range
Roda et al. (2012)	<i>Cocos nucifera</i> cv Jamaican Tall	Trinidad	RPM / leaflet	5 plants, 3 fronds/plant, 3 leaflets/frond. Surface area of the basal and middle areas of each pinna measured by taking the width and height of the area were measured and multiplied. The apical portion of the pinna was calculated using $\frac{1}{2}$ base $\times$ height.	max mean 573.31/ leaflet (August 2008)
Roda et al. (2012)	<i>Cocos nucifera</i> cv Malayan dwarf	Puerto Rico	RPM / leaflet	5 plants, 3 fronds/plant, 3 leaflets/frond. Surface area of the basal and middle areas of each pinna measured by taking the width and height of the area were measured and multiplied. The apical portion of the pinna was calculated using $\frac{1}{2}$ base $\times$ height.	max mean 458.89/ leaflet (February 2008)
Taylor et al. (2012)	<i>Cocos nucifera</i>	India (Kerala)	RPM/cm ²	2 survey areas, 20 plants per area, 1 frond, 3 leaflets /frond. Leaf area were taken by measuring the length and width (latter at widest point), then the area was converted using a standard multiplier ((Prasada Rao & Sebastian, 1994), which was 0.63 for coconut.	max c.a. 0.16 RPM/ cm ² in Palakkad (April 2009) max c.a. 0.04 RPM/ cm ² in Peechi (March 2009)
Taylor et al. (2012)	<i>Areca catechu</i>	India (Kerala)	RPM/cm ²	2 survey areas, 20 plants per area, 1 frond/plant, 3 leaflets /frond. Leaf area were taken by measuring the length and width (latter at widest point), then the area was converted using a standard multiplier (Prasada Rao & Sebastian, 1994) which was 0.74 for areca.	max c.a. 0.4 RPM/ cm ² in Kunnankulam (March 2009) max c.a. 0.4-5 RPM/ cm ² in Nilambur (May/June 2009)

5.1.2 Review of natural enemies

To date, the reported predator complex associated with RPM consists of generalist insect and acarine predators (see Table 2.3 and 2.4, Chapter 2), however there are no direct comparisons of diversity between the two regions in relation to RPM. Such a study could give insights into the nature of the predator complex associated with RPM in both ranges and allow for direct comparison. Further knowledge relating to the nature of the natural enemy complex would provide information to assess whether the apparent increase in population densities of RPM are related to the release of RPM from control by co-evolved natural enemies (enemy release hypothesis). This information would add to the current body of knowledge as it would give insight as to whether a classical biological control programme would be effective against RPM.

Generalist predators which have been reported in association with RPM in Trinidad previously are *Amblyseius largoensis*, *Aleurodothrips fasciapennis*, *Bdella* sp., *Cheletomimus* sp. and species from the insect families Cecidomyiidae (Diptera) and Chrysopidae (Neuroptera) (Peña et al. 2009), however all apart from *A. largoensis* were observed to have no numerical response in association with RPM populations. Taylor et al. (2012) identified *A. largoensis* associated with RPM in India, along with *Amblyseius channabasavanni*. *Amblyseius channabasavanni* has been reported previously in association with RPM in India (Daniel, 1983) however, Taylor et al. (2012) highlighted that the description of *A. channabasavanni* was very similar to *Amblyseius tamatavensis* (a species commonly reported in the New World) therefore further taxonomic studies should be carried out to elucidate if they are synonymous. Other reported generalists preying upon RPM in India have been *Stethorus keralicus* (Daniel, 1976), *Amblyseius raoiellus* (Acari:Phytoseiidae) (Gupta, 2003), *Phytoseius* sp. (Acari:Phytoseiidae) (Somchoudhury & Sarkar, 1987) and a species of Stigmaeidae (Acari) (Daniel, 1983).

Several authors have explored the potential of classical biological control to bring RPM populations down to an acceptable level in the New World. Hoy (2012) carried out surveys in Mauritius and found *A. largoensis* to be the predominant predator associated with RPM, as did Moraes et al. (2012) in La Reunion and Taylor et al. (2012) in Kerala, India; however this predator is also found in the New World and has been studied to explore its potential as a control agent (Carrillo et al., 2012b; Carrillo et al., 2012c; Carrillo et al., 2014; Carrillo & Peña, 2012; Carrillo et al., 2010; Domingos et al., 2013; González et al., 2013; Rodríguez et al., 2010) and morphological or molecular differences which may be present between different populations found in the Old and New World (Bowman & Hoy, 2012; Domingos et al., 2013; Navia et al., 2014). No comparative studies have assessed *A. largoensis* from

India with those found in the New World to date, thus it is unknown whether the species found within both regions are analogous. A comparison of the complex of generalist predators between regions could help to understand the potential predation pressure RPM may be under within each region and contribute to understanding whether RPM face less predation pressure in the New World. McMurtry & Croft (1997) highlighted that some Phytoseiid predators such as *Amblyseius* spp. may not be preadapted to prey upon specific species of mite as would be the assumption of classical biological control (CBC), but may be more adapted to host plants, thus highlighting the importance of broadening predator surveys away from just *C. nucifera* or the reliance on one species as a ‘golden bullet’.

5.2 Objectives

The work presented here included a baseline study firstly to compare and quantify the densities of RPM populations on *C. nucifera* and *Musa* spp. in Kerala India and Trinidad; and secondly, to investigate whether there was any relationship between high population densities on *C. nucifera* and RPM populations on *Musa* spp. (in Trinidad only as this is where RPM is reported on *Musa* spp.). The study used a common method for assessing density in both regions and provided a quantitative comparison of population densities between the Old World and the adventive range in relation to two major hosts. This work built upon surveys conducted in Kerala in Chapter 4, where very low populations of RPM were reported on *Musa* spp. and observations underpinned further study of the predator complex associated with RPM populations in both countries; with view to define the principle predator types and species found in both India and Trinidad. The primary associations identified were further studied to compare the morphology of common predators reported between India and Trinidad. Preliminary laboratory experiments were conducted to assess RPM performance *in vitro* in both India and Trinidad to examine whether there were underlying basic differences in RPM performance *in vitro*. A degree day model was applied to generalised RPM performance data to explore the effect that climatic conditions would have on population development within each region prior in the 9 months prior to the surveys.

Based on the above, three hypotheses are suggested:

1. It is hypothesised that RPM populations will be significantly denser in the adventive range compared to the naturalised range on both *C. nucifera* and *Musa* spp., given the reports from the literature
2. There will be a relationship between RPM densities on *C. nucifera* and *Musa* spp. found on

the same site- those with high population densities on *C. nucifera* will have higher RPM densities on *Musa* spp.

3. Predator diversity will be poorer in the adventive range (Trinidad) compared to the naturalised range (India).

Factors which may be hypothesised to drive the observed density differences such as predator performance/biotype, performance of RPM and general climatic conditions will be explored in a series of baseline assessments.

5.3 Materials and Methods.

5.3.1 Study sites and seasons

To conduct RPM and predator comparison studies, two survey regions were chosen, one in Trinidad (the adventive range) and one in Kerala, India (the naturalised range). In Trinidad, the Maraccas Royal road, in northern Trinidad was chosen for the 5km transect between Luango Village 10.708356,-61.414009 and 10.672522,-61.411389, and in Kerala a 5km transect was initiated at Vadakkenchery village heading eastwards (no GPS data). This is the same area as chapters 3 and 4. Working between regions can potentially cause difficulty as abiotic and biotic factors (abiotic: temperature, humidity, rainfall, day length (latitude), host cultivar) (biotic: predation pressure) may affect population comparisons (Shea & Chesson, 2002). To allow important environmental factors to be considered, the timing and locations of studies were chosen to minimise these differences. Studies in India were carried out during March 2012 (7th-8th March 2012), and studies in Trinidad were carried out in early April 2012 (7th, 8th and 11th April 2012). These study periods were chosen as they coincided with peaks in RPM populations during the dry season in both ranges. Local site temperatures were not taken at the time of sampling as these are only indicative of conditions at time of sampling, not those relating to population build up; however, a generalised comparison of temperatures during the studies period was taken from weather station data and this showed that the mean temperature during sampling periods in India was 29.7 °C and 52.5% RH, and in Trinidad mean conditions were 27.4 °C and 77.5% RH. A simple degree day model was applied to weather station data to compare potential RPM development speeds in section 5.3.5.

5.3.2 Survey methodology

Fifteen sites were randomly chosen to sample along a 5 km transect in both Trinidad and India. Sampling sites in India were chosen by generating a random sequence of the numbers 1:100

representing the houses in the 5km stretch. The first 15 numbers were then chosen and these plots were chosen as sampling sites. If there was no suitable host plant, a coin was tossed and the plot either to the left or right was chosen. Only sites with both *C. nucifera* and *Musa* sp. growing on the same plot were chosen. Sampling sites matching those in India were more limited in Trinidad therefore 15 sites were randomly chosen along the 5km when there were both coconut and banana plants available to sample.

At each site, one leaflet was removed at the rachis from a lower frond of a coconut palm, (but not lowest level frond as these were often damaged/dying). This was in line with sampling techniques developed by Roda et al. (2012), whose conclusions for sampling RPM from *C. nucifera* were to sample one pinna (leaflet) per tree from as many trees as possible for plantation level population estimates. Only palms up to 5m in height were sampled. For *Musa* spp., one lower leaflet was removed. Each leaflet/leaf was inspected on site for predator groups known to prey on mites and these were collected in 80% alcohol using a fine paintbrush. Dimensions of the leaflets/leaves were taken and the leaflets/leaves were wrapped in newspaper and returned to the lab for further inspection under the microscope. Width was measured at the widest point. Length x width measurements were converted using standard multipliers [0.63 for *C. nucifera*- (Prasada Rao & Sebastian, 1994) and 0.8 for *Musa* spp. (Vásquez et al., 2016)] to give an accurate estimation of leaf area. The total RPM numbers were counted (all mobile lifestages) using a stereo microscope and remaining acarine predators found on the leaflet were collected for identification in 80% ethyl alcohol. Samples were mounted using Heinze media (apart from insect predators which were identified using the appropriate methodology/keys) and identified as far as keys available would allow. Only specimens recorded as predators of mites were counted in this analysis. Where possible for banana, varietal information was noted.

5.3.3 Predator diversity

To investigate the predator species diversity, the Shannon Weiner diversity index was applied to data.

5.3.4 Comparison of biological performance of RPM between Trinidad and India

A survival analysis was set up in the laboratory at CABI, Curepe, Trinidad to act as a comparator for RPM performance between regions using the methodology outlined for survival analyses in Chapter 4 (30th October -6th November 2014). Five separate *C. nucifera* leaf sections (collected from separate palms) were collected from the grounds of CABI, Curepe. Coconut leaf sections were 2.5 x 5cm as leaf shape would not allow 4 x 4 cm sections. Survival and number of eggs laid per day were

observed at 9am over a period of seven days. Plates were kept at ambient conditions in the lab (27°C min, 31°C max., no RH taken). *Musa* spp. assays were also undertaken, however the leaf quality during assays was poor so results are not shown.

5.3.5 Simple degree day model predicting RPM development in each range

To consider the influence of prior local temperature on the development of RPM populations in both regions, meteorological station data for nine months prior to the surveys were downloaded from the nearest available meteorological stations to the field sites (01/09/2011-30/04/2012). These stations were at Coimbatore, India and Piarco Airport, Trinidad. Developmental rates were taken from the literature and a degree day model for RPM development was fitted to predict potential population densities, given local temperatures to which RPM had been exposed. The assumption was made that all biotypes of RPM were the same. The lower base threshold temperature was estimated as 10°C using data from the USDA RPM pest risk analysis (Borchert, 2007). To estimate the degree days required for RPM to develop from egg to adult, the equation given by Andrewartha & Birch (1982) whereby:

$$K = y (x - a)$$

Where K is the thermal constant, y is the number of days required to complete development at temperature x, and a is the lower threshold development temperature. Cumulative degree days to which RPM were exposed were calculated from the weather station data where degree days were calculated as:

$$DD = [(T_{max} - T_{min}) / 2] - TL$$

Where DD were degree days, T_{max} and T_{min} were maximum and minimum daily temperatures and TL was the thermal base threshold (10°C). The numbers of degree days to which RPM were exposed subsequently calculated and compared.

5.3.6 Morphological comparison of *Amblyseius largoensis* between India and Trinidad

The only common predator found between India and Trinidad during the surveys was *Amblyseius largoensis*, however only two specimens were collected during the comparative surveys. To increase the number of *A. largoensis* specimens, other specimens collected from both India and Trinidad were used. Specimens of *A. largoensis* were collected from coconut plants in India during CABI field work in Palakkad, Kerala during 2009 (6-16th December), 2011 (6-13th April) and 2012 (4-15th March)

(n=11) using a fine paintbrush then stored in 80% alcohol prior to mounting. These specimens were slide mounted using either Hoyers or Heinze media and slides were heated until cleared. Specimens from Trinidad were collected using a fine paintbrush during fieldwork in 2012 (6th -13th April) and 2013 (9th-25th April) from Maracas Pass, Manzanilla and CABI, Curepe and stored in 80% alcohol prior to mounting in Heinze media and heated and cleared as above (n=12). Chapter 3 tested to see if there was a difference in measurements from the two different mounting media and none was found. Navia et al. (2014) investigated whether the populations of *A. largoensis* from Mauritius and Reunion were the same species as those found in Brazil, Trinidad and Tobago and USA. To enable comparison between results of Navia et al. (2014) many of the same morphological features were measured (Appendix 12) on the collected specimens. A multivariate MANOVA was applied to these data to test for significant differences.

5.3.7 Statistical analysis

As densities are a form of count data (strictly bounded as they cannot be <0), RPM densities were log (x+1) transformed as per Roda et al. (2012) and a two-way ANOVA was fitted to these data to compare densities between countries and host plants. Differences were considered significant where $p < 0.05$. Generalised Linear Models were not suitable for this analysis as the Poisson distribution does not deal with densities/non-integers in R. Presence/absence scores of RPM and predators (1 or 0) were allocated to each site and a GLM using binomial errors was run to assess any significant differences in occurrence on sites between countries and between plants in country. Where over-dispersion was observed in the fitted model, quasibinomial errors were used. To compare RPM densities on *Musa* spp. and *C. nucifera* on the same site Spearman's rank correlation was used as per Otero-Colina et al. (2016).

5.4 Results

5.4.1 Survey results: Baseline study comparing RPM population densities between India and Trinidad

A two-way ANOVA showed that there was a significant interaction between country and host ($F=4.2$, $d.f.=1$, $p < 0.05$), therefore this term was retained in the model. Overall, RPM densities were significantly higher in Trinidad compared to India ($F= 4.65$, $d.f.=1$, $p=0.04$), and significantly higher on *C. nucifera* compared to *Musa* spp. ($F=6.54$, $d.f =1$, $p=0.01$). A Tukey HSD post hoc showed that there were significantly higher densities of RPM on *C. nucifera* in Trinidad compared to India

($p=0.03$; Figure 5.1), with a mean density of 0.51 ± 0.24 RPM per cm^2 in Trinidad and 0.03 ± 0.02 RPM per cm^2 in India. There was however, no significant difference in RPM densities on *Musa* spp. between the two countries ($p>0.05$; Figure 5.2), although mean densities were much higher on *Musa* spp. in Trinidad with a mean density of 0.007 ± 0.004 RPM/ cm^2 in Trinidad compared to 0.0002 RPM/ $\text{cm}^2 \pm 0.00009$ in India. There were also significantly more *Musa* spp. plants infested with RPM in Trinidad compared to India (14 compared to 4; GLM with binomial errors: $z=3.07$, $p=0.002$, $\text{d.f.}=28$). Multigenerational colonies were observed on *Musa* spp. in Trinidad, however no colonies were observed in India. There was not a significant difference in abundance on *C. nucifera* between the two countries (10 compared to 8, Trinidad and India respectively; GLM with binomial errors: $t=0.72$, $p>0.05$, $\text{d.f.}=28$). On *Musa* spp. the range of densities in Trinidad ranged from 0-0.071 per cm^2 , compared to 0-0.001 per cm^2 in India. Of the *Musa* spp. cultivars surveyed in Kerala, India, six were cv Palayan kodan (AAB, subgroup Mysore), three cv Mysore (AAB, subgroup Mysore), two Nendran (AAB subgroup plantain), two Poovan (AAB, subgroup Mysore)), one Robusta (AAA) and one Kunnan (AB). Those with RPM individuals were two x cv Palayan kodan, one x Nendran and one x Kunnan. Of the *Musa* spp. cultivars surveyed in Trinidad were five x Lacatan (AAA, subgroup Cavendish), one x Silk fig (AAB, subgroup Silk), one x Gros Michel (AAA, subgroup Cavendish), one x Grand Nain (AAA, subgroup Cavendish) and six x unknown cultivars. Those with RPM individuals were three x Lacatan, one x Gros Michel, one x Grand Nain, one x Silk fig, two x unknown (specified as cooking bananas) and one x 'unknown'.

Within country in India, there was no significant difference between RPM densities on *C. nucifera* and *Musa* spp. ($F=3.70$, $\text{d.f.}=1$, $p=0.07$), however in Trinidad densities were significantly higher on *C. nucifera* than *Musa* spp. ($F=5.4$, $\text{d.f.}=1$, $p=0.03$). In both countries densities observed were much higher on coconut than banana.

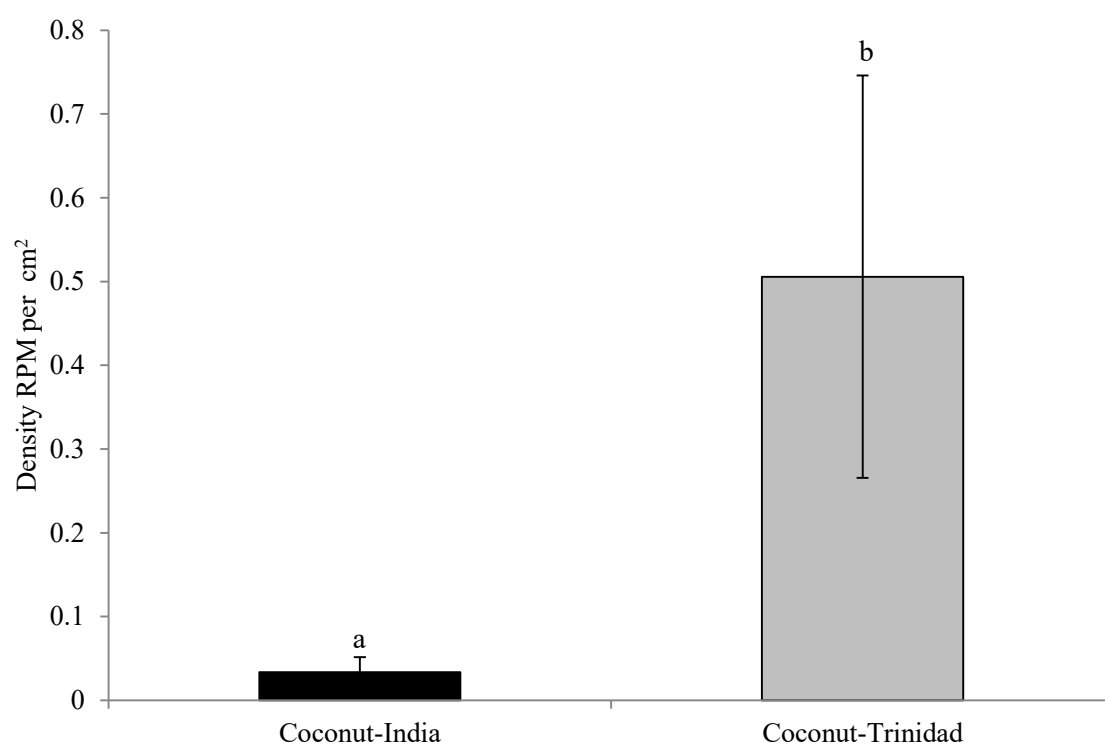


Figure 5.1 Mean density of RPM on *Cocos nucifera* (coconut) palms between India and the Caribbean per cm² ($\pm 1SE$).

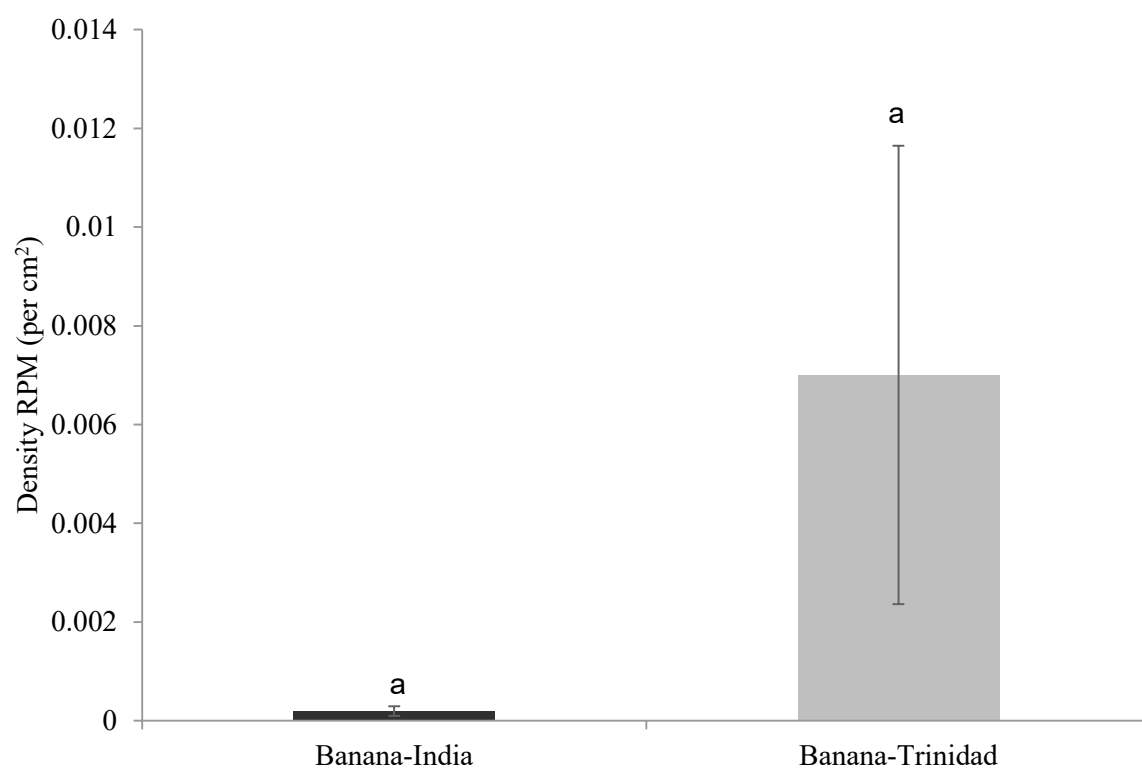


Figure 5.2 Mean density of RPM on *Musa* spp. (banana) plants between India and the Caribbean per cm² ($\pm 1SE$)

5.4.2 Survey results: Predator density and diversity in India and Trinidad

A two-way ANOVA showed that there was no significant interaction between predator densities between country or host ($F=1.88$, d.f.= 1, $p>0.05$) therefore this term was removed from the model. The resulting model showed there was no significant difference in predator density between India and Trinidad ($F=0.13$, d.f.=1, $p>0.05$; Figure 5.3) or hosts ($F=2.36$, d.f.=1, $p>0.05$), however the mean predator density was 2.2 times higher in Trinidad compared to India on *C. nucifera* (0.0018 predators/cm² in Trinidad compared to 0.0008 predators/cm² in India) (Figure 5.3). On *Musa* spp., the mean predator densities were 0.0001 predators/cm² in Trinidad compared to 0.0007 predators/cm² in India- just over five times higher per cm² in India compared to Trinidad (Figure 5.3). There were also no significant differences in the numbers of sites predators were present on *C. nucifera* and *Musa* spp. between Trinidad and India (z ratio=0.42, d.f.=28, $p=0.68$; z ratio=1.05, d.f.=28, $p>0.05$ respectively).

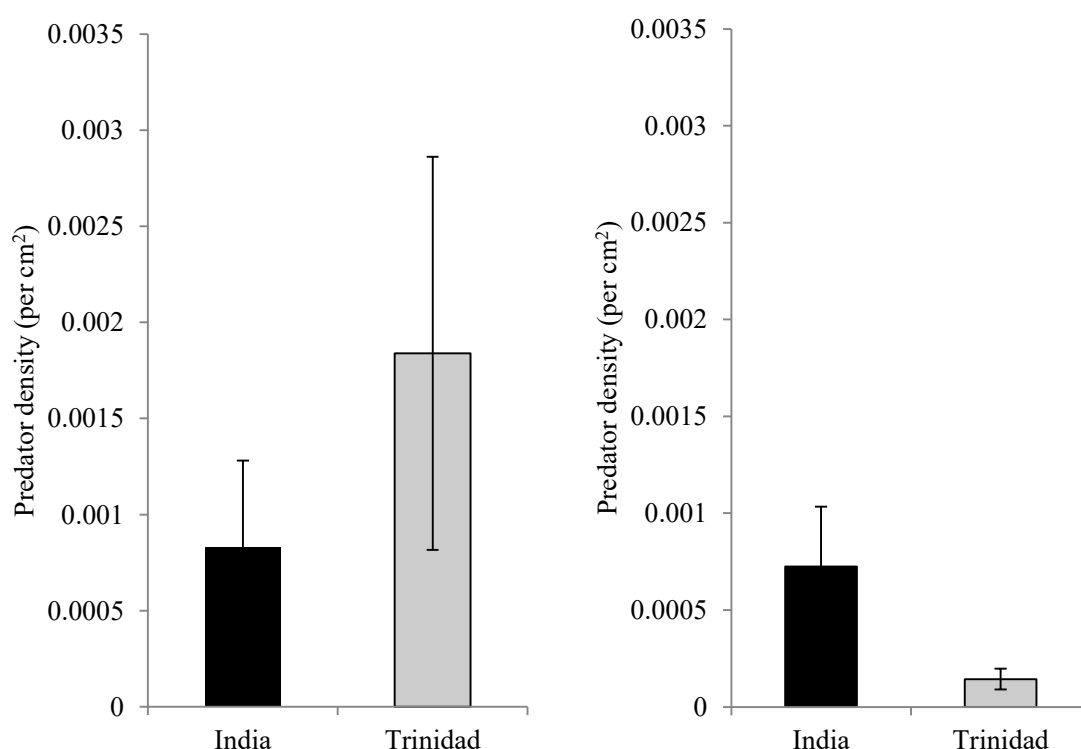


Figure 5.3 Mean density of predators on *Cocos nucifera* palms (left) and *Musa* spp. (right) between India and the Caribbean per cm² (± 1 SE).

On *Cocos nucifera* in India there were only four species of predator collected during the surveys which included *Amblyseius largoensis*, *Cunaxidae* sp., a Cecidomyiidae larva and a predatory Hemipteran, all apart from the Hemipteran were associated with RPM (Table 5.2). The diversity in Trinidad was similar with only three predatory species identified but these were found in higher

numbers. All three species found associated with RPM in Trinidad were Phytoseiid mites (*Amblyseius largoensis*, *Amblyseius coffeae* and *Iphseiodes* sp.). On *Musa* spp. there were many more species of predator identified from samples collected in India with a total of 10 species of predator collected compared to only six in Trinidad (Table 5.2) Predators found in association with RPM in India were *Euseius ovalis*, *Scolothrips* sp., *Neocunaxoides* sp., *Astigmus* sp. and a species of Erythraeidae and in Trinidad, *Amblyseius tamatavensis*, *A. largoensis*, *Amblyseius* sp. (obtusus group), *Iphseiodes* sp., and two species of Cunaxidae. The Shannon Weiner Diversity Index for predators only on *Musa* spp. in India was 1.6793 compared to 1.7329 in Trinidad, however on *C. nucifera* the diversity scores were 1.3863 in India compared to 1.0114 in Trinidad. Herbivorous insects and mites were not included in the diversity calculations as the densities of these were not taken as part of the sampling regime.

Table 5.2 Predators collected during surveys on *Musa* spp. and *Cocos nucifera* in India and Trinidad.
Those marked with * were found in association with RPM.

Host plant	Country	Order	Family/subfamily	Genus/sp.
<i>Cocos nucifera</i>	India	Hemiptera	Reduviidae	
	India	Mesostigmata	Phytoseiidae	<i>Amblyseius largoensis</i> *
	India	Prostigmata	Cunaxidae:Cunaxoidinae	<i>Neocunaxoides</i> sp.1 *
	India	Diptera	Cecidomyiidae *	
	Trinidad	Mesostigmata	Phytoseiidae	<i>Amblyseius coffeae</i> *
	Trinidad	Mesostigmata	Phytoseiidae	<i>Amblyseius largoensis</i> *
<i>Musa</i> spp.	Trinidad	Mesostigmata	Phytoseiidae	<i>Iphseiodes</i> sp.*
	India	Coleoptera	Coccinellidae	
	India	Coleoptera	Coccinellidae	<i>Stethorus</i> sp.
	India	Mesostigmata	Phytoseiidae	<i>Amblyseius largoensis</i>
	India	Mesostigmata	Phytoseiidae	<i>Euseius macrospatulatus</i>
	India	Mesostigmata	Phytoseiidae	<i>Euseius ovalis</i> *
	India	Mesostigmata	Phytoseiidae	<i>Amblyseius</i> sp.
	India	Thysanoptera	Thripidae	<i>Scolothrips</i> sp.*
	India	Prostigmata	Erythraeidae*	
	India	Prostigmata	Stigmaeidae	<i>Agistemus</i> sp.*
	India	Prostigmata	Cunaxidae:Cunaxoidinae	<i>Cunaxoides</i> sp.1 *
	Trinidad	Mesostigmata	Phytoseiidae	<i>Amblyseius tamatavensis</i> *
	Trinidad	Mesostigmata	Phytoseiidae	<i>Amblyseius largoensis</i> *
	Trinidad	Mesostigmata	Phytoseiidae	<i>Iphseiodes</i> sp.*
	Trinidad	Mesostigmata	Phytoseiidae	<i>Amblyseius</i> sp. obtusus group*
	Trinidad	Prostigmata	Cunaxidae	sp3*
	Trinidad	Prostigmata	Cunaxidae	sp4*

5.4.3 Relationship between RPM densities on *C. nucifera* and *Musa* sp. found on the same site in Trinidad

Spearman's rank correlation showed that there was no significant correlation between the densities of RPM found on *Musa* spp. and *C. nucifera* sampled from the same site (data from Trinidad only; S = 421.5, p=0.37, rho = 0.25; Figure 5.4)

When survival of RPM on *C. nucifera* leaf sections from three survival bioassays undertaken in Chapter 4 were compared to survival of RPM on *C. nucifera* leaf sections in Trinidad, no significant difference in survivorship was observed between RPM from Trinidad on *Cocos nucifera* and results obtained from *C. nucifera* in assays 1-3 in India ($\chi^2 = 0.34$, d.f.=3, $p = 0.95$; Figure 5.5).

136

7: $F(3,14)=0.248$, $p=0.10$; Figure 5.6). Over the 7-day period, a mean of 0.76 eggs per female per day were laid by RPM in Trinidad, compared to 0.50, 0.84 and 0.43 eggs per female per day for each of the three different bioassay occasions in India (Figure 5.7).

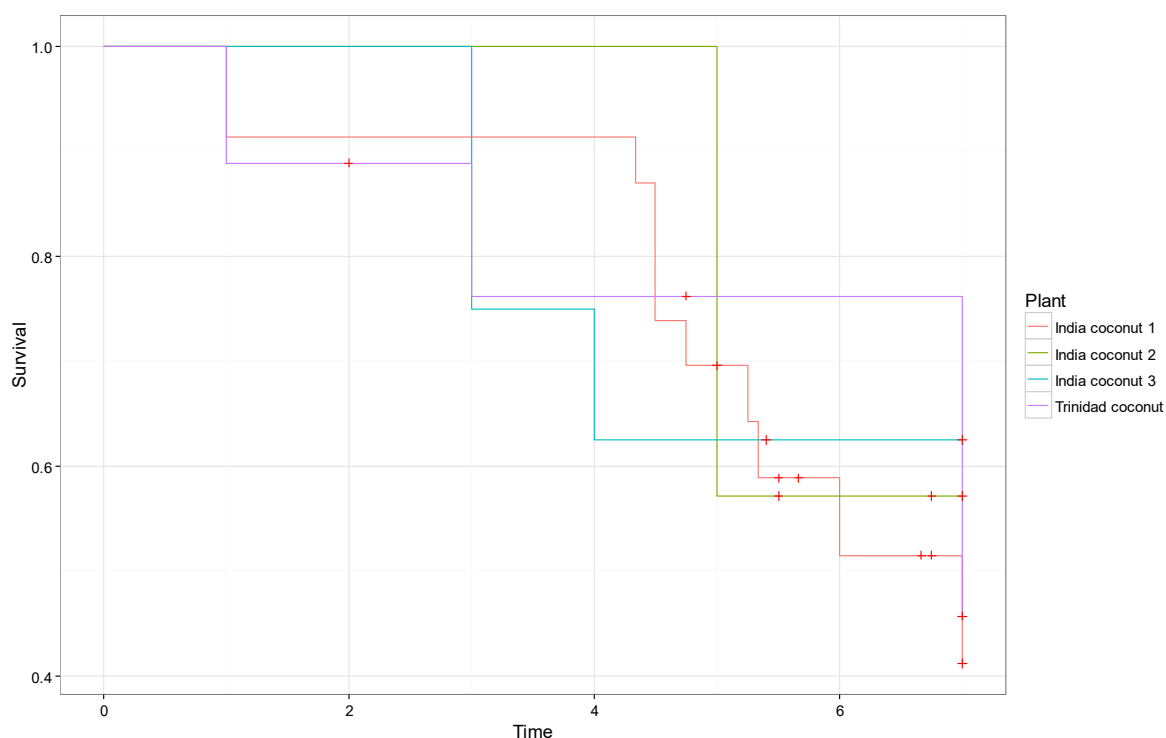


Figure 5.5 Comparison of survival of RPM on coconut leaf sections between India and Trinidad. Data for India taken from RPM collected on *Cocos nucifera* during survival assays 1, 2 and 3.

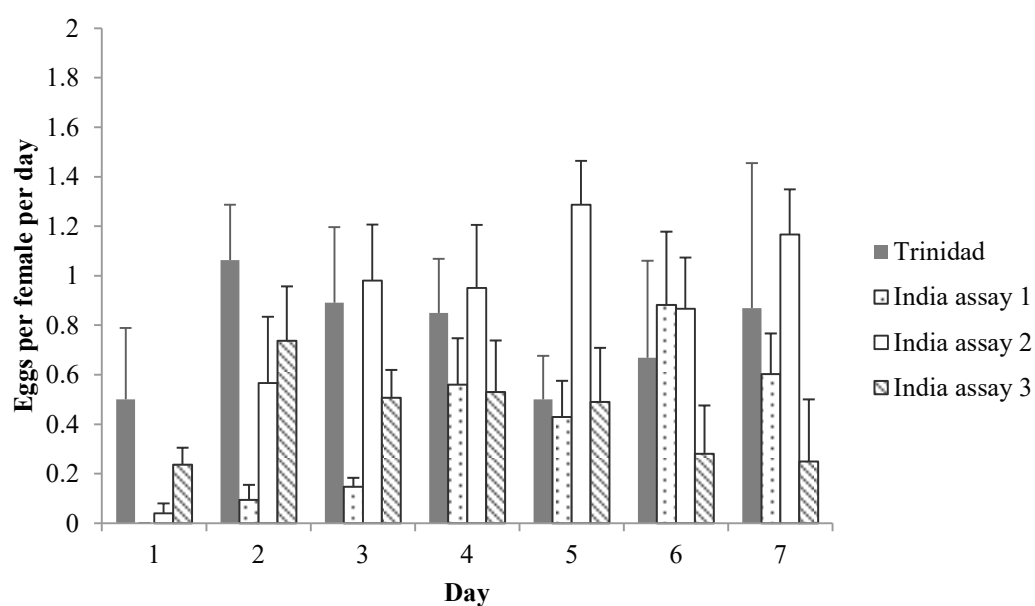


Figure 5.6 Mean number of eggs laid per female per day from survival assay conducted in Trinidad on *Cocos nucifera* compared to the *C. nucifera* controls from assays 1-3 in India (repeated data). No significant differences were found between eggs laid per female on any of the days (days were analysed separately).

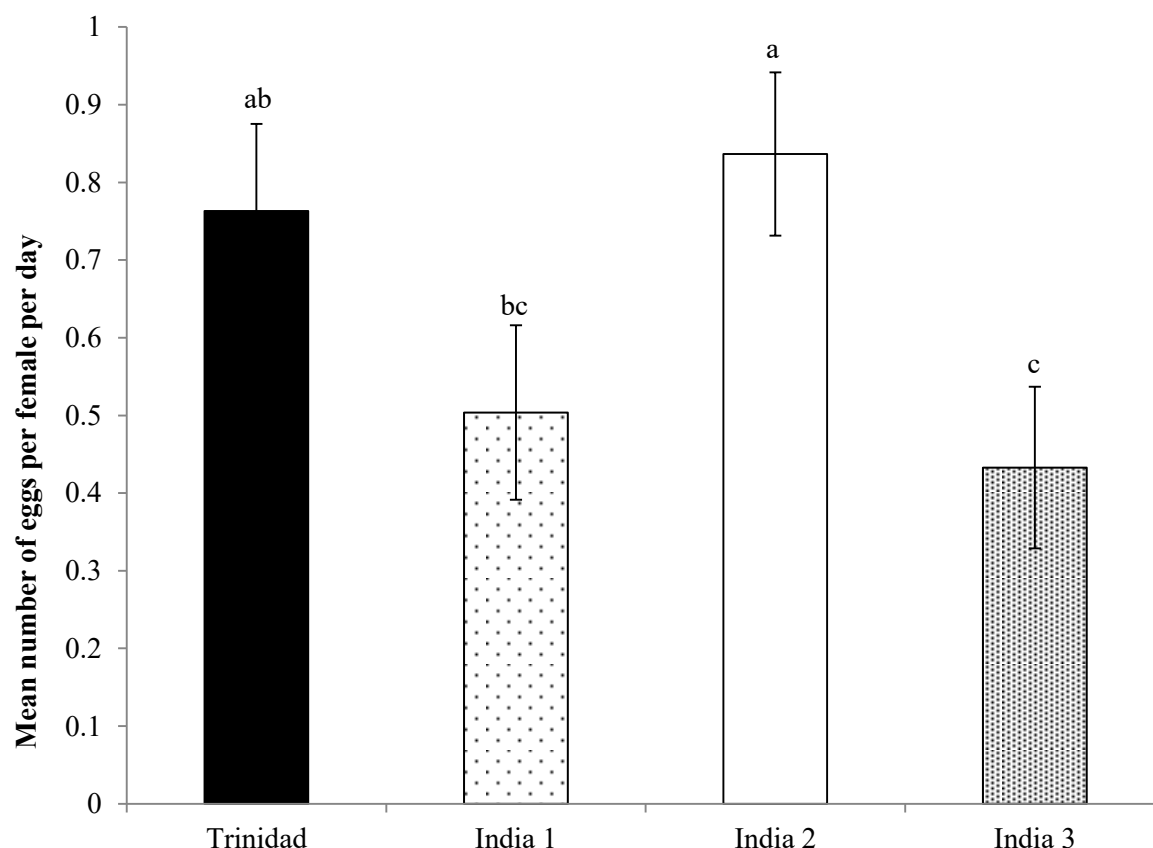


Figure 5.7 Mean eggs laid per RPM female per day, over 7 days in Trinidad and India on *Cocos nucifera*.

5.4.5 Simple degree day model predicting RPM development in each range

The climate data downloaded from the meteorological stations in both regions prior to the sampling events showed that mean daily temperature range was larger in Coimbatore, India compared to Piarco, Trinidad with the mean range of 11.4°C in Coimbatore compared to 9.7°C in Piarco, Trinidad (Figure 5.8). The humidity in Trinidad was generally higher throughout the season compared to that in Coimbatore (Figure 5.9). When the number of resulting degree days above the 10°C lower threshold temperature were calculated for each region (using the data presented in Table 5.3), the number of degree days was higher in Trinidad compared to India (4361.2 compared to 4130.8). Based on the average egg-adult degree day calculation, this resulted in 14.3 generations between September-April in Trinidad compared to 13.5 in Coimbatore. The monthly rainfall for each region was higher in Trinidad for 6 out of the 9 months analysed, with no rainfall falling between December and March in Kerala (Figure 5.10).

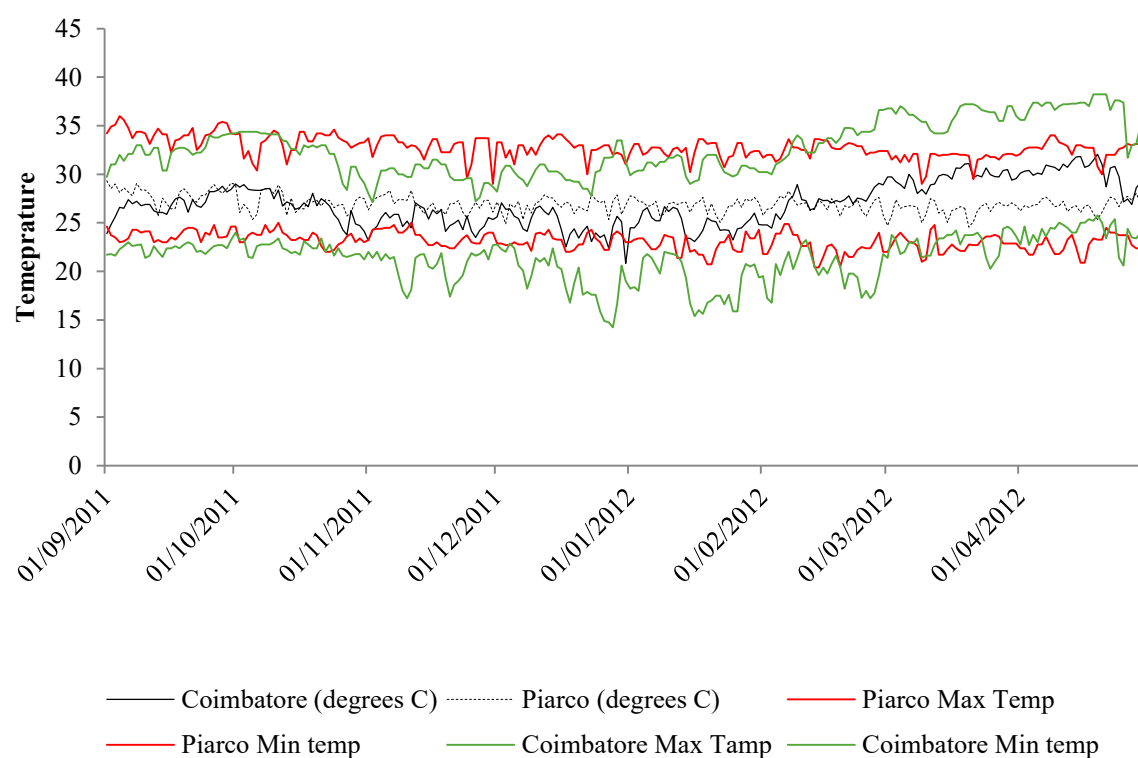


Figure 5.8 Mean, minimum and maximum temperatures for Coimbatore, India and Piarco, Trinidad for 7 months prior to field surveys in March/April 2012.

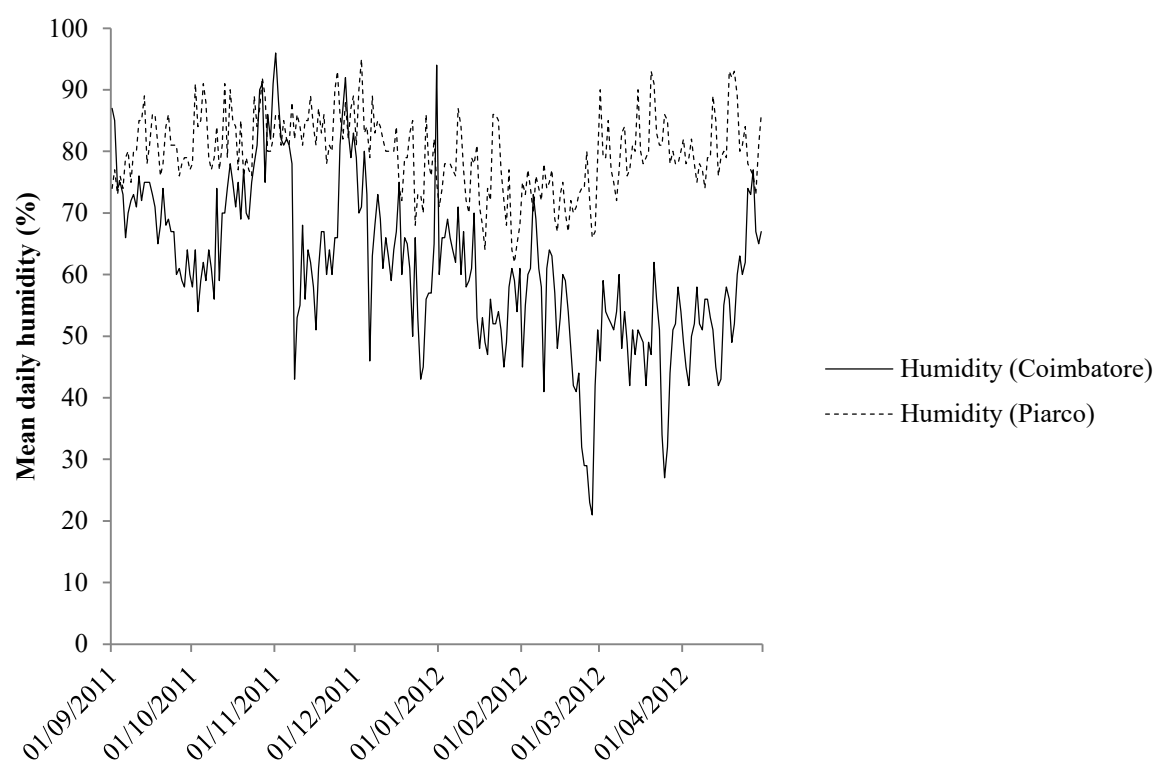


Figure 5.9 Mean daily humidity in Coimbatore, India and Piarcó, Trinidad for 7 months prior to field surveys.

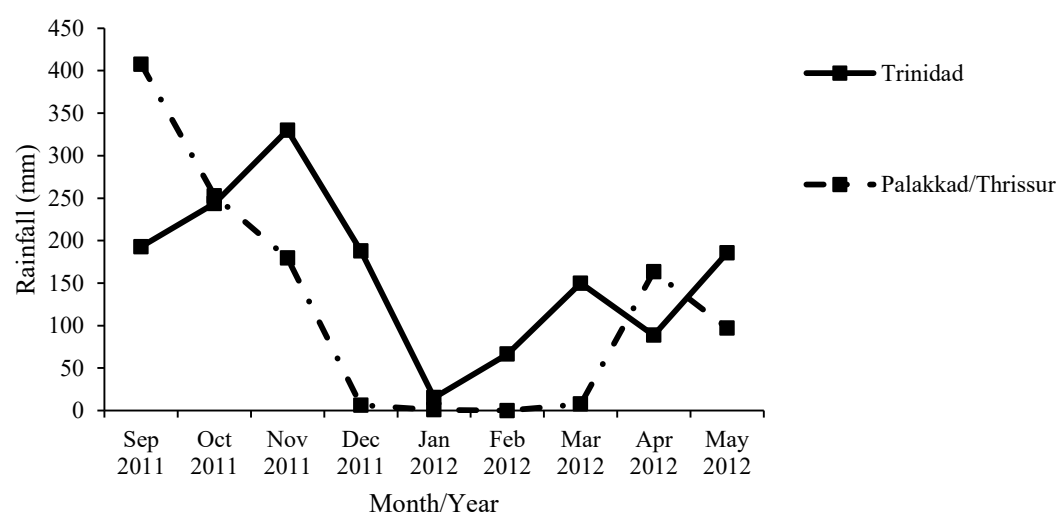


Figure 5.10 Rainfall data for Kerala and Trinidad between September 2011 and May 2012. (source for Kerala data: Knoema, 2018; source for Trinidad: Global Climate Change Portal, 2018)

Table 5.3 Developmental times of RPM taken from literature

Mean temp (°C)	Mean development time egg to adult (y) (days)	x-a (degrees above developmental zero)	Degree days (k(y(x-a)))	Source	Host
24.2	22	14.2	312.4	Moutia, (1958)	<i>Cocos nucifera</i>
17.9	33	7.9	260.7	Moutia, (1958)	<i>Cocos nucifera</i>
26.1	21.4	16.1	344.54		
MEAN			305.88		

5.4.6 Morphological comparison of *Amblyseius largoensis* between India and Trinidad

A MANOVA applied to all *Amblyseius largoensis* measurements showed that there were significant differences in the lengths of dorsal seta Z5 (mean $258.8 \pm 4.10 \mu\text{m}$ in India compared to $275.2 \pm 5.18 \mu\text{m}$ in Trinidad; $F(1,14)=6.6$; $p<0.05$) and the length of SGe IV (mean $116.0 \pm 2.99 \mu\text{m}$ in India compared to $127.7 \pm 2.02 \mu\text{m}$ in Trinidad; $F(1,14)=8.99$; $p<0.01$). All other measurements were not significantly different (Figure 5.11).

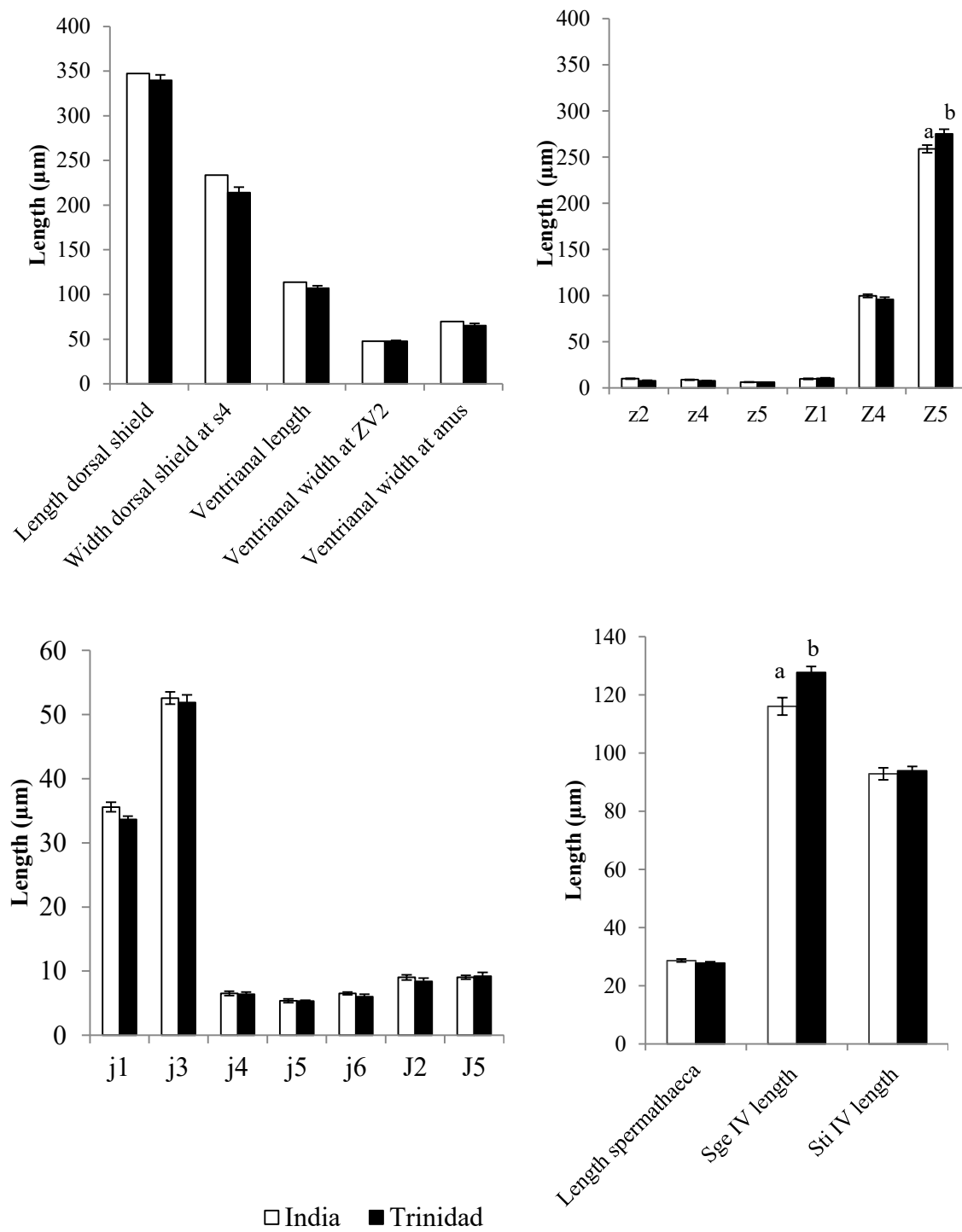


Figure 5.11 Measurements of diagnostics features of *Amblyseius largoensis* specimens collected from India and Trinidad (measurements in μm ; $\pm 1\text{SE}$). Significant differences between specimens from India and Trinidad are marked using different letters

5.5 Discussion

Baseline field surveys confirmed that RPM densities were significantly higher in Trinidad compared to Kerala, India on *C. nucifera*, even though the presence/absence of RPM on host plants did not significantly differ between countries. Although RPM densities were not significantly higher on *Musa* spp. in Trinidad compared to India, mean population densities across all plants surveyed were 35 times higher in Trinidad than in India. There were also significantly more sites where *Musa* spp. were infested with RPM in Trinidad compared to India. Breeding, multigenerational colonies of RPM were observed on *Musa* spp. in Trinidad, however only individuals were found on *Musa* spp. in India, reconfirming findings by Taylor et al. (2011) that *Musa* spp. does not appear to be a host on which RPM are able to establish in this region of India. This contrasts with findings from chapter 4 that RPM can breed in the laboratory on some of these cultivars therefore the incidence and abundance of the natural enemy complex is of great interest to see whether this plays a role in keeping populations under control on *Musa* spp. in the field in Kerala.

The enemy release hypothesis states that one reason for the success of an invasive species may be explained by a reduction of impact natural enemies have in the newly invaded range when compared to the native range. The species of predators collected during the surveys on *C. nucifera* between the two regions were compared and it was found that the predator species diversity was lower in Trinidad compared to India (1.01 compared to 1.39), and the species and family composition of predators did differ somewhat between the two regions. On *Cocos nucifera* in India, the predators were comprised of both Insecta and Acari including, Hemiptera, Cecidomyiidae: Diptera and two predatory mites- *A. largoensis* and a species of Cunaxidae, although the Hemipteran was not found associated with RPM. In Trinidad on *C. nucifera*, the predators collected were comprised of only Phytoseiid mites including *A. largoensis*, *A. coffeae* and *Iphiseiodes* sp., however all were found associated with RPM.

On *Musa* spp. the same pattern was observed as on *C. nucifera*- all predators collected and identified in surveys in Trinidad were Acarine predators -both Phytoseiidae and Cunaxidae, however in India, there were also Erythraeidae, Stigmaeidae, Coccinellidae and Thysanoptera observed indicating potentially a wider diversity of generalists present. The diversity scores however were similar in each region (1.68 in India compared to 1.73 in Trinidad). As sampling was undertaken within a short period of time within each country, the study could be further strengthened through additional surveys throughout different timepoints in the season. Additional surveys could confirm whether this apparent difference in predator complex is a true representation of species composition in different communities. Given the results to date, however given the lack of colonies found on *Musa* spp. it appears that this abundance and diversity of natural enemies found on *Musa* spp. could be keeping

RPM populations under control in India and further studies would need to be carried out to elucidate which are the key predators in this system.

Of the predators found India and Trinidad, there appeared to be some species which were similar/same. *Amblyseius largoensis*, was found in both Trinidad and India in association with RPM. *Amblyseius largoensis* was first described by Muma (1955) from specimens collected in Key Largo, Florida and has been reported in 54 countries/territories globally (Moraes et al., 2004) and re-described by a further 23 authors (Moraes et al., 2004). *Amblyseius largoensis* has been reported to be a Type III generalist predator, most likely able to prey on a wide range of microarthropods, supplementing its diet with pollen and is more likely to be co-evolved to the plant host than the arthropod prey (McMurtry & Croft, 1997). It is unlikely that the species is a Tetranychid specialist due to the lengths of its dorsal setae and their arrangement (McMurtry & Croft, 1997), therefore its presence in association with RPM populations is unsurprising. Prior to the invasion of RPM into Florida, Peña et al. (2009) showed that the microarthropod community inhabiting coconut leaflets included *A. largoensis* and that possible prey prior to the invasion of RPM were the scale *Aonidiella orientalis*, *Aleurocanthus woglumi*, *Tetranychus* spp. and *Tetranychus gloveri*. *Amblyseius largoensis* has also been recorded feeding on *Aceria guerreronis*, *Tetranychus urticae* (Galvão et al., 2007), *Polyphagotarsonemus latus*, *Oligonychus punicae* (Hirst.) (Sandness & McMurtry, 1970; 1972) and *Panonychus citri* (McGregor) (Tanaka and Kashio, 1977; cited in Rodrigues et al. (2010)). In the present study, *A. largoensis* was found in association with RPM, however no work was carried out to establish population dynamics over time, although strong evidence that *A. largoensis* tracks RPM populations has been shown in Cuba and Florida (Pena et al., 2009; Ramos-Lima et al., 2011) and it has been hypothesised that *A. caudatus* reported in association with RPM in Mauritius by Moutia (1958) could actually have been *A. largoensis* (Carrillo et al., 2012c).

During the study, there was also a specimen of *A. tamatavensis* found in association with RPM in Trinidad on *Musa* sp.. Taylor et al. (2012) noted that when the species description for *Amblyseius channabasavanni* commonly reported in association with RPM in India was studied, it was very similar to the description on *A. tamatavensis* therefore comparison of Indian specimens of *A. channabasavanni* and Trinidadian *A. tamatavensis* may elucidate similarities/differences in taxonomy and also confirm whether these are common predators found in association with RPM in both regions. *Amblyseius channabasavanni* (Gupta, 1978) was first described in India from a specimen collected from *Chrysanthemum* sp., in Trivandrum, Kerala, but has also been reported in *Areca catechu* in association with RPM (Daniel, 1983). The female predator reportedly consumed on average between 11-42 RPM eggs per day, although all stages of RPM could be attacked by this predator. Daniel

(1983) also mentioned that this predator appeared to be a specific predator of RPM.

Species in India which were distinct from those found in Trinidad were *Scolothrips* sp., *Euseius* spp., *Astigmus* sp., Cunaxidae, Coccinellidae and Erythraeidae. *Scolothrips* sp. are of potential interest as Mound et al. (2010) reported a new species of *Scolothrips* (*Scolothrips ochoa*) feeding on the genus *Raoiella* in Australia. The specimens recovered during this survey were checked carefully and not found to be *Scolothrips ochoa*- the only *Scolothrips* species recorded to feed on mites species other than those found in the Tetranychidae (Goldsmith, 2009). The species is likely to be *Scolothrips asura* due to its strong bi-colouration (dark pigmented thorax and pale abdomen) (Goldsmith, 2009) and is a likely predator of Tetranychidae, however its status as a predator of RPM is unconfirmed.

Euseius spp. and *Astigmus* sp. are likely to be generalist predators feeding on mites such as Tetranychids/Tarsonemids/Eriophyids (McMurtry & Croft, 1997; Sheeja & Ramani, 2009) therefore may prey on RPM, although they have not been reported as predators of RPM in the literature. The specimens of Erythraeidae and Cunaxidae are likely to exert little influence on the populations of RPM – Erythraeidae are generally found in low abundance (Sheeja & Ramani, 2009) and only one species of Cunaxidae has been shown to feed to any great extent on Tetranychidae in India (Sheeja & Ramani, 2009). As generalist predators, Coccinellidae are likely to prey on RPM. *Stethorus keralicus* has been shown to prey preferentially on RPM (Daniel, 1976) however it was not possible to identify specimens collected in this study to species level.

During the surveys in Trinidad, it was surprising that insect predators were not sampled during the surveys as previous authors have found *Aleurothrips fasciapennis* and species of Cecidomyiidae, and Chrysopidae associated with RPM in Trinidad (Pena et al., 2009) and in Cuba, *Chrysopa cubana* Hagen (Neuroptera: Chrysopidae), *Stethorus* sp., *Scymnus* sp. (Coleoptera: Coccinellidae) and *Orius insidiosus* Say (Hemiptera: Anthocoridae) have all been reported frequently associated with RPM host plants (González et al., 2013). It is possible that improvements to the sampling procedures used here may increase the frequency that insect predators are identified within samples. Collection of leaflets may disrupt adult insect predators which are able to fly away from samples during collection; however, this does not explain the absence of larvae. Again, season long sampling would enable a more complete picture of predator associations throughout the season and should be considered for future work. During more recent experimental work in Trinidad, observations are that a Neuropteran is laying eggs in association with RPM colonies and it is possible that this species is responding to RPM populations, thus further work needs to be conducted to explore this association. The difference observed in RPM densities between regions could be related to the efficacy of these macro-predators

such as Coccinellidae as the micro predators such as *A. largoensis* have been shown to have a preference for feeding on the eggs of RPM, rather than adults. Daniel (1976) demonstrated that *S. keralicus* was able to consume on average 282 RPM during development which would have a significant effect on the growth of populations, and the lack of an effective macro predator such as this in the adventive range could underpin the dramatic population growth of RPM in the New World. Future work should also aim to increase the number of survey sites or the time period over which studies were conducted as the low numbers of predators from the 15 sites in this survey recovered could bias diversity indices and further collections may increase the numbers of collections of wider groups of predators. This was not possible during this survey due to time and resource restrictions. Additionally, during the surveys only the densities of predators and RPM were noted and for a more rounded approach, subsequent analyses should take into account the densities of other phytophagous mites on the host plants as these may affect the presence of predators found. Further surveys focussing on the collection and enumeration of macro predators should be undertaken as these may be key in controlling RPM population growth.

It was hypothesised that the presence of RPM on secondary hosts such as *Musa* spp. may be related to the density on the major host *C. nucifera*. With such high densities on *C. nucifera* in Trinidad mites could be dispersing from crowded low quality leaflets on coconut palms to establish on hosts such as *Musa* sp., however no evidence to support this was found during this study. When density results were compared to those reported from Cuba (Ramos-Lima et al., 2011), RPM densities in Trinidad were much lower than those reported at their peak in Cuba on *Musa* spp. (2.15/ square centimetre, compared to 0.007 RPM per square centimetre in Trinidad), however sampling methodologies differed greatly, in Cuba 10cm² leaf sections were sampled as opposed to whole leaves used in this study and it was not clear if this is a 10 x 10 section of leaf of 10 square centimetres.

For results from India, RPM densities reported in this study were lower than those reported by Taylor et al. (2012) on *C. nucifera* using a similar sampling method to the one in the present study. The authors reported a mean peak of just over 0.1 RPM per cm² and 0.15 RPM per cm² from *C. nucifera* in the same survey region during March and April 2009, compared to 0.03 per cm² in March 2012 reported during this survey. This highlights the importance of using standardised survey techniques between regions and standardised timings to assess comparative densities, as false assumptions related to abundance may be made. A further survey in April 2012 may have shown higher densities of RPM on *C. nucifera* in India, however given the magnitude of increase between March and April in Taylor et al. (2012), it is unlikely that the densities would have equalled those in Trinidad. Season long surveys carried out in both regions increasing the site replication would strengthen the current

observations as this would take into account seasonal fluctuations in density. A power test carried out showed that using a two-way ANOVA only had a power value of 0.65 with α at the 0.05 level, with a large effect size (0.35 as per Cohen (1992)) during this study. To increase the power level to 0.8, the numbers of samples per country would have needed to be 21. For a one-way ANOVA, the power was calculated as 0.75 with the current sample size.

To help explain the difference in densities between regions reported, the effect of local climate was considered, whereby meteorological station data for nine months prior to the surveys (01/09/2011-30/04/2012) was used to build a simple degree day model with information taken from the literature on RPM development in response to temperature. The model showed over a 9 month period prior to the survey, there would have been 14.3 generations between September-April in Trinidad compared to 13.5 in Coimbatore. These figures are not different enough to warrant the differences in the populations observed based on temperature alone. A possible explanation is that RPM populations in Kerala, India are observed to go through a “boom-bust cycle” whereby the populations are barely detectable between June-December, however between January and May populations build up to significant numbers (Taylor et al, 2012; Yadavbabu & Manjunatha, 2007). The annual population crash occurs around the same time as the onset of monsoon rains in this region, thus it could be that the intensity of rains during this period act to dislodge mites and dramatically reduce the populations to levels where it is difficult to build back up to high population densities. Between 2011 and 2015 in the Palakkad region of Kerala, peak monthly rainfall was recorded between 387mm-797mm and exceeded 250mm on 16 occasions (Knoema, 2018). Peak rainfalls occurred between June and August annually. In comparison, the peak rainfall experienced by Trinidad in the same time period only exceeded 250mm on one occasion (Climate Change Knowledge Portal, 2018). The implication of this could be that RPM populations are never dramatically reduced in Trinidad, perhaps allowing population growth over a period of time and hence denser populations. In a sense, RPM populations may be benefitting from a ‘monsoon release’ rather than an enemy release, whereby rains have a lesser impact on populations in the adventive range. In India as the monsoon is a regular phenomenon which occurs every 12 months, the populations are only able to build to a finite level before the dramatic reduction occurs once again. Studies in Cuba and Puerto Rico have shown that the population increase and dramatic decrease occur in the adventive range but the decreases are to a lesser extent. Peña et al. (2009) presented RPM population dynamic data from Puerto Rico which showed a dramatic increase in RPM populations in March 2008, but also with a dramatic decrease in April 2008, although populations only fell to around 500 RPM/leaflet. Rainfall in February and March was <100mm, however during April this increased to 189mm which coincided with a population drop (rainfall data taken from Climate Change Knowledge Portal, 2018). Ramos-Lima et al. (2011) showed

that RPM populations increased rapidly in Cuba on *Musa* spp. from January until April 2009, however there was a decline in RPM populations densities with the onset of heavier rains in this case from lower rainfall levels at <30mm/month to 80-100mm at the end of April/start of May 2009. However, once again the decline was only to just under 1000 RPM/10cm² from >2000/10cm². When compared to figures presented in Taylor et al. (2012), it is possible to see that appreciable rainfall occurred in May/June 2009 (around 600mm) which coincides with populations on *C. nucifera* dropping from around 0.12 RPM/cm² to almost zero. It could therefore be hypothesised that intensity or duration of rainfall may be a limiting factor in RPM population increase.

There was no significant difference in survival observed between RPM from India and Trinidad on *C. nucifera* leaf sections and no significant difference in oviposition rate between RPM from either region. Results from these assays were in line with other reported oviposition rates- Cocco & Hoy (2009) reported a mean oviposition rate of 0.93 eggs per female per day for RPM in Florida on *C. nucifera* leaf sections which is in line with mean results obtained in Trinidad (mean 0.76/day) and the second assay in India (0.84/day). Two of the assays in India reported lower eggs per female per day (0.50 and 0.43 respectively), but only the latter was significantly different to those reported from Trinidad. These results indicated that *in vitro*, given constant conditions, reproductive rates could be expected to be similar between regions using this methodology. Further assays should investigate this on attached leaves in the field to assess whether this is a true reflection of activity when predators are excluded. Additionally, gathering data on total oviposition, oviposition period and longevity would give a more robust comparison. Ramos-Lima et al. (2011) followed RPM females for their entire oviposition period in Cuba and noted that eggs per female per day were generally much higher 3-7 days after emerging as adults, with mean oviposition rates between 1.5-4 per day in this period, but dropping following on from this. The time period in each country during the present study did not allow for a generational cohort of RPM to be reared and studied using this method, however this may explain some of the variability in the results from India when replicated on different occasions as different aged adult females may have been tested. The randomisation method applied to the experimental set up should have eliminated any biases of selection of RPM from similar age cohorts, therefore this should negate any effects of female age in the experimental design.

Comparisons of specimens of *A. largoensis* from India and Trinidad showed that the only significant difference between *A. largoensis* specimens morphologically were the lengths of the dorsal seta Z5 and the length of the genu on leg IV. In the study by Navia et al. (2014), seta Z5 was shown to be significantly longer in Trinidad when compared to *A. largoensis* specimens from La Reunion and Brazil. A comparison with results in the present study showed that the Z5 measurements in this study

were much shorter than those from the Navia et al. study indicating that this is a variable feature which differs within and between regions. Results from Navia et al. (2014) showed that specimens from La Reunion had longer j1, Z4 and s4 compared to those in the Brazil and Trinidad, however no significant differences between these measurements were found during the present study. Tixier (2012) (cited in Navia et al. 2014) presented a guide to distinguish between interspecific and intraspecific variability among the Phytoseiidae, whereby if the mean seta length for one group of specimens is $< 65\mu\text{m}$, a difference of $10.58\mu\text{m}$ on average needs to be observed to relate to inter-specific variability, and for setae $>65\mu\text{m}$ a mean difference of $33.99\mu\text{m}$ needs to be observed. Following these guidelines, the differences in setae length observed between India and Trinidad show intra-specific variability in these characters and thus is likely to constitute the same species. It was not possible to conduct molecular analyses on collected samples as they were prepared as permanent slides, so further work should be conducted to demonstrate this at a molecular level. A preliminary study was carried out to assess eggs consumption of *A. largoensis* in India, and this showed mean daily consumption rates of 48.4 ± 7.39 eggs per day ($n=5$ specimens, data taken over 3d) (data not shown –see Appendix 13 for details) which is in line with findings by Carrillo et al. (2012b) who showed that *A. largoensis* reared on RPM consumed on average 47.2 ± 2.7 eggs per day, indicating the performance in terms of eggs consumption may be similar between countries.

This is the first time that RPM and associated predator populations have been compared using the same survey methodology between regions, and for the first time confirm the extent of the severity of RPM outbreaks in Trinidad; however, the reasons behind these differences are less clear. Given the laboratory performance data, it would be expected that RPM performance would not differ dramatically in the field which points to extrinsic factors which may be influencing RPM populations, namely predator associations or predatory species richness, or potentially abiotic factors such as rainfall. The presence of *A. largoensis* in both India and Trinidad, on both *Musa* spp. and *C. nucifera* indicates the importance of this predator, however even though higher densities of the predator are observed in the field in Trinidad, it seems to be having little effect on population suppression. Studies comparing the morphology, and preliminary studies investigating eggs consumption indicated the specimens collected in India and Trinidad were of the same species and performed similarly in terms of egg consumption. Alternative hypotheses for the difference in population densities of RPM should therefore centre on the combined impact of the different groups of predators associated with RPM in India or investigate further whether the differences in the RPM morphology demonstrated in Chapter 3 are having an effect on predatory efficacy. Additionally, further studies should take an intra-season approach to studying the fluctuations in RPM populations as this study only captures a snapshot of RPM populations when they are at their reported highest. Work by other authors indicates that the

populations of RPM crash in both Trinidad and India and this chapter does not take into the account the effect of these population crashes on seasonal abundance of RPM or what could potentially cause the population crashes. Other possible causes not studied during this chapter are the effect of acaropathogens on mite populations.

In conclusion, at the outset of this set of studies, three hypotheses were suggested. Results confirmed that RPM populations were significantly denser in the adventive range compared to the naturalised range on *C. nucifera* and were higher and significantly more abundant on *Musa* spp. in the adventive range. The second hypothesis stated that there would be a relationship between RPM densities on *C. nucifera* and *Musa* spp. found on the same site- however results from this study did not support this. Finally, predator diversity was shown to be poorer on *C. nucifera*. in Trinidad compared to India, however results on *Musa* spp. were similar, with a marginally higher diversity observed in Trinidad.

6.0 The effect of RPM lateral setae and secreted droplets on predation by *Amblyseius largoensis*

6.1 Introduction

In chapter 5, it was shown that a range of generalist predators were found in India and Trinidad, but the species compositions differed, apart from the presence of the predator *Amblyseius largoensis* (Acari:Phytoseiidae) which occurred in both countries. Furthermore, it was shown that population densities of the RPM were significantly higher in Trinidad compared with those in India. Although the composition of the predator complex was shown to differ and this will have an important impact on RPM populations in both regions; *A. largoensis* remains the focal organism in biological and classical biological control programmes due to its strong association with RPM populations in several countries in the Old and New World (Hoy, 2012; Moraes et al., 2012; Navia et al., 2014; Silva et al., 2014). Given the differences observed in RPM densities, several questions arise about the efficacy of the predator complex in the New World versus the Old World. Although there will be several factors underlying these differences, the efficacy of *A. largoensis* as a predator may be one contributory factor. Findings in chapter 3 showed that RPM lateral setae lengths differed between Kerala, India and the Caribbean, and one avenue of exploration may be, that although *A. largoensis* is present in both regions, the efficacy of the predator may be reduced by these morphological differences, as RPM with longer lateral setae may be ‘better defended’. These differences may contribute to the success of RPM in the adventive range. The following section outlines literature about the relationship between *A. largoensis* and RPM, efficacy and mode of action of Phytoseiid predators and prey defence mechanisms.

Population increases of *A. largoensis* are positively correlated with RPM population increases in Trinidad (Pena et al., 2009), and many authors have reported *A. largoensis* as the primary predator in other parts of the adventive range (González et al., 2013; Pena et al., 2009; Ramos-Lima et al., 2011). It has also been reported associated with RPM throughout the Old World (Hoy, 2012; Silva et al., 2014; Taylor et al., 2012), indicating the importance of the association. In Florida, USA, Carrillo & Peña (2012) found that *A. largoensis* females had a marked preference for consuming RPM eggs over adults and observations by Rodríguez et al. (2010) showed contact with RPM setae and droplets on the end of adult RPM setae caused a change of direction of *A. largoensis*. These results indicated that dorsal setae and droplets may act to repel predators, however no studies have formally investigated their role. In addition, there are no studies which have investigated the effect seta length could have in

repellence of predators. This could be important given the observed differences in lateral seta length of RPM from the populations studied in the adventive range and India.

In the first section of this chapter, literature on predator and prey morphology is presented, then two background studies were undertaken to establish whether lateral seta length could potentially cause a barrier to predation through comparison of attacking structures on *A. largoensis* and defensive setae on RPM. Then, the nature of droplet expression was explored using data collected during survival studies in chapter 4 to establish whether droplet expression was similar between regions and under what conditions droplets are present. Following these studies, experiments testing the effect that lateral setae and droplets have in relation to RPM defence were undertaken and presented, with the hypotheses that both lateral setae and droplets will play a role in defence of RPM.

6.1.1 Mode of action of Phytoseiids

Predation of prey mites by a predator will depend on several factors. Firstly, the predator must be able to choose and locate the prey effectively, then capture and seize the prey successfully and thirdly consume the prey. Prey mites however, often have evolved defensive features or behaviours to evade predators. Symondson et al. (2002) highlighted that the prey choice of predators may be restricted either physically, physiologically or behaviourally; and prey size is likely to be an important contributory factor where the prey range is likely to be determined by the predator: prey size ratio especially preying on those which are smaller and less mobile (Sabelis, 2009). Studies have shown that chelicerae are involved in feeding and are used to catch, seize, tear and manipulate prey, and that large chelicerae are related to arthropod prey diets (Buryn & Brandl, 1992). For Phytoseiids, the mode of action of predation is often through ‘grasping’ with chelicerae, with prey tending to be smaller and less mobile when ‘graspers’ do not reach far or are absent (Sabelis, 2009). This is indicative that the size of chelicerae versus size of prey is important. Usher & Bowring (1984) showed that *Gamasellus* sp. (Acari:Ologamasidae) used forelegs to immobilise prey, then chelicerae were used to pierce and excavate the body of the prey mite.

6.1.2 RPM defence

To ward off attack by predators, prey species often have evolved defences to repel the predator. Prey defences outlined by Evans (1992) included the production of ‘distasteful chemicals’ and enlarged setae which may be erected in response to predator attack. Evans (1992) stated that *Tullbergia granulata*, a springtail, produced a secretion which was distasteful to predators when attacked, and evidence suggests that the droplets on the distal end of RPM setae may have a defensive role. As mentioned, Rodríguez et al. (2010) observed that contact with RPM setae and droplets on the end of

setae caused a change of direction of *A. largoensis* and authors also observed repellence from colonies where there were many exuviae. Droplets at the end of setae have been reported for all member of the genus *Raoiella* (Beard et al., 2012) however little is known of their function, although they are hypothesised to be defensive secretions. From observations, these droplets are expressed constantly and are present on all life stages- including the stipe of the egg (Beard et al., 2012).

Setae have also been demonstrate to be important in defence against predation; Yano & Shiotsuka (2013) were able to demonstrate using *Panonychus citri* (Acari:Tetranychidae) that dorsal setae play a role in defence against predators, with almost 100% of mites predated when dorsal setae were removed compared to 10-20% when setae were left *in-situ*.

6.2 Baseline studies

6.2.1 Investigating and characterising the theoretical barrier to predation of RPM posed by long lateral setae on RPM specimens in Trinidad compared to India when compared to ‘attacking structures’ of *Amblyseius largoensis*

Methods

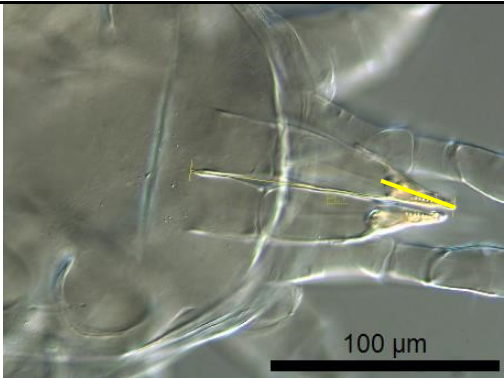
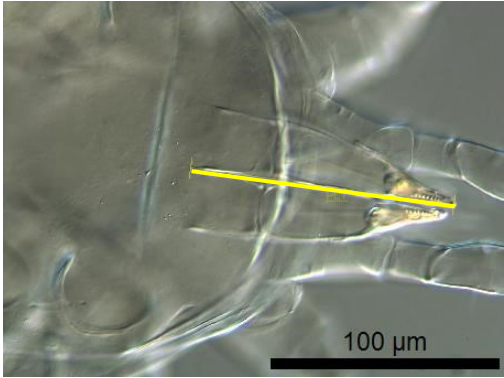
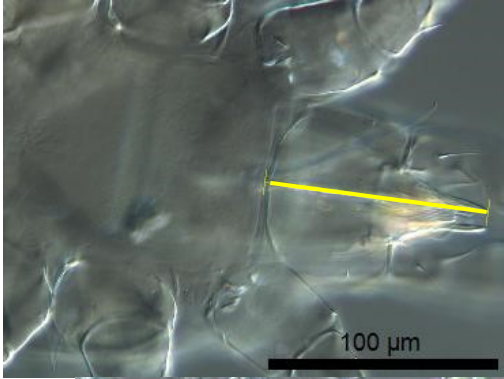
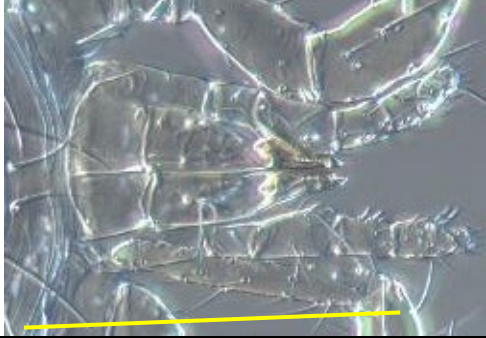
Given the results in chapters 3 and 5, it was clear that the morphology of RPM between regions differed significantly, however the morphology of the structures of *A. largoensis* measured did not differ significantly. The study in chapter 5 however did not investigate in detail the structures involved in attack of RPM. To date it is not known to what extent this will have on the ability of *A. largoensis* to attack adult female RPM as little has been established about the mechanism of attack and whether a small difference in seta length could help ‘better defend’ RPM. This baseline study aimed to establish in theory whether there could be a barrier to attack of RPM posed by long lateral setae. To define the theoretical barrier to attack posed by lateral setae, an understanding of the predator attack mechanism was required; thus, first a study was undertaken to describe the attack sequence of an adult female specimen of *A. largoensis* in conjunction with an adult female RPM specimen; this is summarized in Appendix 14. The attack sequence showed that the predator contacted the RPM specimen with its front leg then lunged using its chelicerae to attempt to seize the RPM specimen. Assuming the chelicerae are the main method of seizing and accessing prey, then the physical dimensions of these and their accessory structures need to be defined in relation to the length of RPM lateral setae. The chelicerae sit on the gnathosomic base (chelicerae plus gnathosomic base are referred to as the cheliceral process here) and are ‘pushed out’ using hydrostatic pressure from

within two membranous tubes of which are attached to the body of the chelicerae when the predator attacks its prey. The gnathosomic base is separated from the main body cavity by a membrane (Evans 1992). The pre-oral channel works in conjunction with the labrum (opposite it) to sieve solid food particles from their food, therefore this requires close contact with the prey for successful consumption (Evans, 1992). Following successful attack, the prey is either brought towards the salivary stylus or this is protruded so that fluids may be imbibed. Palps may act as sensory appendages or may play a role in seizing and holding prey. The underlying theoretical basis for repellence would be that the predator is repelled when it contacts a lateral seta of RPM, thus if the structures involved in attack are longer than the lateral setae, successful attack is likely to occur; if the attacking structures are shorter than the lateral setae, repellence is likely to occur.

Using this logic, the length of ‘attacking structures’ of specimens of *A. largoensis* and ‘defensive structures’ of RPM were measured and compared between regions. Measurements of the mean length of one chelicera, chelicera+cheliceral sheath (cheliceral process), hypostome+gnathosomal base and palp length (as shown in Table 6.1) were measured on 22 specimens of *A. largoensis* from Trinidad and 19 specimens of adult female *A. largoensis* from India. These were then plotted against mean lengths of lateral setae (c3, d3, e3 and v2) of RPM collected from *C. nucifera* in India (n=69) and Trinidad (n=128).

For both Indian and Trinidadian specimens, mean lengths and standard errors of lateral setae (RPM) and predator mouthparts (*A. largoensis*) were calculated from data and plotted on scatter plots. A control line was added to plots showing where the length of lateral setae would equal the length of the measured mouthparts. An ANOVA was fitted to data for predator mouthparts to show any significant differences in morphology present between regions.

Table 6.1 Mouthpart measurements taken on *Amblyseius largoensis*.

Mouthpart	Description of Measurement	Picture
Chelicera	Base to tip	
Chelicera plus cheliceral sheath (cheliceral process)	Base on membrane to tip of chelicera	
Hypostome and gnathosomal base	Tip of hypostome to gnathosomal base	
Palp length	From gnathosomal base to tip of palp	

Results

Results showed that there was no significant difference in the length of chelicerae ($F(1,39)=0.4$, $p>0.05$), chelicerae and base ($F(1,39)=2.3$, $p>0.05$), length of palps ($F(1,39)=2.7$, $p>0.05$) and length of the base of the chelicerae ($F(1,39)=1.6$, $p>0.05$) between specimens from India and Trinidad, however, there was a significant difference in the length of the hypostome between countries with the mean length of $100.7\mu\text{m} \pm 0.64$ in India compared to $97.2\mu\text{m} \pm 0.74$ in Trinidad ($F(1,39)=12.31$, $p<0.05$; Figure 6.1).

Figure 6.2 shows the comparison with mean lateral setae lengths of RPM taken from each region in comparison to lengths of corresponding attacking structures of *A. largoensis* from the same region. The control lines shown represent the point where the lateral setae length would equal that of the measured body part, with the theory being that if the body part of *A. largoensis* involved in the attack behaviour is longer than the lateral setae, there is a lower chance that *A. largoensis* would be repelled as the predator would be able to contact the RPM body *before* the lateral seta has contacted the body of *A. largoensis*. Thus, if the body part is shorter, there is a higher chance that *A. largoensis* would be repelled. Figure 6.2 showed that the length of chelicerae would not affect the behaviour of *A. largoensis* to any degree, however since chelicerae are pushed out on the cheliceral process the length of this chelicera base plus chelicera was measured. Mean readings showed that *A. largoensis* preying on RPM in India would in theory *not* be repelled, however the results for the *A. largoensis*:RPM combination in Trinidad are on the borderline depending on which lateral seta the predator approached (from the rear would mean repellence, from the anterior contacting v2 would not). These results were more pronounced for the hypostome length: lateral setae length in both regions- with a likelihood that *A. largoensis* would be more likely to be repelled in Trinidad than India given this hypothesis.

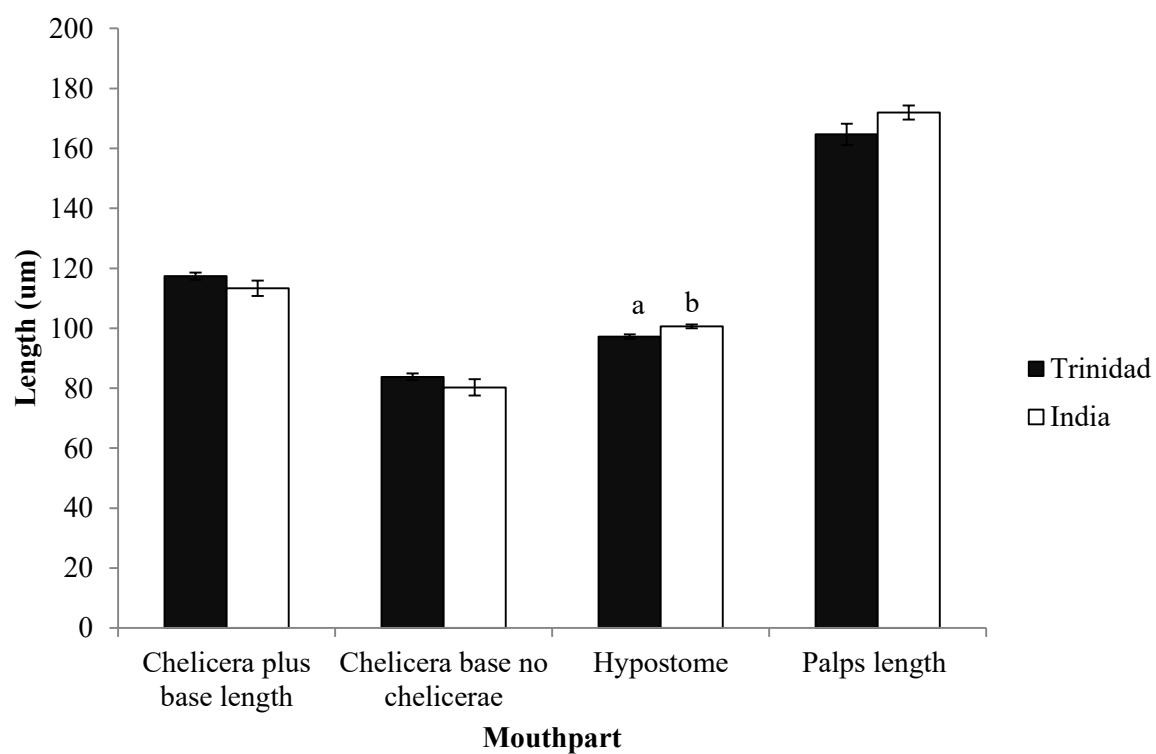


Figure 6.1 Comparisons of lengths of mouthparts and palps of *Amblyseius largoensis* originating from India and Trinidad which may be used to attack prey.

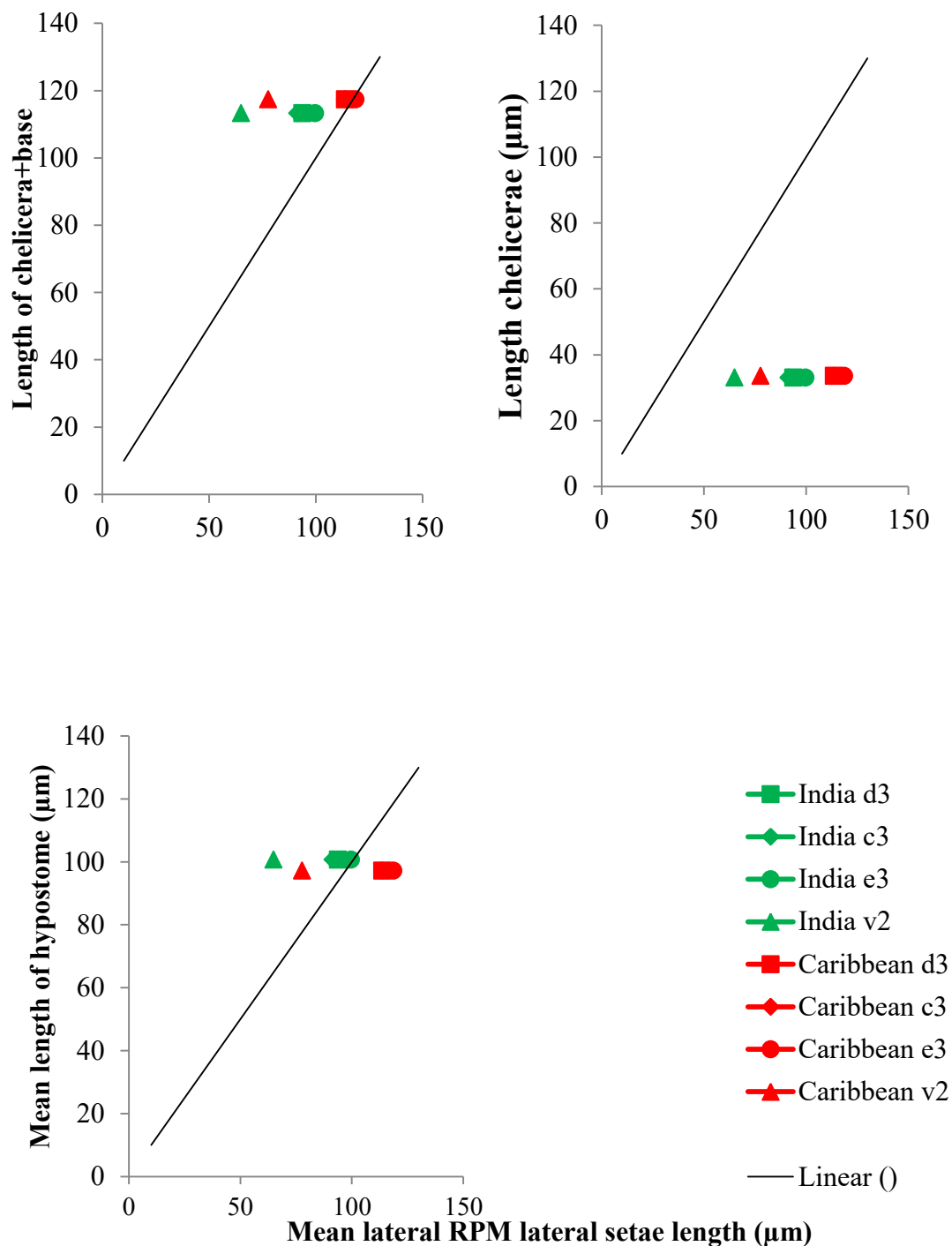


Figure 6.2 Length of *Amblyseius largoensis* mouthparts and accessories involved in attacking behaviour during predation, compared to the length of the lateral setae of RPM. Data are shown for India and Caribbean (i.e. *A. largoensis* and RPM dimensions relating to each region). The control line represents the point where the length of the lateral setae would equal the length of the measured body part of *A. largoensis*. Points appearing in the top left section of plot area are shorter than the lateral setae length, and those in the bottom right are longer than RPM lateral setae.

6.2.2 Investigating and characterising droplet expression between RPM cultured on different host plants and regions

Methods

To date there has been no information published as to when and why RPM express droplets at the distal tip of the dorsal setae, although it is hypothesised that these droplets play a role in defence. During survival analyses conducted in chapter 4, data were collected on the presence /absence of droplets on RPM adult females on predator free leaf discs from different hosts in India and on both *C. nucifera* in both Trinidad and India. These data were reanalysed to see if there was a difference in expression of droplets on RPM individuals between different host plants (in India) and on coconut leaf discs between regions (Trinidad and India). For the assessment between different host plants, RPM were collected from *C. nucifera* palms in Kerala India, and placed onto cut leaf sections of *A. catechu*, *C. nucifera*, *Musa* sp. cv Palayam kodan, *Heliconia* spp., *C. renda*, *B. flabellifer* and *Pandanus* sp. using the methodology described in Chapter 4. Red Palm Mite were assessed daily and the numbers of live RPM with and without droplets were noted over a period of six days. For each day, there were five replicates of each host plant, however data between days were temporally pseudo-replicated as the same individuals were followed for six days, therefore a generalised model using binomial errors was applied per day to analyse the results. Models were compared to the null model and a chi-squared test was applied to the full and null model to test for significance. For a comparison between regions, the same methodology was applied, however leaf sections analysed were *C. nucifera* only from both Trinidad and India, with RPM originating from *C. nucifera* in each region. For the regional comparison, data were taken from two separate assays in India and one assay in Trinidad. Due to poor leaf condition, one replicate was removed from the Trinidad analysis.

Results

The differences in the numbers of droplets produced by RPM were inspected daily over the duration of the experiment. Compared to RPM on *C. nucifera* (due to its strong association with RPM populations in several countries in the Old and New World), the proportion of RPM with droplets on the end of dorsal setae was significantly different on all days apart from day 7 (day 1: difference in deviance=118.9, difference in d.f.= -8, $p < 0.001$; day 2: difference in deviance= -26.9, difference in d.f.= -8, $p=0.01$; day 3: difference in deviance= -41.9, difference in d.f.= -8, $p < 0.001$; day 4: difference in deviance= -45.5, difference in d.f.= -8, $p < 0.001$; day 5: difference in deviance= -34.6, difference in d.f.= -8, $p < 0.001$; day 6: difference in deviance= -25.0, difference in d.f.= -8, $p < 0.001$; day 7 difference in deviance=-5.3, difference in d.f.= -5 $p=0.46$). The proportion of RPM with droplets was significantly lower ($p < 0.05$) when kept on leaf sections of *Heliconia* sp. 2, *Pandanus* sp.

and *C. renda* on day 2; *Pandanus* sp. and *C. renda* on day 3; *B. flabellifer*, *Pandanus* sp. and *C. renda* on day 4; *A. catechu* and *Pandanus* sp. on day 5 and *Musa* sp. cv Palayam kodan and *B. flabellifer* on day 6 (Figure 6.3).

On *C. nucifera* in India and Trinidad, on days 4-7 following transfer onto *C. nucifera* leaf sections there was no significant difference between proportion of RPM expressing droplets (day 4: difference in deviance= -3.7, difference in d.f.=2, $p=0.13$; day5: difference in deviance= -5.3, difference in d.f.=2, $p=0.07$; day 6: difference in deviance= -7.8, difference in d.f.=2, $p=0.19$; day 7: difference in deviance=-8.98, difference in d.f.=2, $p=0.15$; Figure 6.4). On days 2 and 3 the model was significant compared to the null model (day 2: difference in deviance= -11.2, difference in d.f.=2, $p=0.004$; day 3: difference in deviance = -6.5, difference in d.f.=2, $p=0.02$), but contrasts did not show any significant difference between regions or assays. Trinidad 100% of live females expressed droplets for the first five days of the experiment compared to a mean of 86.8% in assay 1 and 72.3% in assay 2 in India, only reducing on day 6 and 7.

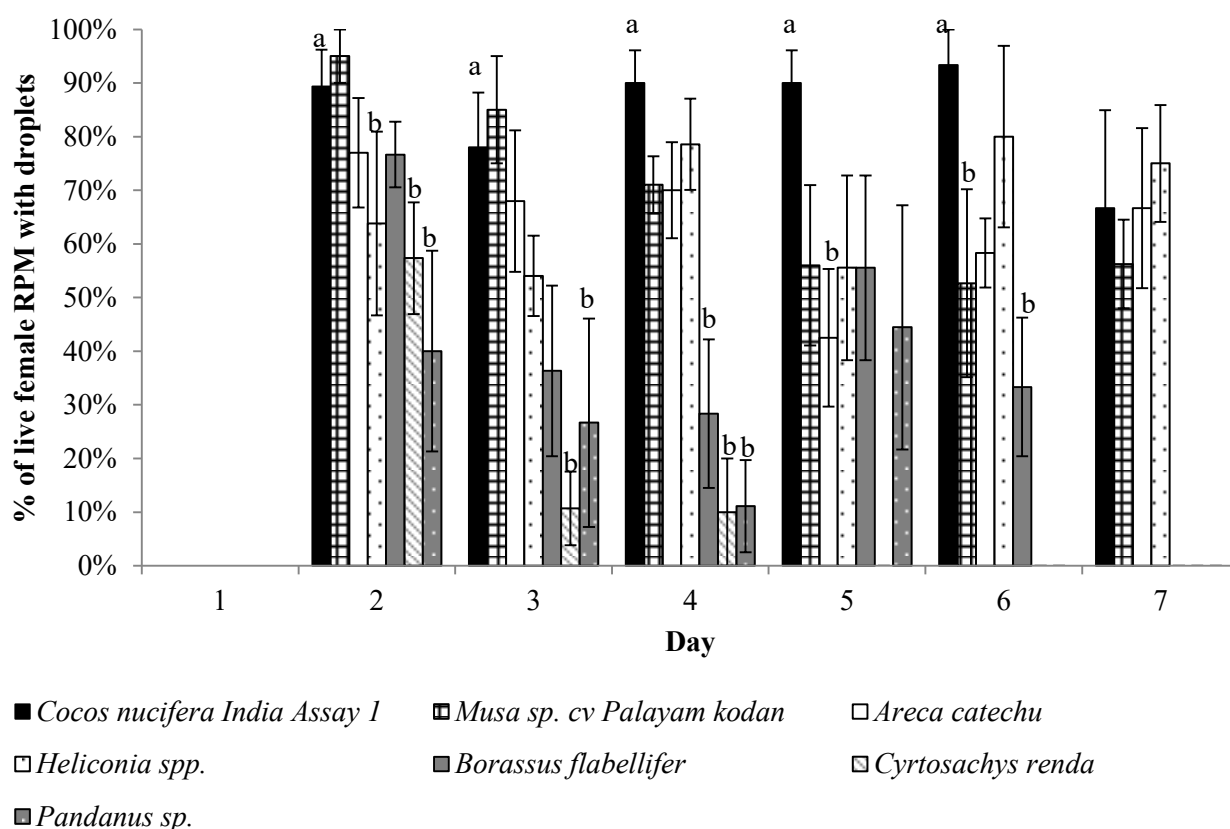


Figure 6.3 Proportion of live RPM females with droplets on the distal tip of dorsal setae on days 2-6 after transplanting onto leaf disc sections of 7 different plant species. Proportions significantly different to *Cocos nucifera* are shown ($p < 0.05$), $\pm 1SE$. NB droplet data not taken on day 1.

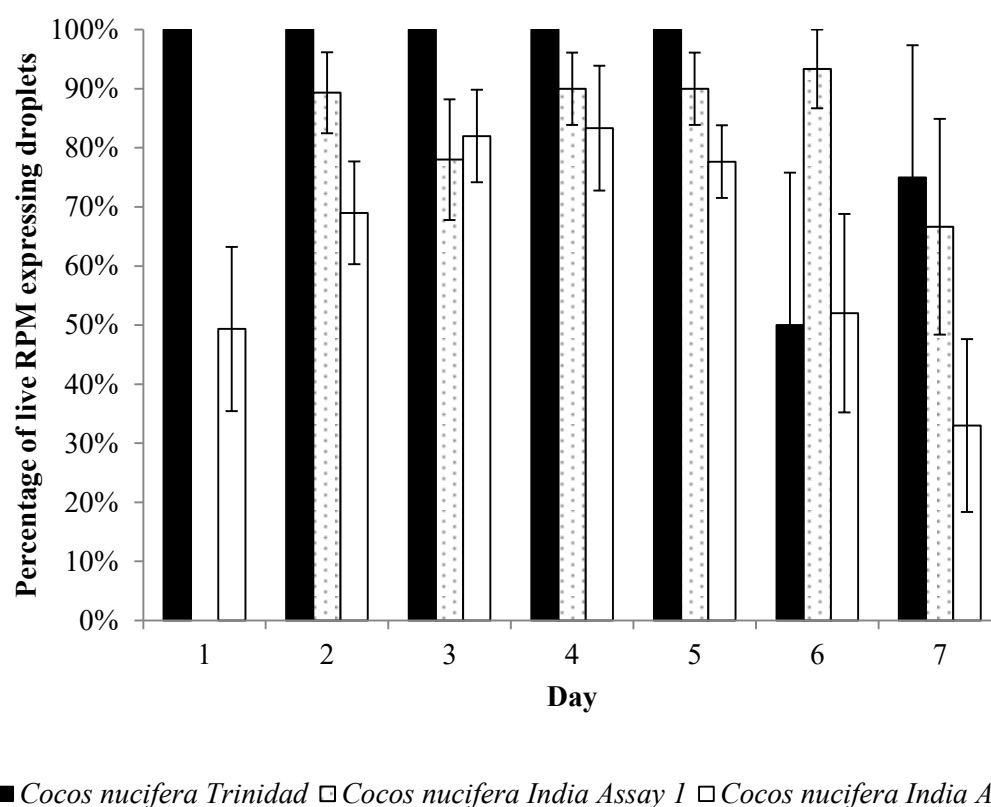


Figure 6.4 Percentage of adult RPM females expressing droplets during survival analysis experiments (see Chapter 3). NB droplet data not taken on day 1 of assay 1 in India. No significant differences were observed between regions.

The relationship between droplet presence and eggs laid per female was explored (Figure 6.5). Data from the different host plants in India were used as there were variable numbers of eggs laid across the different host species. To avoid problems with temporal pseudo-replication, data from each day were analysed separately. The proportion of RPM with droplets and the numbers of eggs laid per live female that day were analysed using a GLM with quasi-binomial errors. Results showed that there was a significant relationship between proportion of RPM with droplets and the numbers of eggs laid by the female (day 2: difference in deviance= -11.4, difference in degrees of freedom=-1, $p=0.004$ for the model; $t=2.41$, $p=0.02$, day 3: difference in deviance= -22.6, difference in degrees of freedom=-1, $p<0.001$ for the model, $t=3.37$, $p=0.002$; day 4: difference in deviance= -17.8, difference in degrees of freedom=-1, $p= 0.0001$; $t= 3.40$ $p=0.002$;and day 5: difference in deviance= -17.29, difference in degrees of freedom=-1, $p= 0.0001$, $t=2.99$, $p=0.006$); however not on day 6 (difference in deviance= -3.97, difference in degrees of freedom=-1, $p= 0.07$, $t= 1.62$, $p=0.12$), or day 7 (difference in deviance= -0.51, difference in degrees of freedom=-1, $p= 0.48$, $t=0.70$, $p=0.49$).

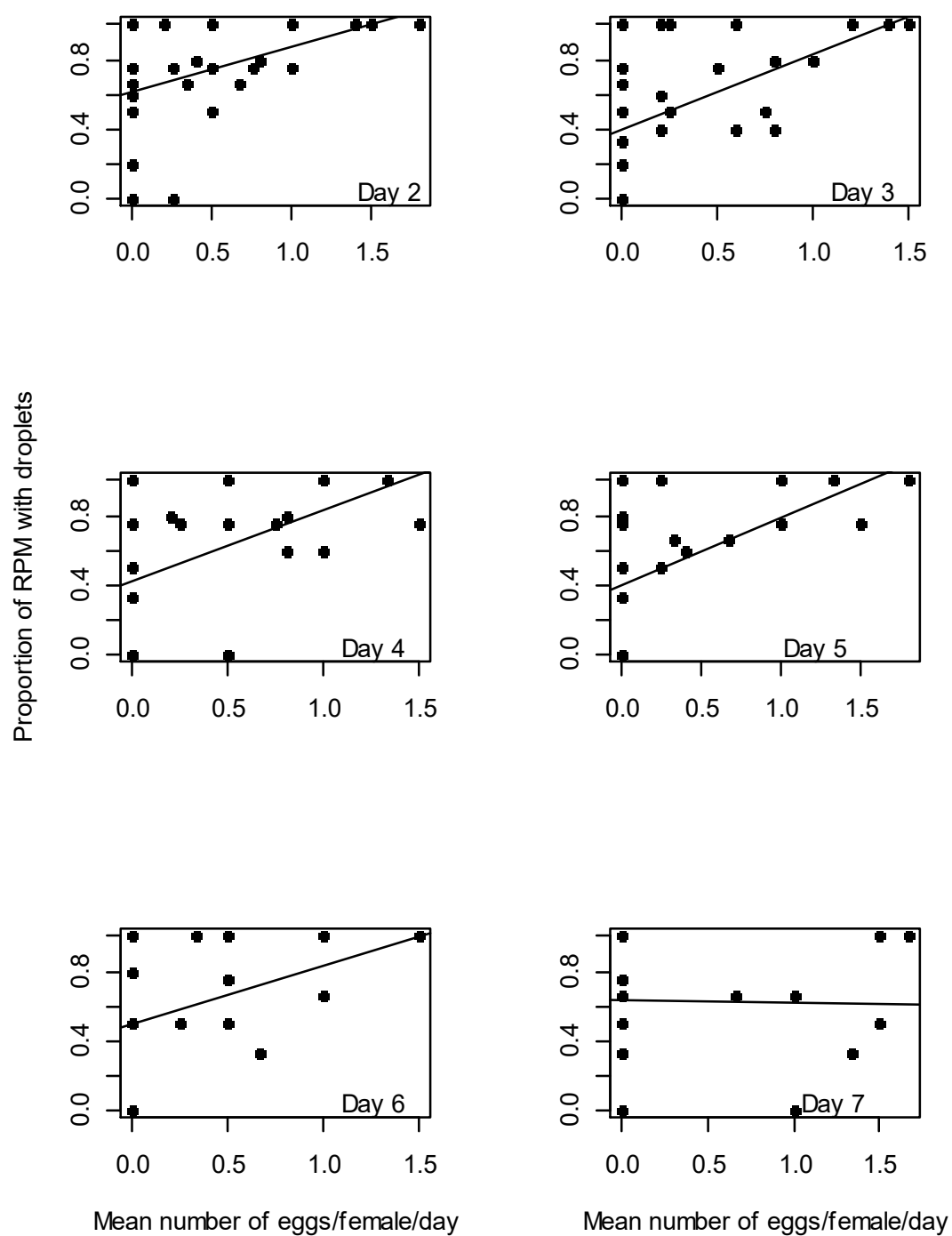


Figure 6.5 Mean number of eggs laid per female per day compared to the proportion of RPM with droplets on setae.

Baseline studies discussion

Baseline studies aimed to explore the nature of the relationship between RPM lateral setae and *A. largoensis* attacking structures; in addition to exploring the nature of droplet expression. The first baseline study showed that there were no significant differences in the morphology of the ‘attacking’ structures of *A. largoensis* between Trinidad and India, apart from a small difference in the length of the hypostome. Given the significant differences in the lengths of the lateral setae of RPM between India and Trinidad however, there were two theoretical differences demonstrated which could potentially enhance repellence during the attack process in Trinidad but not India- the length of the cheliceral process (which was borderline) and hypostome, depending on the direction of approach of *A. largoensis* – if the predator approaches from the rear it is likely to encounter longer setae than those at the front. These results indicated that shorter setae could in theory lead to lower repellence, however this needs to be tested experimentally. In theory, the longer the setae are, the longer the handling time of an adult RPM could be. If the predator orientated itself to approach RPM from the most favourable direction (from the anterior where setae are shorter), the predator may not be repelled.

With regards to when RPM express droplets, results showed that the proportion of RPM with droplets on the end of dorsal setae was significantly lower when kept on leaf sections of hosts which were shown to be unsuitable for RPM development in Chapter 4 i.e. *Pandanus* sp., *C. renda*, *B. flabellifer* in the first four days of the assays compared to *C. nucifera* as a host. Between regions, there was no significant difference in droplet expression, however in Trinidad, 100% of RPM had droplets for the first five days of the assay compared to a mean of 87% and 72% from assays on coconut in India, perhaps indicating a slight difference in droplet production between regions. Assays to quantify droplet production over time and characterise compounds produced would indicate whether similar production rates and droplet composition could be expected between regions. A significant relationship was also observed between the proportion of RPM with droplets and the numbers of eggs laid by the female on the first five days of the assay. Those expressing fewer droplets tended to lay fewer eggs.

Results for droplet expression showed that RPM constantly expressed droplets. Constant expression may indicate that droplets are not produced as a *responsive* defence mechanism, however their role in defence should be investigated. Results showed a higher proportion of RPM expressed droplets when on a favourable host compared to non-favourable host, and the relationship between droplet expression and fecundity indicated that droplets were produced when RPM were feeding and reproducing, and with a reduction observed when unable to feed/reproduce. If shown to have a

defensive role, the implications of this are that adult RPM on non-preferred host plants may be more prone to predation. The proportion of RPM with droplets on the tips of setae on 'secondary' host plants was lower than those on *C. nucifera* (*Musa* sp. cv Palayam kodan, *A. catechu*, *Heliconia* spp.). Although these results were not significant, this again may indicate that on a secondary host, RPM may be more prone to predation.

These baseline studies have shown that in theory the lateral setae and droplets may have a defensive role for RPM, however there is no experimental evidence to date to support this view; thus, the following experiments were undertaken to explore the role lateral setae and their length, and droplets have in RPM defence against *A. largoensis*.

6.3 A test of the effect of RPM lateral setae length and droplet expression on predation by *A. largoensis*

6.3.1 Methods

Hypothesis 1: Long lateral setae will affect the efficacy of *A. largoensis* when attacking adult female RPM- RPM with longer lateral setae will not be predated, RPM with shorter lateral setae will be predated

To test these predictions, 53 replicates of one adult female *A. largoensis* and one adult female RPM were placed into arenas for 24h. Arenas were constructed using plastic rings and filter paper, held together by two bulldog clips on either side. Plastic rings were cut with a 30mm diameter and a 15mm diameter hole in the centre and a section of Whatman filter paper was sandwiched between two rings and held with the bulldog clips. Arenas were enclosed using a glass coverslip placed on top of the rings. The seal was sufficient to prevent predators and RPM escaping. Whatman filter paper was used in place of plant material so plant olfactory or gustatory cues did not distract predators (Figure 6.6). Adult female *A. largoensis* were collected from freshly cut coconut palm material infested with RPM (collected from trees at CABI, Curepe, Trinidad) and placed individually in arenas and starved for 24h. After 24h an adult female RPM was placed into the arena and the interactions between predator and RPM were observed for 30min. After 24h the predator and RPM were removed and it was noted whether the RPM and predator were alive or dead and additionally whether the RPM had been predated. This was clear from the colour of the predator's gut (turns red when it has eaten RPM). Both predator and RPM were placed in 70% alcohol (chosen due to airline regulations for transport of alcohol) and preserved for slide making. To control for presence/absence of droplets, this experiment

was conducted using RPM with droplets, and with droplets removed (using a piece of filter paper to soak up exudate). A series of control arenas (to test to see whether predators could consume prey under these conditions if given the choice) were set up using five RPM eggs placed onto the filter paper. In total, there were 21 replicates where RPM had droplets removed, 17 replicates where RPM had droplets and 15 controls using RPM eggs. Predators and RPM were initially identified by sight; however, slides were prepared using Heinze media to confirm species, sex and lifestage of both predator and RPM after the experiment. Slides were prepared mounting the predator and RPM on the same slide and placing a drop of Heinze media onto an inverted circular glass coverslip and specimens were placed ventral side up. A glass slide was then lowered onto the coverslip and the slide was inverted. Mites were checked to ensure they were dorsal side up using a stereo microscope, then, slides were placed onto a slide heater until the specimens had cleared sufficiently. Once slides were prepared, the dimensions of RPM and *A. largoensis* were measured as follows using x400 magnification and image pro insight software. From RPM, length, width, and lengths of c1, d1, e1, c2, d2, e2, f2, c3, d3, e3, f3, h1, v2, Sc1 and Sc2 setae were made. For the corresponding predator, body length and width at S4 were measured along with the length of Z4, Z5 and S4 setae, chelicera length, length of palps, length of cheliceral process (including chelicera), cheliceral process minus chelicera and length hypostome+ gnathosomal base (referred to as hypostome in results section). Dimensions were compared to predation status.

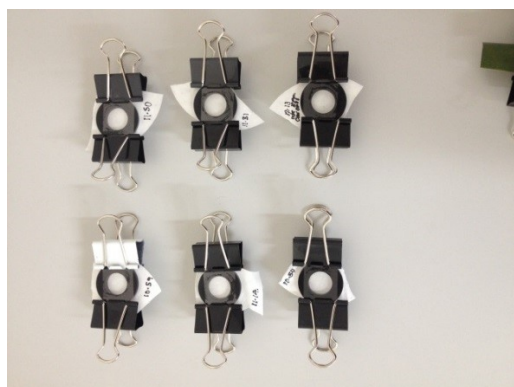


Figure 6.6 Arenas used for hypothesis 1

For statistical analysis, the numbers of treatments where the RPM/ RPM eggs were predated and those which were not were calculated, and a vector of numbers of predated, and total tested was composed. A binomial test was applied to these data and this gave proportions of RPM predated and a probability of success. A χ^2 test was also applied to test for significant differences. A GLM using binomial errors was fitted to these data to test for significant differences between treatments. Differences were considered significant when $p < 0.05$. For mean relative lengths and standard errors of the dorsal setae, length and width of RPM specimens for live and predated specimens were compared using a

MANOVA. Only treatments where the predator was alive after 24h were tested (apart from one treatment where RPM was predated during the first 30min observation).

Hypothesis 2: Droplets will enhance the defence of RPM against *A. largoensis* attack- RPM with droplets will be attacked on fewer occasions compared to those without droplets.

Behaviour of *A. largoensis* was observed for the first 30mins of each replicate set up in hypothesis 1. Behavioural observations included the numbers of times the predator contacted RPM and the amount of time spent stationary, moving and eating. For statistical analysis, the number of occasions where the predator contacted either the RPM female or egg were noted during the assays. To these data, a GLM using poisson and quasipoisson errors (where data were overdispersed) of residuals were fitted. Models were compared to the null model using a chi-squared test to test for significance. Individual sessions where the predator was grooming, searching, eating or stationary were timed and the total time spent undertaking these activities was calculated for each replicate. Differences in length of time were tested using ANOVA, and difference in the numbers of occasion a predator displayed an activity was tested using GLM with poisson/quasipoisson errors were fitted to these data.

From the above observations, there appeared to be an effect caused by the predator physically contacting the droplets i.e. increased grooming/stationary time. To further investigate the behaviour caused by contact with droplets, a second arena experiment was set up. Arenas were constructed as above but instead of using Whatman filter paper, a section of coconut palm, placed ventral side up was used. This was because the RPM were more likely to stay *in situ* on plant material creating more realistic conditions of contact/no contact (RPM were observed to be more mobile in filter paper assays). To increase the chances of contact with the droplets, 12 adults female RPM were placed in the arena either with or without droplets on the end of their setae. Again, a separate control treatment using 12 eggs was set up to assess whether predators would eat under the given conditions. Predators were taken fresh from leaf material and placed in the arena with the RPM/RPM eggs. Un-starved individuals were used- this was again to replicate real life contact conditions (increased contact may be observed for starved individuals). For RPM with droplets and no droplets there were 14 replicates each and for the RPM eggs assays, there were 15 replicates.

Once placed in the arena, predators were observed for 10 minutes and the number of contacts with RPM / RPM eggs, the number of predation events (consumption of RPM/ RPM eggs), the amount of time searching, grooming and spent stationary were measured. Sessions were videoed using a Dinolite microscope camera which fitted into the stereo microscope eyepiece. After the ten-minute

sessions, videos were analysed and behaviours were recorded. Grooming was defined as when the predator was stationary; however, there was movement of the chelicerae, hypostome and/or the first two front legs contacting the gnathosome. If these behaviours were not present, the predator was deemed stationary. When the predator was moving, this was termed searching behaviour, and when the predator was consuming the prey, this was termed 'eating'. Once the 10-minute time period was completed, predators were placed into 70% alcohol so that species, sex and life-stage could be confirmed.

Data for predator contact with RPM/ RPM eggs and behaviour were analysed using a GLM, however as the total times of sessions varied (they were not precisely 10 min to the second) these data were analysed as proportion data using binomial errors. A vector was constructed of the time displaying the particular behaviour and the time not displaying the particular behaviour, and a GLM using binomial and quasibinomial errors to account for overdispersion of the model where required were fitted. A chi-squared test was used to test to see whether the treatment effect was significant compared to a null model. Mean proportions of time spent displaying a given behaviour were calculated by summing the total time spent doing the activity between all replicates for that treatment and dividing by the total time in assessment for that treatment. The numbers of occasions where RPM/RPM eggs were contacted were also analysed over time using a LMER model, then numbers of contacts per mite were analysed per minute using a GLM with poisson/quasipoisson errors to test for significant differences in numbers of contact. Differences were considered significant when $p < 0.05$.

6.3.2 Results

Hypothesis 1: Results

When the lateral setae and dimensions of RPM were compared between those which were predated and non-predated, there was no significant difference in the setae lengths; however, the individuals which were predated measured significantly shorter in length than those which were not predated ($F(1,21)=55.3$, $p < 0.001$; Figure 6.7).

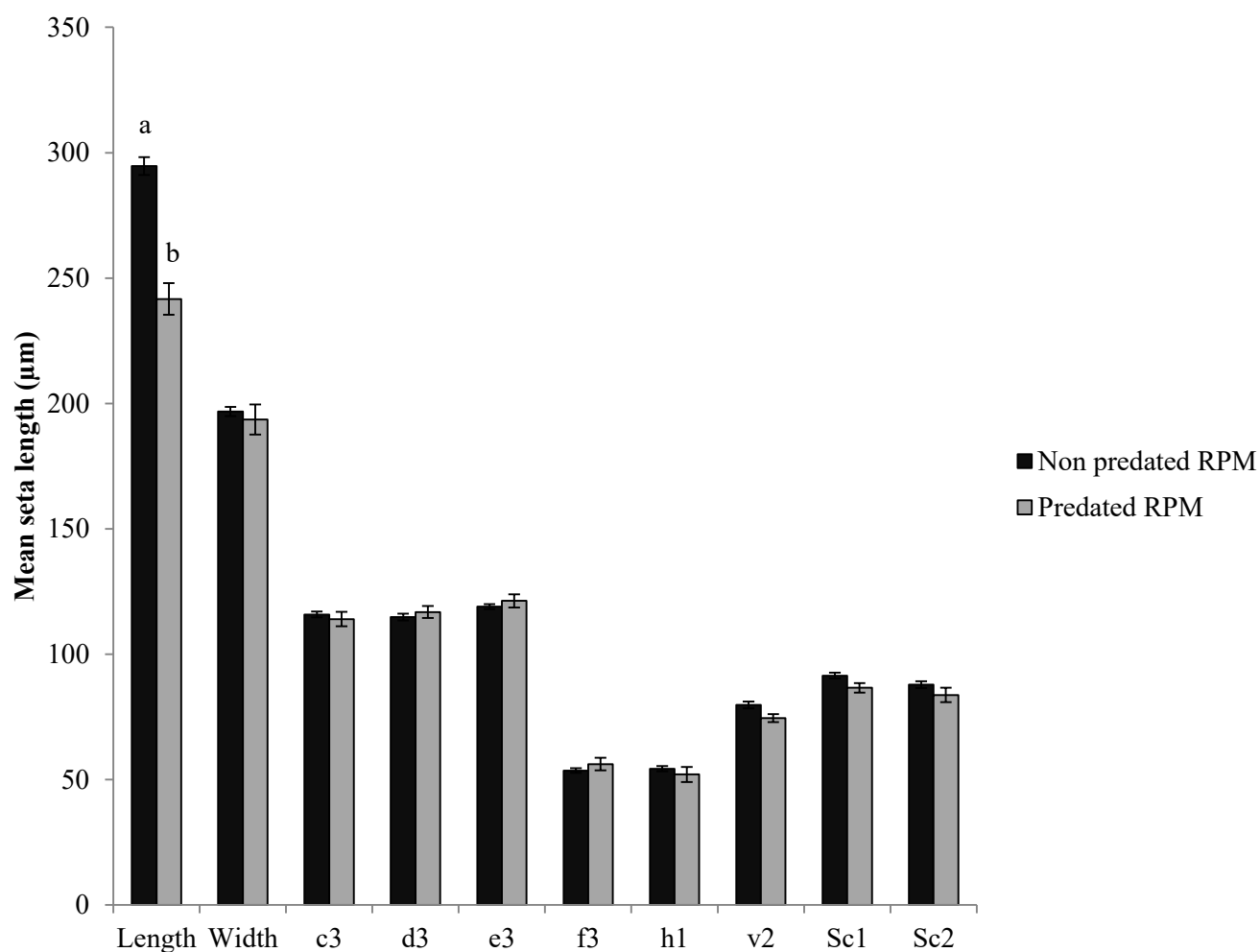


Figure 6.7 Comparisons of setae length between predated and non-predated RPM.

In total, there were only six out of the possible 34 replicates where RPM were predated (droplets and no droplets), of which five were measured (1 RPM were missing from collection tube when slides were prepared). Figure 6.8 and 6.9 show the comparison of lengths of lateral setae in comparison to the lengths of the cheliceral process and hypostome of corresponding predators. There was no observable pattern which split the predated from the non-predated RPM in terms of lengths of lateral setae.

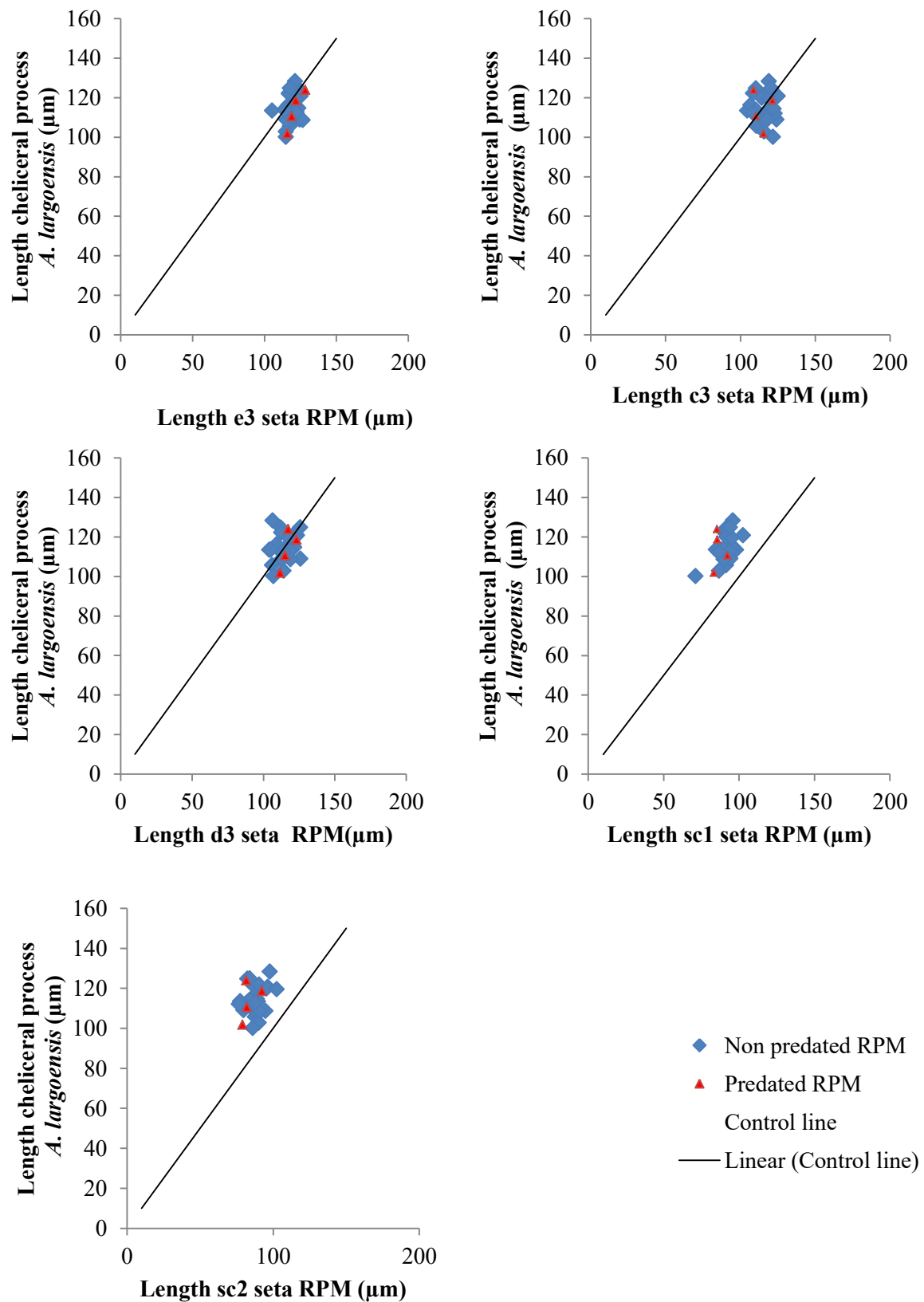


Figure 6.8 Comparison of lengths of cheliceral process of *A. largoensis* to the lateral setae of RPM which have been predated and those which were not predated.

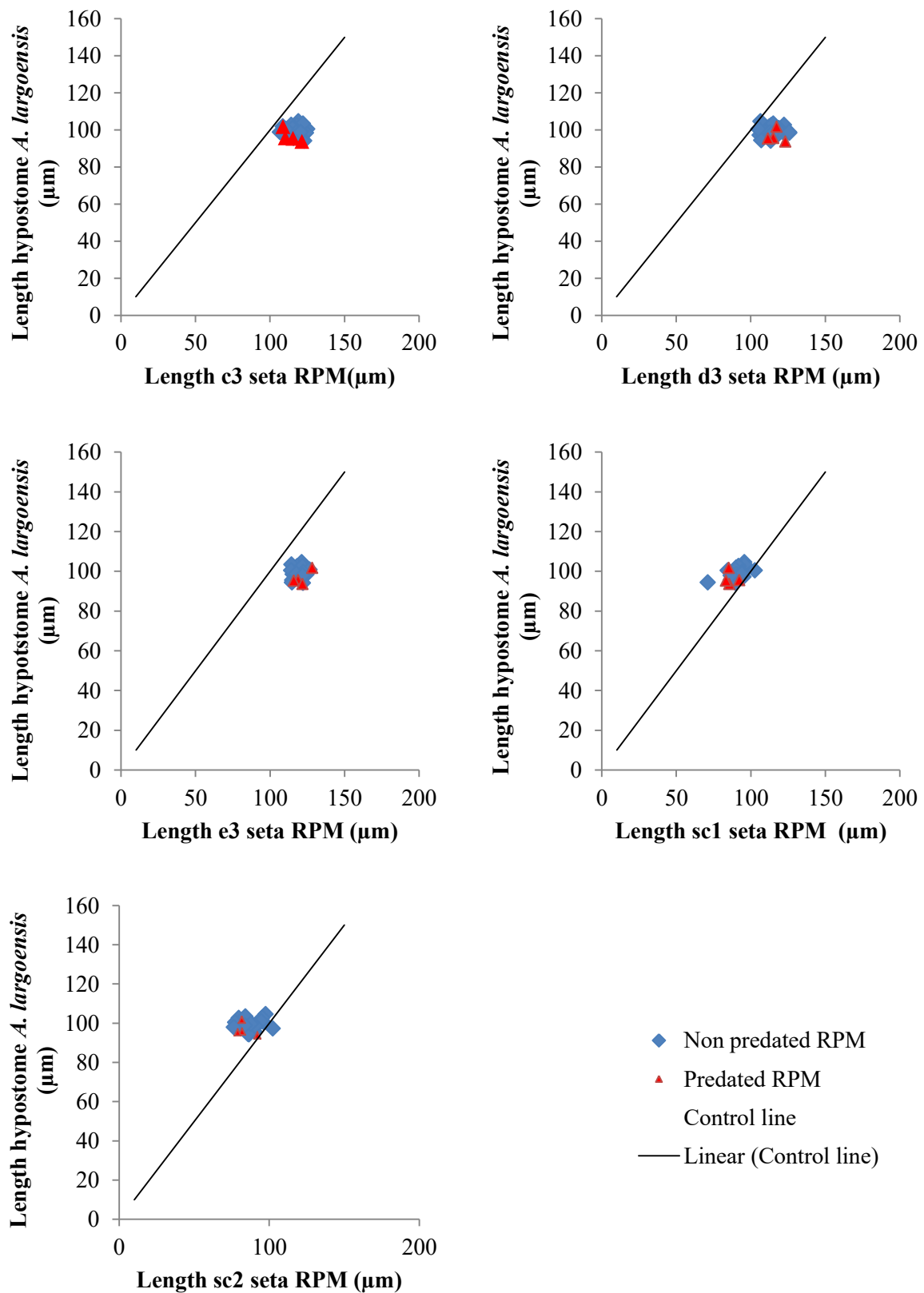


Figure 6.9 Comparison of the length of hypostome on *Amblyseius largoensis* specimens to length of lateral setae on RPM which were predated or non-predated during 1:1 24h challenges.

Hypothesis 2: Results

Figure 6.10 shows the proportion of treatments where the RPM/RPM eggs were predated. A binomial test estimated that there was a significant difference in the numbers of RPM/RPM eggs predated ($\chi^2=22.2$, d.f. = 2, $p<0.001$) where only in 6.7% of the cases RPM with droplets were predated compared to 26.3% for RPM without droplets and 91.7% of cases for the egg treatments. There was no significant difference between the numbers of adult female RPM predated with or without droplets ($\chi^2=1.1$, d.f. = 1, $p=0.3$), however both differed significantly from the numbers of eggs predated (with droplets: $\chi^2=16.2$, d.f. = 1, $p<0.001$; without droplets: $\chi^2=10.1$, d.f. = 1, $p=0.001$).

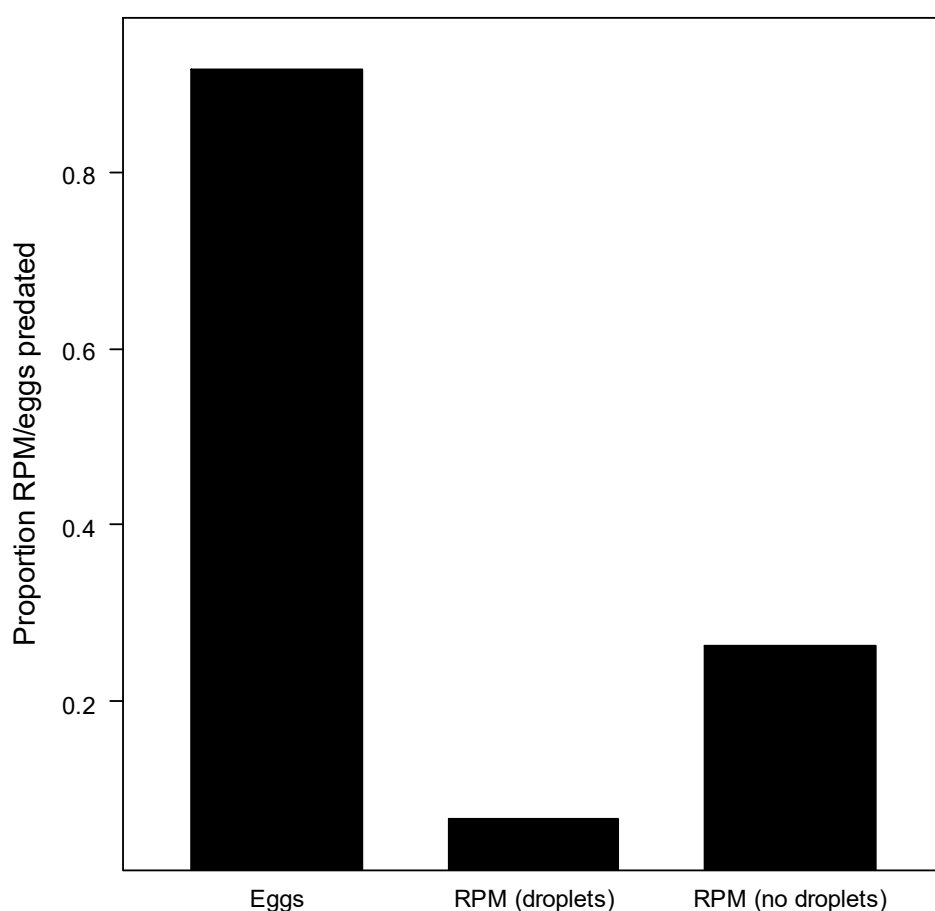


Figure 6.10 Results from the 24h one-one challenges with adult female RPM and adult female *Amblyseius largoensis* (starved for 24h prior to introduction). n= 15 for droplets, n=19 for no droplets and n=12 for eggs.

During the 1:1 challenge assays, the behaviour of the predator in relation to RPM was observed for the first 30 min period when the predator was first introduced into the arena (Figure 6.11). The number of ‘contacts’ were observed and it was found that there was no significant difference in the

number of times the predator contacted RPM with droplets, without droplets or eggs (compared to null model difference in deviance= -8.0, difference in d.f.=2, $p=0.50$). As the number of contacts was reliant on the moving time of the predator in that period, the number of contacts per moving time of the predator was established. This found that there were fewer contacts made with RPM with droplets than those without, however the difference was not significant ($F=0.58$, d.f.=2, $p>0.05$).

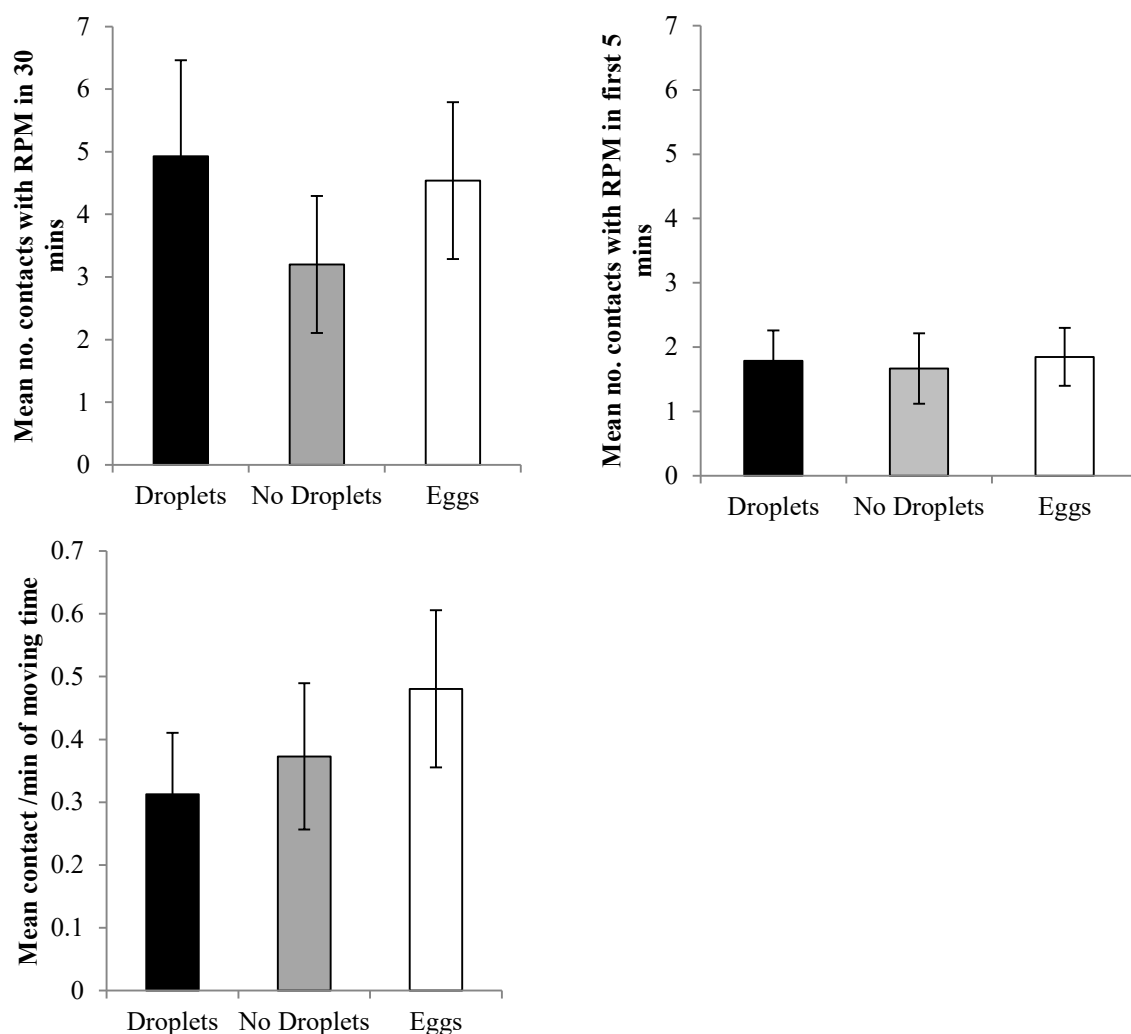


Figure 6.11 The behaviour of *Amblyseius largoensis* adult female in response to adult female RPM when observed over 30 minutes (unless specified) when 1:1 in an arena.

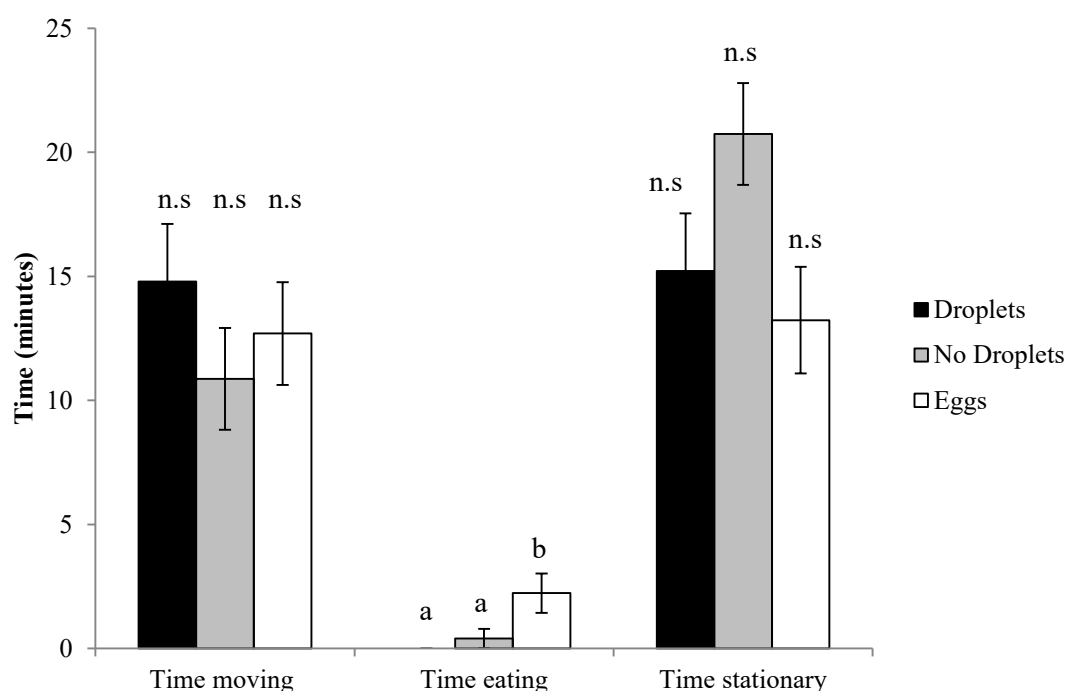


Figure 6.12 Behaviour of *Amblyseius largoensis* when observed for first 30 minutes in an arena with one adult female RPM.

There was no significant difference in the time spent moving or stationary between all three treatments ($F=1.21$, $d.f.=2$, $p=0.31$; $F=2.23$, $d.f.=2$, $p=0.12$ respectively) however, the predator spent significantly more time eating when placed in the arena with eggs, than with adult RPM with or without droplets ($F=5.82$, $d.f.=2$, $p=0.006$). During these observations, there appeared to be an effect caused by the predator physically contacting the droplets i.e. increased grooming/stationary time, however the number of contacts made in 1:1 challenges meant that results were not significant, thus challenges were set up whereby the predator was introduced into an arena with 12 adult RPM either with or without droplets, or eggs as a control. Results showed that there was a significant difference between the number of contacts made by the predator between adult RPM with droplets, without droplets and eggs (compared to the null model difference in deviance = -211.8, $d.f. = 40$, difference in $d.f. = -2$, $p < 0.001$; Figure 6.13). The predator contacted the adult RPM females with no droplets on significantly more occasions (mean 17.6 times; $t=3.12$, $p=0.003$) than those with droplets (mean 10.2 times), and in turn the predator contacted both the adult stages with and without droplets on more occasions than the eggs (mean 1.93 times $t=-4.97$, $p<0.001$). The predator did not consume any of the adult female RPM with or without droplets during observations, however on average, there were 0.8 ± 0.29 occasions per assay where the predator consumed an egg (Figure 6.13). To relate this to the number of contacts, the ratio between the number of contacts and the number of prey consumed was 2.4:1.

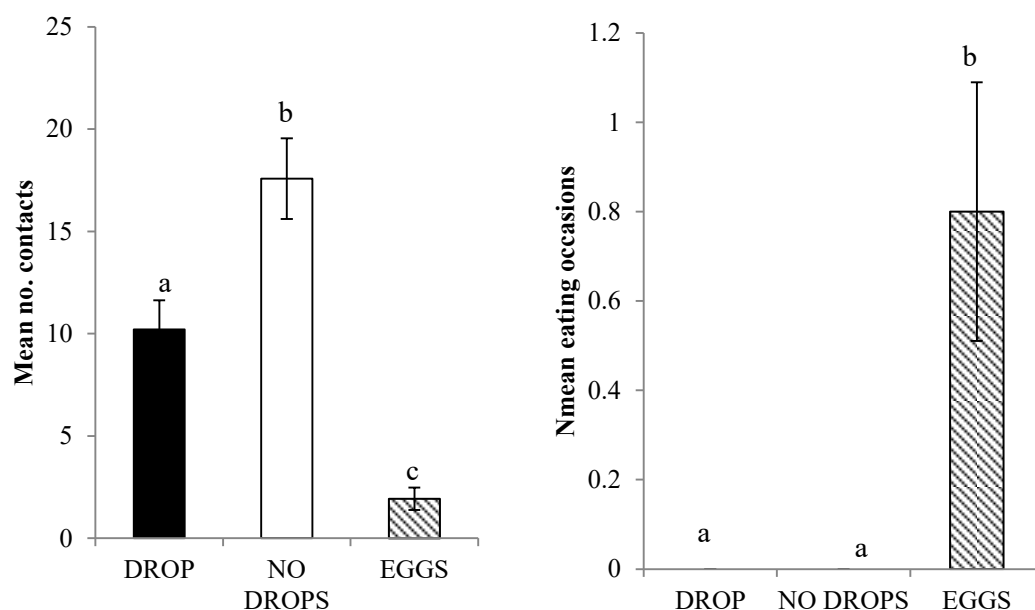


Figure 6.13 Average number of contacts made by the predator *Amblyseius largoensis* to 12 adult female Red Palm Mite with a) droplets on the end of setae (n=14) b) no droplets on the end of setae (n=14) and with 12 eggs (n=15) within a 15mm diameter arena in timed 10 minute challenge sessions. Different letters show significant differences.

A linear mixed effect model showed there was a significant difference in the numbers of contacts made over time between treatments ($\chi^2=73.2$, d.f.= 2, $p<0.05$; Figure 6.14). Individual models applied to these data on minutes 1,2,3 and 4 showed that the numbers of times eggs were contacted was significantly lower than contacts made with adults over each of these minutes ($t= -2.71, -2.80, -2.87$ and -2.85 respectively, $p<0.01$). There was no significant difference between the number of contacts made with adults with/without droplets therefore these factor levels were combined. During minute 5 significantly more contacts were made with RPM with no droplets, compared to those with drops and eggs alone ($t=4.27$, $p<0.001$).

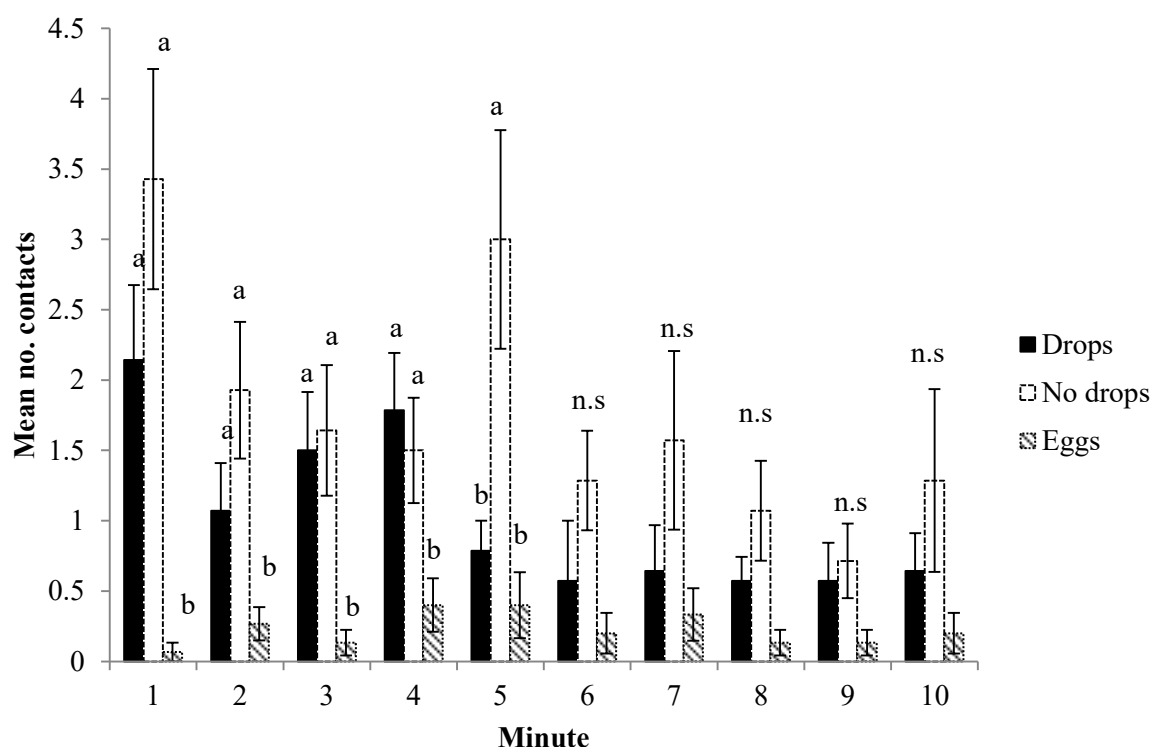


Figure 6.15 Mean number of contacts made per minute (± 1 SE) with either arenas containing 12 adult RPM with droplets, 12 adult RPM without droplets or 12 eggs, during a 10-minute assessment.

Grooming

There was no significant difference found between the number of grooming occasions in each of the treatments (Figure 6.16, difference in deviance = -2.63, d.f.=40, difference in d.f.=2, $p=0.36$), however the proportion of time spent grooming was found to differ significantly depending on whether the predator was in an arena with eggs or adult RPM (difference in deviance = -1566.8, d.f.=40, difference in d.f.=2, $p<0.001$). No significant difference was found between grooming time for predators exposed to RPM with droplets or without droplets (z ratio= 0.84, $p= 0.68$), however predators spent significantly longer grooming when exposed to adult RPM regardless of whether droplets were present on the end of the setae or not (z ratio= 4.10, $p=0.0001$ and z ratio= -3.33, $p=0.003$ respectively) when compared to grooming time in the presence of RPM eggs (Figure 6.16). There was evidence to suggest that grooming time was longer for individuals which had contacted RPM with droplets but this was not significant (z ratio= 0.84, $p=0.68$).

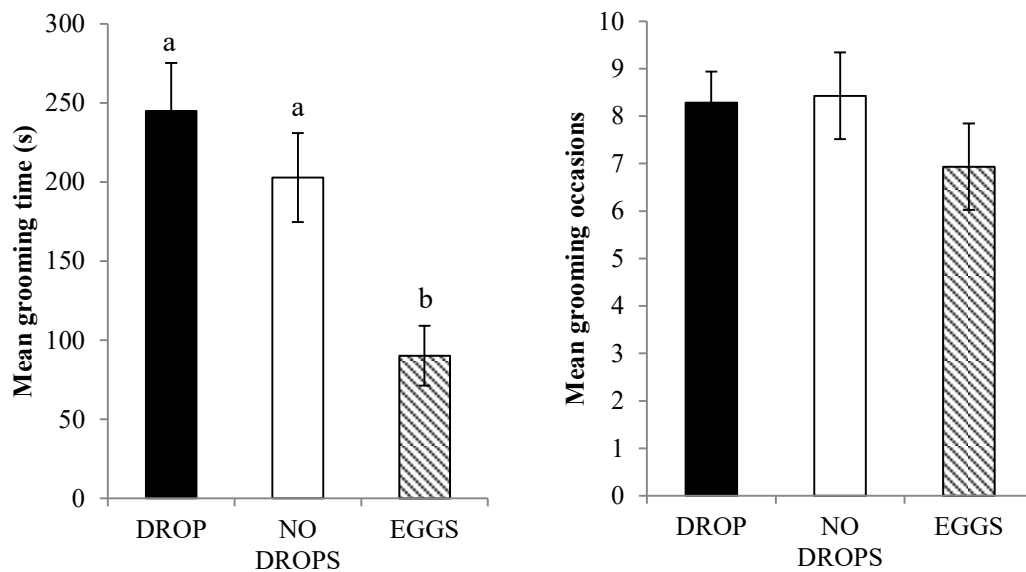


Figure 6.16 Mean time spent grooming for predators contacting RPM adults with droplets, without droplets and eggs ($\pm 1SE$). Different letters indicate significant difference ($p < 0.05$) b) number of occasions where RPM stopped to groom. Mean grooming time between treatments

Searching

There was a significant difference between time spent 'searching' by the predator in different treatments (when compared to the null model difference in deviance = -1521.8, d.f.=40, difference in d.f.=2, $p=0.002$). No significant difference was found in the proportion of time spent 'searching' between predators in an arena with RPM with droplets compared to those without droplets (z ratio = -1.88, $p=0.14$; Figure 6.17) however the searching time when the predator was in the presence of RPM with droplets was significantly lower compared to those in arenas with eggs (z ratio= -3.49, $p=0.001$). Factor levels for adults with and without droplets were combined and the model was re-run, and the results showed there was a significant difference between proportions of time spent searching when in the presence of RPM adults and eggs (difference in deviance to the null model= -1512.6, d.f.=41, difference in d.f.=1, $p < 0.0001$; z ratio= 4.14, $p < 0.0001$).

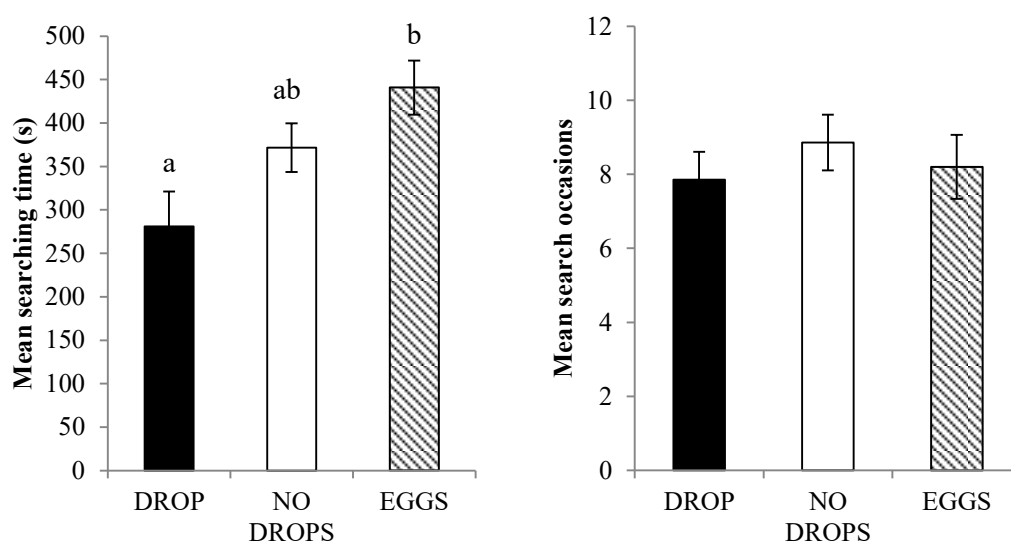


Figure 6.17 Mean amount of time the predator spent searching within the arena in each treatment ($\pm 1SE$). Different letters indicate significant differences ($p < 0.05$) b) the mean total number of search occasions in each treatment ($\pm 1SE$).

Stationary

There was no significant difference in the number of times or the amount of time the predator was stationary in each of the treatments (difference in deviance=-4.0, d.f.=40, difference in d.f.=2, $p=0.40$; difference in deviance=-668.7, d.f.=40, difference in d.f.=2, $p=0.18$ respectively; Figure 6.18).

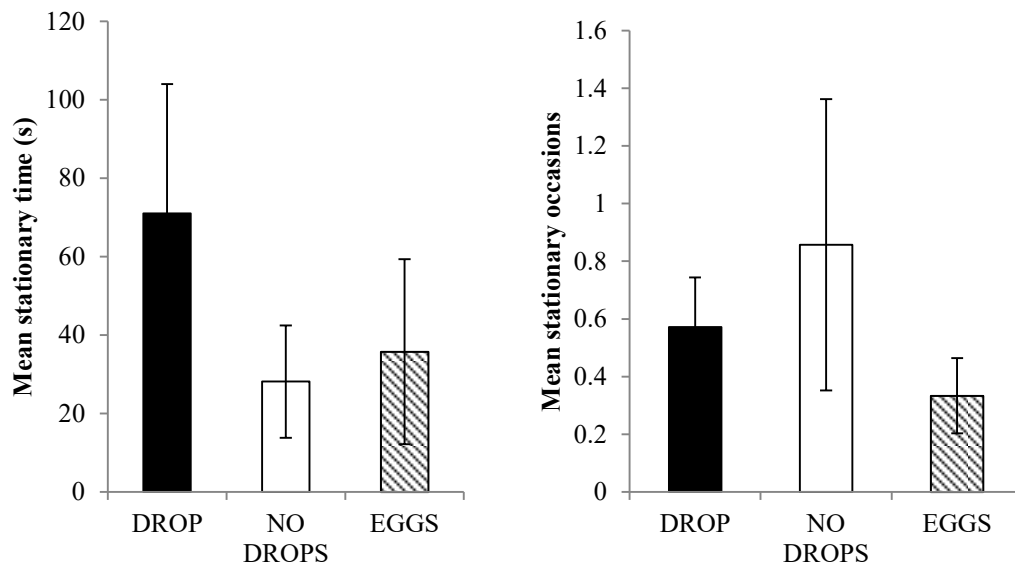


Figure 6.18 a) The mean amount of time the predator spent stationary in each of the individual treatments
b) the mean number of occasions the predator was stationary in each of the treatments.

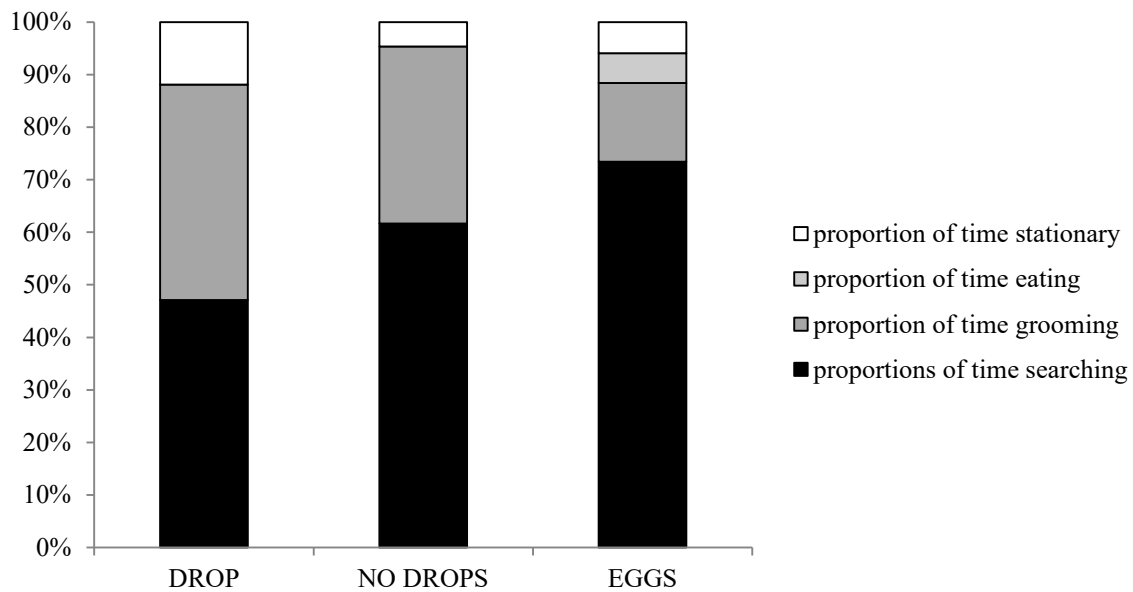


Figure 6.19 A summary of the proportion of time the predator spent doing the 4 activities measured during the 10-minute behaviour assays, in relation to whether they were in arenas with RPM adult females with droplets, without droplets or eggs.

6.4 Discussion

The experiments presented aimed to explore the extent to which lateral setae and secreted droplets act as a constraint to predation of RPM by *A. largoensis*. No evidence was found to support the

hypothesis that predation success was inversely related to increasing length of the lateral setae. The only significant difference observed between those predated and not predated was in the length of the RPM individual as opposed to the lateral setae length. It was uncertain however, whether some of the RPM individuals measured smaller as they had been predated, thus shrunken (as they are soft bodied mites) or whether they were smaller to begin with. Increased frequency of RPM predation by *A. largoensis* has been shown for RPM nymph stages compared to adult RPM so size could be an important factor (Carrillo & Peña, 2012).

Difficulty was encountered obtaining a range of RPM with differing lengths of setae from Trinidad only and given the low numbers of individuals predated, the hypothesis proved difficult to test using Trinidad specimens of RPM alone. It was not possible to undertake the bioassay in India as the Memorandum of Understanding (MOU) had expired with Kerala Forest Research Institute (KFRI). The shortest lateral setae measured during the 1:1 challenges were c3:105, d3:104, e3:105, v2:80, sc1:98, sc2:90, compared to mean lengths of counterparts in India of c3:91, d3:91 and e3:99 thus analogous studies in India would enable further interpretation of the role of setae length. Results from the morphological baseline studies presented here suggested that *A. largoensis* may handle RPM more easily in India so further assays should be carried out in India to test this.

In addition to measuring predation only, different measures of predation efficacy may help to further test the impact that lateral seta length has on predation efficacy. For example, there may not be a relationship between seta length and predation success, however the length of setae may affect handling time which may affect overall numbers of RPM predated from a population by an individual predator. During observations in this study of one aborted and one successful attack, (Appendix 14), *A. largoensis* had difficulty in approaching the adult females RPM from the rear where the extended lateral setae are found. During the aborted attack the predator shrank back immediately upon contact with the e3 seta. The successful attack counted 16 aborted attack attempts before a successful attack was made and the predator was observed initiating attack attempts at the c3 position on the right hand side then circling the RPM on the posterior side past d3, e3, on right hand side to e3,d3,and c3 on the left hand side. The successful attack attempt was finally made at the sc1 seta position where feeding commenced- at the front of the mite. These behavioural observations support the theory that the posterior lateral setae c3, d3 and e3 may play a role in repellence, and that successful attacks are made from approaching the anterior side of RPM where lateral setae are shorter. Carrillo et al. (2012b) observed that populations of *A. largoensis* which had previous exposure to RPM were more likely to prey upon the larval stages of RPM compared to naïve populations, indicating that experience in handling RPM could affect prey choice and thus increase the range of prey life stages which *A.*

largoensis predate.

These results although not yet conclusive, do provide justification for further study. If predator efficacy in terms of predation success or handling time is shown to differ for those with shorter lateral setae, this would highlight the importance of considering both predator and prey during consideration of predators for assessment under classical biological control (CBC) programmes. Classical Biological Control is based around the concept that natural enemies in the area of origin are co-evolved with the prey, and have a larger impact on pest populations. Morphological studies presented here showed that although the functional structures involved in attack by the predator do not differ, RPM morphology between regions does, and this could lead to theoretical barriers to efficacy. If the difference in lateral seta length is caused by a genetic bottleneck, it could mean that even predators from the area of origin may not be as efficient in handling the prey as there would be a wider diversity in seta lengths where it has co-evolved with RPM. Conversely, if seta length does confer an advantage against predation by *A. largoensis* in the area of origin, then theoretically, individuals with shorter lateral setae would be more vulnerable and thus rarer in these populations. Hypotheses produced from the results of chapter 3, raised the question of whether the observed lateral seta lengths of both populations in the adventive range in the New World and those from RPM in Kerala India resulted from genetic bottlenecks created when they were introduced into each region, and that populations in Kerala were not those from the area of origin. It was hypothesised that the traits were highly conserved between generations and were not influenced by selection pressures from exogenous sources such as natural enemies. This may explain the lack of selection towards individuals with longer setae in India as these individuals would not be present, with the implication being that the population in India is innately more vulnerable to predation by *A. largoensis* if seta length were to be proven to affect predator efficacy.

Results from the consumption experiments to test hypothesis 2 showed that droplets enhanced the defence of RPM with only 6.7% of RPM with droplets predated compared to 26.3% for RPM without droplets and 91.7% of cases for the egg treatments. The further behavioural studies aimed to tease out the differences in predator behaviour attributable to droplets and setae and setae alone, and although differences were observed between individuals with droplets and without droplets, significant results were not obtained. Trends in the 1:1 behaviour assay suggested that fewer contacts were made with RPM with droplets than those without, therefore this assay was redesigned and repeated to increase the encounter rate the predator would have with RPM individuals. The second assay, where 12 RPM individuals were placed in the arena as opposed to the 1:1 assays, was able to demonstrate that the number of contacts that the predator made with RPM individuals with droplets was significantly

lower than those without droplets. However, no significant differences between RPM with droplets/without droplets were observed when each minute of the assay was taken individually, although the number of contacts was consistently higher for those without droplets at all time points apart from one. During studies the deliberate transfer of droplets from RPM female to egg was observed, indicating the presence of droplets may play an important role.

Other behavioural observations were made to see if contact with setae and droplets affected behaviour of the predator. Results showed that the proportion of time spent grooming was significantly higher when the predator was in an arena with adult RPM, regardless of droplet status when compared to eggs alone. No statistical difference was shown between individuals with or without droplets but data suggested that those which encountered droplets groomed for longer periods than those which encountered setae alone. Further replication could elucidate whether these differences could be significant. The time spent grooming, impeded the amount of time the predator could spend searching- the proportion of time spent searching was higher for predators in the egg treatment as opposed to treatment with adults however this was not significant. These results were similar to results demonstrated by Smedley et al. (2002) and Shiojiri & Takabayashi (2005) who demonstrated that when *Formica japonica* (Hymenoptera:Formicidae) came into contact with oil droplets secreted at the end of setae of *Pieris rapae* (Lepidoptera:Pieridae) this induced increased grooming behaviour and decreased the proportion of time in contact with the prey secreting droplets. The authors of both studies concluded that these droplets played a role in defence against predators.

When in the presence of eggs, the predator spent significantly more time eating compared to the treatments with adult RPM, even though the encounter rate was significantly lower for eggs. The predator often encountered an egg and consumed it immediately, whereas during an encounter with adults it was often repelled and then spent time grooming. Carrillo & Peña (2012) showed that *A. largoensis* preferred consuming RPM eggs, and the likelihood that an individual would be predated reduced with each life stage (with adults the least likely to be predated). These data presented here show experimentally that when *A. largoensis* contact the adult RPM, the behaviour is affected inducing more grooming and less searching time. Indications were that over time the predator avoided contact with RPM after an initially higher number of contacts thus, the predator was learning to avoid the adult RPM.

Assays in the present study did not explore the olfactory effect droplets alone on *A. largoensis* behaviour, although preliminary assessments and exploration of experimental procedures were made (data not shown). Previous research by Sabelis (1981) considered prey stage preference exhibited by a

Phytoseiid predator to arise when contact was made with the prey, thus it may be that the role of contact with droplets is a key factor rather than olfactory cues from them.

The wider implications of the findings of this study are that healthy RPM feeding on primary host plants produce droplets consistently whilst feeding and laying eggs; and although setae alone act as repellent structures, the combination of setae and droplets acted to change the behaviour of *A. largoensis*. It is likely this repellence caused by droplets and setae is responsible for the avoidance of adult RPM, but what are the implication of the low levels of predation of the adult stage? Carrillo et al. (2014) demonstrated that inundative releases of *A. largoensis* (1:10 and 1:20 *A. largoensis*: RPM) significantly reduced RPM population increase compared to treatments where *A. largoensis* was excluded. Over a period of three months, the treatments with the highest densities of predators released had significantly fewer RPM eggs compared to treatments where no predators released; and all treatments where *A. largoensis* was released (at ratios of 1:10, 1:20 and 1:30 *A. largoensis*: RPM) had significantly fewer adults. These results indicated the effectiveness of *A. largoensis* as a predator of RPM, and that apparent mortality can be directly attributed to *A. largoensis* predation, however RPM populations still increased over this time period. Treatments with 1:10 *A. largoensis*:RPM increased from 41 to 125 RPM/pinna compared to 43 to 1000 RPM/pinna where *A. largoensis* were excluded. Evidence from the field supports these observations. Ramos-Lima et al. (2011) observed RPM and *A. largoensis* populations on *Musa* spp. over a period of 12 months in Cuba and the populations of *A. largoensis* tracked those of RPM with a slight time-lag but did not act to reduce populations. The same observations were made by Peña et al. (2009) in Puerto Rico. The tracking of the RPM populations show that *A. largoensis* is an important factor in RPM mortality, however other control agents may be required to reduce relative RPM population densities. It can only be hypothesised the effect that additional predation of mobile RPM stages could make. Further studies are required in India to ascertain whether other stages of RPM are actively predated by *A. largoensis* and to assess whether there are other key mortality factors which act to reduce RPM populations.

During assays in Trinidad (although not observed during field collections reported in chapter 5) a species of Neuroptera was observed feeding on all life stages of RPM, and eggs were observed within RPM patches on material collected for these experiments. It is possible that this could be the same species reported by González et al. (2013), *Chrysopa cubana* (Neuroptera: Chrysopidae). Observations showed that the Neuropteran larva could handle the prey without interference from the lateral setae (see Appendix 15) and could consume a considerable number of RPM mobile stages in a short period of time. Classical Biological Control programmes underway are exploring the potential use of *A. largoensis* from other regions and although studies to date have been promising in terms of

higher intrinsic rate of increase of predator populations fed on RPM nymphs (Domingos et al., 2013), these exotic populations should also be tested for their ability to handle the 'New World' RPM , as even though the predator may be effective in the field in the Old World, there is a possibility that if RPM populations are similar to those in Kerala India, the efficacy in these regions could be due to higher RPM susceptibility rather than higher predator efficacy. Further studies should be conducted to test these hypotheses.

7.0 General discussion

The study of factors contributing to the success of an invasive species can provide information about how best to manage the pest. Few studies however, benefit from studying the invasive species in both the adventive and native range, and Hierro et al. (2005) argued that a comparative biogeographical approach is required to test hypotheses such as enemy release hypothesis, evolution of invasiveness, empty niche and novel weapons hypotheses. Throughout this thesis, where possible, the studies have been undertaken in both India (the naturalised range) and Trinidad (the adventive range) and although the focal points of studies have largely been in two focused areas, results have contributed to the body of knowledge relating to RPM. India was chosen as the country of study in the Old World, as the area of origin for the mite has not yet been established, thus it was not possible to confirm the native range. India however, was the area where the mite was first described (Hirst, 1924) and much of the historical work has been undertaken there in relation to RPM (Daniel, 1983; Jalaluddin & Mohanasundaram, 1990; Jayaraj et al., 1991; Kapur, 1961; Ponnuswami, 1967; Puttaswamy & Rangaswamy, 1976; Saradamma, 1972; Sarkar & Somchoudhury, 1988; 1989; Sathiamma, 1996; Somchoudhury & Sarkar, 1987; Yadavbabu & Manjunatha, 2007). In this thesis, RPM was considered naturalised in India because of its longstanding association with *C. nucifera* and *A. catechu* in the country.

The review by Hierro et al. (2005) stated that invasive plants only become super abundant in a recipient community by ‘doing something different’ and this may be a similar case for arthropods. The overall aims of this thesis were to explore factors which may help explain the differences observed between the status and abundance of RPM in the Old and New Worlds; with main qualitative differences reported in the literature prior to the study including the vastly increased host range and reportedly higher RPM densities in the adventive range. To explore the underlying drivers for this, the work was divided into two broad approaches: firstly, studies were undertaken to assess and confirm the observations reported in the literature relating to both host range and density in both the adventive and naturalised range; and secondly studies were undertaken to explore some of the possible drivers underlying these observations. Prior to the commencement of the work here, it was difficult to compare the situations in the two ranges, as a variety of different approaches and sampling methods had been used by different researchers, and it was unclear whether observed differences in host range were due to a lack of sampling in the naturalised range, lack of detailed study of host plant relations in the naturalised range, or whether there is a real host range expansion in the adventive range. In this chapter, the main findings are discussed in the broader context of invasion ecology,

recent research relating to RPM and implications for management of the pest. Future avenues of research are suggested for researchers working towards a solution to RPM outbreaks in the adventive range. The discussion here is divided in relation to the research undertaken to the two broad questions posed in chapter 2.8.1 and 2.8.2: Has RPM expanded its host range in the New World and if so, what are the factors behind this expansion? And, how accurate are the reports relating to the increase in RPM population density between Old and New World regions and if accurate, what factors may be driving the differences?

7.1 Has RPM expanded its host range in the New World and if so, what are the factors behind this expansion?

To answer the above question, it was necessary to clarify the host plant relations of RPM in India. At the outset of this work, little was known about the host plant associations of RPM in India, other than the reports of seasonal infestations of RPM on *C. nucifera* and *A. catechu*. Work undertaken here aimed to clarify the situation in the Old World, so comparisons could be made with the adventive range. In chapter 4 the results of host range leaf experiments and surveys uncovered little evidence that RPM had a wider host range in Kerala than previously reported, with *B. flabellifer*, *C. renda* and *Pandanus* sp. -all commonly grown in the survey area- excluded as possible hosts. *Phoenix roebelenii*, however, was reported as a new host association for the Old World. The landscape of the study area surveyed was comprised of a mix of smallholder farms, coconut plantations and rice paddy fields, thus the diversity of alternative palm species to survey was low, apart from stands of *B. flabellifer* and ornamental palms grown sporadically in gardens of smallholder farmers. Thus, it is likely the under reporting of other host palm species may be due to lack of abundance of alternative palm hosts in the survey area. Further surveys encompassing a wider geographical area may find new host palm associations, as further possible host plant species may be encountered. Nonetheless, the results relating to the use of different host plant taxa in the field indicate that in Kerala, RPM has a relatively narrow host range, in that the only multigenerational colonies observed were on *C. nucifera*, *A. catechu* and *P. roebelenii*.

One of the key observations at the outset of the work was the number of reports of RPM breeding on plants from the order Zingiberales (including *Musa* spp.) in the adventive range compared to sparse reports of RPM on this species in the Old World. These observations raised questions about whether there were in fact different races of RPM present (either host associated or geographic) which were better able to exploit plants from the order Zingiberales, or whether these associations (with *Musa* spp. in particular) were under reported in the Old World. These questions led to two main studies in

chapters 3 and 4. The first set of studies in chapter 3 aimed to assess the molecular and morphological status of RPM collected from different host plants in each region. The aim was to clarify whether there was any evidence of host plant or geographical races in terms of morphological or molecular differences between RPM populations collected from different host plants in India and Trinidad. The second set of studies in chapter 4 aimed to explore the relationship RPM had with hosts found in the field in Kerala, India, (in both the field and the laboratory) to complement those already undertaken in the adventive range.

Results (Chapter 3) found no evidence of morphological differences which could be attributed to host plants between populations of RPM collected from different host plants in either region using Principle Components Analysis (PCA) and Linear Discriminate Analysis (LDA). Importantly, specimens collected from hosts of the Zingiberales and Arecales did not differ morphologically in the adventive range, and evidence from the literature supported this where no molecular differences between RPM collected from *Musa* spp. and *C. nucifera* in Trinidad were found (Dowling et al., 2008).

It was not possible to conclusively compare RPM collected from plants from the Zingiberales and Arecales in India due to a lack of specimens Zingiberales. Furthermore, no significant differences could be attributed to host associations found between specimens collected from the different Arecales species within the country, as may have been predicted given the slight molecular differences reported between RPM specimens collected from *A. catechu* and *C. nucifera* (Dowling et al. (2012). Instead, significant differences between some of the dorso-central and dorso-sublateral setae were found between different sampling locations as well as different hosts (in both regions) indicating that differences were more likely site (and possibly nutrition) associated. These findings were largely in line with those from a recent study conducted in Venezuela (Vásquez et al., 2014) where authors found host level differences in setae length and distances between setae. The authors stated that differences in setae length were more likely due to environment than evidence for host plant races and found that it was the dorso-central and dorso- sublateral setae which were variable among site and host. Phenotypic plasticity in seta length has been attributed to host level, and environmental level differences by several authors (Houck & OConnor, 1996; Skoracka et al., 2002) and has led to the re-description of some species complexes i.e. *Eutetranychus banski* (Acari:Tetranychidae) (Mattos & Feres, 2009). It is possible that differences in nutrition and local conditions influence these seta lengths. Host associated differences may manifest in attributes other than setae length thus further molecular work to underpin these observations should be undertaken. The differences between specimens collected from *A. catechu* and *C. nucifera* in Bangalore, India

(Dowling et al., 2012) may not manifest morphologically.

There was evidence however, that there were distinct morphological differences between the populations of RPM studied in India and the Caribbean, thus it is possible there are geographic races or population subsets of RPM. The lateral setae c3, d3 and e3 were found to be significantly longer in the Caribbean than the population studied in Kerala, India. An analysis of lateral setae lengths in the Caribbean showed that there were no significant changes in length over time (2009, 2012, 2014), therefore it is unlikely that longer setae were selected for over this time. The likelihood is that the lengths of these setae are highly heritable, given the low standard errors for setae lengths within both the Indian and Caribbean populations (c3: India- 0.83, Caribbean- 0.59; d3: India- 0.9, Caribbean- 0.72; e3: India- 0.94, Caribbean- 0.50). It is possible that these differences probably have not evolved *in situ* in country in response to different selection pressures as would be concurrent with the evolution of invasiveness hypotheses (Lee, 2002); instead it is more likely that the source population of RPM arrived with these traits and due to a genetic bottleneck at the point of introduction caused by a relatively small founder population, these traits have been inherited by subsequent populations. This would indicate a single introductory event and that the source populations in the adventive range were not akin to those studied in Kerala, India. Dowling et al. (2012) showed there were molecular differences between RPM collected from two separate locations in India (from *C. nucifera* and *A. catechu*), and RPM collected in Iran and UAE, thus it may be that populations studied in Kerala were analogous to those ex *C. nucifera* in Dowling et al. (2012), although further testing is required to confirm this, as it was not possible to extract enough DNA from specimens from Kerala. The specimens collected ex *A. catechu* and *C. nucifera* in the Dowling study should be further examined to see if there are differences in lateral setae length as these could be indicators for two distinct races of RPM. Similar morphological studies have been carried out on the coconut mite, *Aceria guerreronis*, between America, Africa and Asia (Navia et al., 2009). The authors measured morphological variation of *A. guerreronis* between America, Africa and Asia and found that Principle Component 1 (PC1) and Principle Component 2 (PC2) accounted for 43.5% of total variation between populations when all measurements were analysed. In the present study, when all measurements were analysed for RPM between regions 70.1% of the variation was explained by PC1 and PC2. When a PCA was applied to lateral setae alone, PC1 accounted for 90.8% of the variation suggest an almost perfect partitioning of samples into two distinct geographic races based on these features.

Given the differences between the two populations; assays and surveys were undertaken to explore the relationship RPM had with *Musa* spp. in Kerala, India. Although there was no evidence of host associated morphological races, the presence of geographical morphological differences in

populations raised questions about whether these were different races with different host ranges i.e. can RPM populations in Kerala, India utilise plants from the order Zingiberales as hosts in addition to those from the Arecales? Results (chapter 4) showed that, depending on the species/cultivar there was no significant difference in survival of RPM on leaf discs taken from plants from the order Zingiberales in Kerala when transferred from *C. nucifera* (*Musa* sp. cv Palayam kodan, *Musa* sp. cv Robusta, *Musa* sp. cv Red, *Heliconia rostrata*, *Heliconia psittacorum* and *Heliconia stricta*) and *A. catechu* (*Musa* sp. cv Palayam kodan – other species/cultivars not tested). In terms of numbers of eggs per female per day, significantly fewer eggs were laid on the majority of *Musa* spp. cultivars tested (4.3.1: Robusta, Red, Nendran and Poovan) however, on *Musa* sp. cv Palayam kodan, *H. psittacorum* and *H. stricta*, although fewer eggs were laid when compared to *C. nucifera*, differences were not significant (4.3.1 and 4.3.2). It was not possible to conduct crosses from *Musa* spp. onto palms in India to test performance on plants from the Arecales, as there were no established colonies in the field. The design of assays was such that results could be compared to those published in Florida (Cocco & Hoy, 2009), where the authors transferred RPM from *Musa* sp. onto *C. nucifera* and from *C. nucifera* onto several varieties of *Musa* spp.. The authors showed that the numbers of eggs per female per day were lower when RPM were transferred from *C. nucifera* to *Musa* spp. (between 0-0.3 eggs per female per day [apart from one *Musa* sp. cultivar (Lady finger) where there were 0.75 eggs per female per day] compared to 0.97 ± 0.18 on *C. nucifera*), and results were largely comparable with the results presented here (between a mean of 0.02-0.27 eggs per female per day on *Musa* spp. and 0.43-0.84 on *C. nucifera*) indicating similar *in vitro* performance of RPM on *Musa* spp. between India and the adventive range in terms of egg laying. The similarities in *in vitro* performance indicate that *Musa* spp. are likely to be a secondary host for RPM in both regions, and there is not likely to be a biological difference in how RPM perform on these species between the two regions *in vitro* given the current results. Laboratory evidence showed that in addition, RPM when placed on *Musa* sp. cv Palayam kodan leaf discs – a cultivar commonly grown in Kerala- did not show the same level of run off as it exhibited with more unfavourable hosts (*Pandanus* sp., *B. flabellifer* and *C. renda*), indicating host plant acceptance in the laboratory. Field results however contradicted this, as the only multigenerational colonies observed in Kerala, India were on *C. nucifera*, *A. catechu* and *P. roebelenii*, and breeding colonies of RPM were only found on *C. nucifera*, even when these plants were grown in close proximity to multiple *Musa* sp. cv Palayam kodan plants (4.3.1). Further multisite surveys confirmed the lack of multi-generational colonies on *Musa* spp. in Kerala, India compared to many observations in Trinidad (5.4.1). Results indicated that RPM had either a preference or fidelity to *C. nucifera* in the field, or that other factors were limiting populations on these plants.

A model derived from survival and fecundity data showed that the low abundance in the field may have been due to a lower net reproductive rate on *Musa* sp. cv Palayam kodan when compared to *C. nucifera* and *A. catechu*. The lower numbers of eggs per female per day laid on *Musa* spp. would lead to lower RPM numbers in the field compared to those on *C. nucifera* and *A. catechu*. Study trips did not allow for full generations of RPM to be followed, thus further work should be conducted collating information on duration of life stages, survival of different life stages and total oviposition as has been collected by other authors (Balza et al., 2015; Moutia, 1958; Ramos-Lima et al., 2011; Vásquez et al., 2015a). Using the nymphal survival data, simplistic population models could be constructed to predict the expected increase in population numbers when host plant alone is considered. In the current model, nymphal survival was presumed to be 100% and instead, the adult survival data collected in chapter 4 was used to predict potential field populations. This showed that given adult survival and egg laying alone as a predictor, populations could be expected on *Musa* sp. cv Palayam kodan, albeit at a much lower level than *C. nucifera* and *A. catechu*. Balza et al. (2015) demonstrated the phenolic compounds found in different cultivars may be responsible for resistance mechanisms against RPM, and the presence of these compounds could be limiting RPM populations on local Keralan *Musa* spp. cultivars. The diversity of cultivars grown in India is likely to be higher than those grown in the New World, as India is thought to be the centre of diversity for many of the AAB and AB cultivars (Sharrock et al., 2001); thus it is possible that cultivars in India are innately more resistant to attack by RPM than those grown in the adventive range.

If reduced nymphal survival, lower total oviposition/adult longevity were some of the reasons for the absence of RPM on *Musa* spp. in the field, one would still expect to find evidence of small colonies in the field comprising of eggs and mobile stages as were observed in Trinidad; however only individual mobile stages were ever found. In the adventive range multi-generational colonies on *Musa* spp. have been observed in the field even though oviposition levels were lower and adult longevity was reduced on *Musa* spp. when compared to *C. nucifera* (Ramos-Lima et al., 2011), perhaps indicating that there may be other factors causing these observations. Given the lack of RPM colonies on *Musa* spp., in India it is possible that other factors are contributing to their absence in the field, such as the associated natural enemy complex. Results in chapter 5 showed that the diversity of predators collected from *Musa* spp. was higher than *C. nucifera* in India, however when predator diversity scores were compared on *Musa* spp. between regions predator diversity were similar. It is possible that natural enemies associated with RPM in India have a greater impact on RPM populations than those in the adventive range, as these species have coexisted for a significantly longer period of time. This would be concurrent with the enemy release hypothesis (Keane & Crawley, 2002). Further studies to explore the relationship of RPM and associated predators on *Musa* spp. in India would add

further insight to this theory, as only predators collected ex *C. nucifera* and *A. catechu* have been studied to date in India (Daniel, 1976; 1981; 1983). The efficacy of the natural enemy complex in relation to ERH are discussed further in 7.2.

7.2 How accurate are the reports relating to the increase in RPM population density between Old and New World regions? If robust, what factors may be driving the differences?

By undertaking work in both regions, it was confirmed for the first time in chapter 5, that RPM infestations were significantly denser on *C. nucifera* in the adventive range compared to India, and that RPM was significantly more abundant on *Musa* spp. at the time the surveys were undertaken. Comparative density and abundance studies are rare in the area of invasion ecology (Hierro et al., 2005) and the logistical difficulties in conducting analogous surveys in two regions where similar conditions prevail have contributed to this. It was not possible to conduct surveys at the same time, however, by choosing the peak season for both which had already been established in the literature, confidence in the results can be relatively high, although ideally these studies should be carried out on a season long basis to confirm the differences observed.

To explore factors which may be related to the differences in densities, studies were required to clarify the situation in India in comparison to the adventive range, as to date, selected studies have been carried out individually in each range, but no direct comparisons have been made. Studies aimed to explore possible drivers for these differences and looked both intrinsically at RPM morphology and performance and extrinsically, at host plant relations, predator relations and climatic factors. Findings are discussed in the context of invasion success theories, including evolution of increased competitive ability (EICA) (Blossey & Notzold, 1995) and the enemy release hypothesis (ERH) (Keane & Crawley, 2002), however these hypotheses were not directly tested.

The EICA hypothesis states that a decrease in the allocation of resources to defence (due to reduced predation pressure) allows for an increase in allocation of resources for growth and reproduction (Blossey & Notzold, 1995). An increase in growth and reproductive capacity could be hypothesised to account for denser populations in the adventive range if indeed they are reproducing under conditions of lower predation pressure. Although this hypothesis was not directly tested, preliminary studies were conducted to assess RPM survival and fecundity between regions, and an assessment of the associated natural enemy fauna was made in both regions. In terms of RPM performance, the length of study trips did not allow for all reproductive parameters such as egg to adult time, oviposition period, mean female longevity to be measured, however studies were instead conducted using the

method described by Cocco & Hoy (2009) so results could be compared to published results from the adventive range. Studies comparing survival and fecundity on *C. nucifera* showed no significant differences between RPM studied in Kerala and Trinidad, and results were broadly comparable with those published by Cocco & Hoy (2009); however, more in depth studies should be undertaken to test biological performance between regions, such as comparative lifetable analyses. The only detailed lifetable analyses from the Old World to date have been conducted by Moutia (1958) and Zaher et al. (1969). Moutia (1958) showed that the numbers of eggs per female per day laid by RPM in Mauritius (taken from entire length of oviposition period) were on average 1.0 per day, and other studies have shown the figure to be between 0.93 and 2.1 on average on *C. nucifera* (Balza et al., 2015; Cocco & Hoy, 2009; Ramos-Lima et al., 2011; Vásquez et al., 2015b). The approach in the work here used the methodology outlined by Cocco & Hoy (2009) which did not follow the entire oviposition period thus, averages were slightly lower in comparison to other studies with a mean of 0.76 eggs per female per day in this study in Trinidad and 0.5, 0.84 and 0.43 eggs per female per day in three separate studies in India. Results by Cocco & Hoy (2009) were similar to these in terms of eggs per female per day on *C. nucifera* with an average of 0.93 eggs per female per day on *C. nucifera* over 11 days. In the present study, results from India were more variable with oviposition in two assays lower than those in Trinidad, one significantly so, and lower when compared to the data from Cocco & Hoy (2009), thus reasons for the variability in results should be explored. It is possible that although survival and egg laying are not significantly different, there may be differences in other performance parameters mentioned previously which may affect overall population growth. These parameters should be tested before conclusions can be drawn relating to performance of RPM between regions.

Factors extrinsic to RPM may also affect the density of populations of RPM. Firstly, host plant level differences such as cultivar or environmental conditions could potentially lead to differences in RPM population observations between regions. Red Palm Mite may flourish on certain cultivars compared to others and with differences in plants grown between regions, this may exacerbate observed differences. Lee (2002) outlined that differences observed in invasive populations compared to their native range could be due to selection in response to abiotic (i.e. temperature, photoperiod or climate) or biotic (i.e. local predator or competitor fauna) factors, however an estimation of development speed in association with maximum and minimum temperatures applied using a simple degree day model, assuming identical performance between regions, showed there would be little difference in population growth and numbers of generations, given the mean climatic conditions in both regions. Further lifetable information would enhance these predictions. An analysis of rainfall between regions however did show that although monthly rainfall during times when RPM populations were building up were generally higher in Trinidad compared to India, the peak monthly rainfall annually was much

higher and more frequent in Kerala, India compared to Trinidad. During a five year period in Palakkad, Kerala, India, (2011-2015) peak monthly rainfall exceeded 250mm on 16 occasions between June and August ranged between 387mm-797mm (Knoema, 2018) compared to the same time period in Trinidad where rainfall only exceeded 250mm on one occasion (Climate Change Knowledge Portal, 2018). Further studies investigating the effect of rainfall intensity and frequency are required to ascertain the effects on seasonal RPM population dynamics.

The enemy release hypothesis states that distribution and abundance of an invasive will be increased in the adventive range due to lower impact of natural enemies. This is caused by firstly the absence of specialist co-evolved natural enemies found in the native range, which are not present in the adventive range and secondly, low predation pressure from natural enemies in the adventive range caused by a low propensity to switch hosts to prey upon the invasive (Keane & Crawley, 2002). The hypothesis also states that generalists found in the adventive range will have a greater impact on native species, as opposed to introduced species. Results showed that species within predator complexes studied in relation to RPM were comprised of generalist predators, although the species within these complexes differed in each region, apart from *A. largoensis* (Carrillo et al., 2014). These results supported those already observed in the literature (Daniel, 1983; Peña et al., 2009; Taylor et al., 2012) however results contributed comparative predator diversity scores. Diversity scores showed that predator diversity was slightly higher on *C. nucifera* in India (1.39) than Trinidad (1.01) and marginally lower on *Musa* spp. in India (1.68) when compared to Trinidad (1.73). It is possible that the different species found within the generalist complexes in both regions, differ in their impact against RPM, with those in India- which have had a longer period of time to learn to handle RPM- perhaps more efficacious than those in the adventive range. Certainly, the literature has shown the importance of *A. largoensis*, *S. keralicus* and larvae of Cecidomyiidae (which were all found in association with RPM during surveys in India) as important predators of RPM in the Old World (Daniel, 1976; 1983; Domingos et al., 2013) and their taxonomic equivalent species in the New World such as *S. utilis* and *S. punctillum* (Coccinellidae: Coleoptera) and *Arthrocnodax* sp (Diptera Cecidomyiidae) have been shown to have low efficacy against RPM (Carrillo et al., 2012c). *Stethorus keralicus* has been shown to be an effective predator of RPM in Kerala (Daniel, 1976), however *S. utilis* and *S. punctillum*- which have been recorded in association with RPM in the adventive range- do not feed on RPM and have a preference for preying on Tetranychidae (Carrillo et al., 2012c; Pena et al., 2009). The role of the predatory *Scolothrips* sp. in the complex in India also needs to be established, especially in light of a new closely related species of predatory thrips identified as a predator of RPM (*Scolothrips ochoa*) (Mound et al., 2010).

Although *A. largoensis* has been shown to be an important mortality factor against RPM in the adventive range (Peña et al. 2009, Ramos-Lima et al., 2011, Carrillo et al. 2012c), the literature has also shown that even when inundative releases are undertaken, *A. largoensis* does not act to reduce RPM populations, only the rate of increase of RPM populations (Carrillo et al. 2014). Studies in chapter 6 identified the mechanisms underlying the avoidance of predation of the adult females by *A. largoensis* thus unless populations gain experience in handling other RPM stages, additional mortality factors are required to curb RPM populations. Alternative predators such as Coccinellidae and Neuropterans may be beneficial as they may be able to handle and consume the adult stages of RPM without being repelled (see Appendix 15). It could be hypothesised that the lack of additional key mortality factors in the adventive range (albeit predators or abiotic differences such as rainfall) may underpin the differences observed in the densities of populations.

In relation to predators, there were only predatory mites recorded on plants surveyed in Trinidad, however there is evidence that there are larger insect predators consuming RPM in the adventive range such as *Chrysopa cubana* Hagen (Neuroptera: Chrysopidae), Coccinellids such as *Stethorus* sp. and *Scymnus* sp. (Coleoptera: Coccinellidae), and Hemipterans such as *Orius insidiosus* Say (Hemiptera: Anthocoridae) (González et al., 2013) and efforts to explore their efficacy should be made where data is lacking.

Morphologically, in chapter 3 significant differences were observed in the lengths of lateral setae c3, d3 and e3 between specimens collected in India and Trinidad; and work in chapter 6 aimed to explore the role these setae have. Was there evidence these setae interfere with predation efficacy of *A. largoensis* and if so, what role could setae length have in repellence? The implication being that adult RPM in the adventive range are potentially less susceptible to predation by *A. largoensis* resulting in denser populations of RPM. Results from chapter 6 showed that lateral setae and droplets did repel RPM's main predator *A. largoensis*, however it was not possible to demonstrate a link between predator efficacy and setae length due to the lack of diversity of setae lengths on RPM specimens collected in Trinidad. Analogous studies in India would be able to prove or disprove this theory using the same experimental design. Although no experimental link was shown between the length of setae and predation success, it was possible to demonstrate that these setae and their associated droplets do impact on the behaviour of the predator, although the hypothesis that RPM in the adventive range are *better* defended remains to be answered. This would require comparative assays to be undertaken in India where the lateral setae on RPM are significantly shorter.

The observations that *A. largoensis* was repeatedly repelled when approaching the rear setae and

finally made a successful attack from the anterior where lateral setae were shorter, demonstrated the effect that these setae may have in the reduction of efficacy of *A. largoensis* against adult stages of RPM. The mean length of v2, sc1 and sc2 setae were between 4-5 μm on average shorter on predated than the non-predated counterparts and length of these setae on average were between 74-91 μm long. The means lengths of c3, d3 and e3 setae in India (from *C. nucifera*) were found to be 91.9, 93.1 and 99.4 μm respectively indicating that if this experiment were to be repeated in Kerala, there is a possibility that there would be a higher predation success rate for adult female RPM. If further experiments conducted were able to confirm that the difference in seta length is a significant factor in defence against predation from *A. largoensis*, it may contribute to the understanding as to why RPM colonies are less dense in India compared to the New World, even if India is not the area of origin for RPM. Predator exclusion experiments carried out in Florida, USA (Carrillo et al., 2014) have shown that in the absence of *A. largoensis*, populations of RPM are significantly higher, however the authors highlighted that this was due to egg predation and there is a requirement that mobile stages need to be controlled also to ensure more effective control of RPM. Experiments in Roraima, Brazil have compared the performance of *A. largoensis* from Roraima and La Reunion against RPM and found that the predator from La Reunion did not control the population of RPM more effectively than the resident *A. largoensis* from Roraima, Brazil (Morais et al., 2016); even though the predator from La Reunion has been shown to have a significantly higher net reproductive rate and prey consumption when fed RPM nymphs compared to *A. largoensis* from Roraima, Brazil (Domingos et al., 2013). Studies have shown that although *A. largoensis* from each region are the same taxonomic species, there are distinct genetic differences between the two groups (Navia et al., 2014); thus, there is a situation now where we have seen there are not only differences in the genetic and morphometrics of *A. largoensis* from different regions (Navia et al., 2014), but there are also differences in the morphometrics of RPM between regions. This could have implications for classical biological control programmes if the different lengths of RPM lateral setae are shown to affect the handling of RPM during predation.

The presence of droplets on the end of RPM setae were shown to enhance the defence of RPM against predation through lower levels of contact and predation by *A. largoensis*. Red Palm Mite on a favourable host such as *C. nucifera* were more likely to have droplets present on the ends of their setae than those on unfavourable hosts such as *Pandanus* sp., *C. renda*, *B. flabellifer* and after a period of days, lower levels of droplet expression were observed on *A. catechu* and *Musa* sp. cv Palayam kodan. The implications of this are two-fold. Firstly, droplet expression was related to eggs production, so those expressing droplets were likely to also be laying eggs. Secondly, those on unfavourable hosts were most likely producing lower numbers of eggs and droplets, thus given the

predator behaviour results are more likely to be vulnerable to predation from *A. largoensis*. This result may go some way to explaining the differences observed in the field in India and it would be interesting to run analogous test in Trinidad to see if there is the any difference in the volume of droplets produced between individuals on less favourable hosts.

Although the outcomes of this work have contributed to the body of knowledge relating to RPM. The work also raises further questions. Firstly, further surveys establishing the abundance and diversity of Arecales and Zingiberales within each region may clarify potential host availability for RPM- it was not clear within the work presented whether the absence of RPM on alternative palms was due to a lack of availability of hosts or whether the absence was due to host plant resistance. With regards to the relationship with *Musa* spp. in the Old World, further studies to establish the reproductive performance of RPM on *Musa* spp. *in vivo* would be beneficial to support the outcomes of the *in vitro* experiments. In relation to predator diversity, wider surveys throughout the season using techniques which would sample the insect predators would give insight into the comparative seasonal dynamics of the predators in each range, and more focus should be given to those predators which are able to handle adult stages. Exclusion experiments in India may establish to what extent the insect predators impact on RPM populations. Molecular work is required to explore the theory that there are geographical races and RPM from each proposed race should be compared under identical conditions to establish if there is any difference in their bionomics.

Conclusions

At the outset of this thesis, the overall aims were to investigate the differences in observations made between RPM populations in the Old and New World, and to explore some of the main factors which underpinned these differences. From the hypotheses outlined in the introduction and each of the individual chapters, the following conclusion can be drawn:

1. Although there were some morphological differences found between RPM collected from *C. nucifera* and *A. catechu* in India (setae f3, v2 and c1; $p < 0.05$), these setae were also found to differ significantly between different collection sites. It is likely these differences were caused by extrinsic environmental factors, rather than host level factors.
2. Again, although there were some significant morphological differences found between RPM collected from *C. nucifera* and *Musa* spp. in the Caribbean (setae c2 and f2; $p < 0.05$), these

- setae were again found to differ significantly between different collection sites. It is likely these differences were caused by extrinsic environmental factors, rather than host level factors
3. There were significant differences observed in the morphology of RPM between India and the Caribbean, with lateral setae c3, d3 and e3 significantly longer. These differences led to an almost perfect partitioning of populations using PCA and hierarchical discriminate analysis.
 4. The host range of RPM was not found to be wider than reported for RPM in the Old World- only one further host was confirmed (*Phoenix roebelenii*); however further surveys encompassing a wider area and range of hosts may add to the host list in India.
 5. *Borassus flabellifer*, *Cyrtostachys renda* and *Pandanus* sp. can all be excluded as possible hosts for RPM in Kerala, India.
 6. Adult female RPM survival on different species of Zingiberales was shown to differ depending on species and cultivar in India. No significant difference in survival of RPM was found between RPM on leaf discs of *Musa* sp. cv Palayam kodan, Red and Robusta and *Heliconia psittacorum* and *Heliconia stricta*.
 7. It is unlikely that there are host specific races of RPM. No significant differences were found in survival of RPM transferred from *A. catcechu* and *C. nucifera* onto *A. catcechu*, *C. nucifera* and *Musa* sp. cv Palayam kodan. It is likely that *Musa* spp. are a secondary host and RPM survival and performance in terms of egg laying is cultivar dependent.
 8. RPM populations were significantly denser *C. nucifera* and denser on *Musa* spp. (but not significantly so) in Trinidad compared to India. Red Palm Mite were significantly more abundant on *Musa* spp. in the adventive range in Trinidad.
 9. There was no relationship between RPM densities on *C. nucifera* and *Musa* spp. found on the same site in Trinidad.
 10. Arthropod predator diversity was not poorer in the adventive range (Trinidad) compared to the naturalised range (India).
 11. *Amblyseius largoensis* was the only predator observed in both regions, thus confirming its importance as a predator of RPM.
 12. It was not possible to demonstrate whether long lateral setae affected the efficacy of *A. largoensis* when attacking adult female RPM. No relationship was observed between seta length and predation success. Further assays are required with RPM with a wider diversity of lateral setae lengths.
 13. The presence of droplets on the distal tips of RPM setae significantly reduced the number of occasions *A. largoensis* contacted RPM individuals compared to those without droplets and eggs.
 14. Droplets and setae were both shown to affect the behaviour of *A. largoensis*, with *A.*

largoensis spending longer periods of time grooming when it had contacted droplets or setae. These differences were not significant however.

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9.0 Appendix

Appendix 1 Fieldwork dates, locations and activities

Fieldwork Location	Dates	Activities
Trinidad, St. Kitts, Dominica, Nevis, Antigua	End of May 2009	Chapter 3: Morphological comparison of RPM- RPM samples from <i>Cocos nucifera</i> and <i>Musa</i> spp. on my behalf by Dr. D. Moore
Kerala, India (hosted by KFRI, Peechi)	6 th -16 th December 2009	Chapter 5: Morphological comparison of <i>Amblyseius largoensis</i> between India and Trinidad -Collections of <i>A. largoensis</i> for morphological study. Season long sampling commenced in Kerala, and wind traps deployed. These data were not presented in the thesis.
Kerala, India (hosted by KFRI, Peechi)	9 th - 20 th March 2010	Chapter 3: Morphological comparison of RPM- Collections of RPM made from various sites between 10 th – 19 th March 2010; Chapter 4, Hypothesis 1: host plant survey in the wider area, field survey of bananas of different ploidies, local <i>Musa</i> spp. cultivars survival and fecundity analysis; further field studies on RPM dispersal
Kerala, India (hosted by KFRI, Peechi)	4 th - 17 th July 2010	Wind trap samples identified (data not shown in thesis)
Kerala, India (hosted by KFRI, Peechi)	2 nd - 15 th April 2011	Chapter 3: Morphological comparison of RPM ; Chapter 4, Hypothesis 1: Laboratory survival and fecundity assays on alternative host species 6 th -13 th April 2011, Appendix 1 A preliminary study to assess the egg consumption of <i>Amblyseius largoensis</i>

		between Trinidad and India. (India <i>A. largoensis</i>). Chapter 6: Investigating and characterising droplet expression between RPM cultured on different host plants and regions (India assays)
Kerala, India (hosted by KFRI, Peechi)	4 th -15 th March 2012	Chapter 3: Morphological comparison of RPM; Chapter 5 surveys on <i>Musa</i> spp. and <i>C. nucifera</i> surveys conducted 7 th – 8 th March 2012. Survival analysis hypothesis 2, chapter 4: Survival and reproduction of RPM on natal host compared to alternative host. Laboratory experiments on RPM dispersal (data not in thesis)
CABI, Curepe, Trinidad	06/04-13/04/2012	Chapter 3: Morphological comparison of RPM. Chapter 5: surveys on <i>Musa</i> spp. and <i>C. nucifera</i> 7 th – 8 th April 2012 and 11 th April 2012; Chapter 5 Morphological comparisons of <i>A. largoensis</i> ; Initial <i>A. largoensis</i> : RPM challenges for chapter 6 undertaken. Data not shown in thesis but experience used for methodology improvements. Dispersal experiments undertaken in CABI grounds and laboratory (data not shown in thesis)
CABI, Curepe and Manzanilla, Trinidad	09/04/2013- 25/04/2013	Chapter 5 <i>A. largoensis</i> collections for morphological comparison
CABI, Curepe, Trinidad	27/10/2014-11/11/2014	Chapter 5: Comparison of biological performance of RPM between Trinidad and India survival analysis commenced 30 th October- 6 th November 2014;

Chapter 6 Hypothesis 1: Long lateral setae will affect the efficacy of *A. largoensis* when attacking adult female RPM; Hypothesis 2: Droplets will enhance the defence of RPM against *A. largoensis* attack- RPM with droplets will be attacked on fewer occasions compared to those without droplets. Experiments commenced 28/10/14

Appendix 2 Host plant, country and location within country of RPM specimens collected and measured for the study

Sample	Host	Region	Sub region ID	Site description	Latitude	Longitude	No. plants	No. Females
I1	<i>Cocos nucifera</i>	India	Kerala	Vadakkencherry area, Site 1	N 10 35 37.5	E 076 30 13.1	5	1;4;3;5;3
I3	<i>Cocos nucifera</i>	India	Kerala	Vadakkencherry area, Site 3	N 10 35 08.5	E 76 31 10.1	1	4
I4	<i>Cocos nucifera</i>	India	Kerala	Vadakkencherry area, Site 4	N 10 35 32.3	E 76 31 02.3	1	3
I2	<i>Cocos nucifera</i>	India	Kerala	Vadakkencherry area, Site 2			1	3
I4B	<i>Cocos nucifera</i>	India	Kerala	Vadakkencherry area, Site 4B			1	3
I11	<i>Cocos nucifera</i>	India	Kerala	Vadakkencherry area, Site 11			1	1
I13	<i>Cocos nucifera</i>	India	Kerala	Vadakkencherry area, Site 13			1	1
IPAL	<i>Cocos nucifera</i>	India	Kerala	Palakkad area			1	1
I1	<i>Musa</i> sp.	India	Kerala	Vadakkencherry area, Site 1	N 10 35 37.5	E 076 30 13.1	1	1
IK	<i>Musa</i> sp.	India	Kerala	Pattikad-Vadakkencherry Roadside,			1	1
I3	<i>Areca catechu</i>	India	Kerala	Vadakkencherry area, Site 3,	N 10 35 08.5	E 76 31 10.1	2	7;3
IB	<i>Areca catechu</i>	India	Kerala	Kannara, Peechi area, Banana Research Station	N 10°32'12.94"	E 76°19'15.21"	2	2; 4
I13	<i>Areca catechu</i>	India	Kerala	Vadakkencherry area, Site 13			1	3
I4	<i>Phoenix roebelenii</i>	India	Kerala	Vadakkencherry area, Site 4	N 10 35 32.3	E 76 31 02.3	2	4;4
Cd1	<i>Cocos nucifera</i>	Caribbean	Dominica	1	N 15 37 61	W 061 25 69.8	1	6
Cd13	<i>Cocos nucifera</i>	Caribbean	Dominica	13	N 15 22 41.8	W 061 15 20.9	1	3
Cd17	<i>Cocos nucifera</i>	Caribbean	Dominica	17	N 15 35 18.9	W 061 27 84.3	1	5

The ecology of *Raoiella indica* (Hirst) (Acari:Tenuipalpidae) in India and Trinidad

Sample	Host	Region	Sub region ID	Site description	Latitude	Longitude	No. plants	No. Females
CT24	<i>Cocos nucifera</i>	Caribbean	Trinidad	24	N 10 20 49.0	W 060 59 83.8	1	3
CT6	<i>Cocos nucifera</i>	Caribbean	Trinidad	Maraccas royal road, site 6			1	1
CT7	<i>Cocos nucifera</i>	Caribbean	Trinidad	Maraccas royal road, site 7			1	3
CT11	<i>Cocos nucifera</i>	Caribbean	Trinidad	Maraccas royal road, site 11			1	3
CT13	<i>Cocos nucifera</i>	Caribbean	Trinidad	Maraccas royal road, site 13			1	2
CT15	<i>Cocos nucifera</i>	Caribbean	Trinidad	Maraccas royal road, site 15			1	2
CA35	<i>Cocos nucifera</i>	Caribbean	Antigua	35	N 17 03 32.5	W 061 50 96.1	1	2
CA49	<i>Cocos nucifera</i>	Caribbean	Antigua	49	N 17 02 51.2	W 061 52 94.5	1	3
CSK38	<i>Cocos nucifera</i>	Caribbean	St. Kitts	38	N 17 18 05.1	W 062 46 58	1	3
CSK39	<i>Cocos nucifera</i>	Caribbean	St. Kitts	39	N 17 22 70.5	W 062 45 25	1	2
CSK42	<i>Cocos nucifera</i>	Caribbean	St. Kitts	42	N 17 08 69.2	W 062 37 73	1	2
CD7	<i>Musa sp.</i>	Caribbean	Dominica	7	N15 27 52.2	W061 15 97.4	1	3
Cd16	<i>Musa sp.</i>	Caribbean	Dominica	16	N15 26 07.9	W 061 15 59.4	1	3
CT21	<i>Musa sp.</i>	Caribbean	Trinidad	21	N10 05 24.2	W061 53 99.0	1	4
CT29	<i>Musa sp.</i>	Caribbean	Trinidad	29	N10 20 49.0	W 060 59 83.8	1	3
CT1B	<i>Musa sp.</i>	Caribbean	Trinidad	Maraccas royal road, site 1			1	2
CT3B	<i>Musa sp.</i>	Caribbean	Trinidad	Maraccas royal road, site 3			1	2
CT6B	<i>Musa sp.</i>	Caribbean	Trinidad	Maraccas royal road, site 6			1	4
CT9B	<i>Musa sp.</i>	Caribbean	Trinidad	Maraccas royal road, site 9			1	1
CT10B	<i>Musa sp.</i>	Caribbean	Trinidad	Maraccas royal road, site 10			1	3
CT11B	<i>Musa sp.</i>	Caribbean	Trinidad	Maraccas royal road, site 11			1	3
CT12B	<i>Musa sp.</i>	Caribbean	Trinidad	Maraccas royal road, site 12			1	3
CT13B	<i>Musa sp.</i>	Caribbean	Trinidad	Maraccas royal road, site 13			1	3
CT15B	<i>Musa sp.</i>	Caribbean	Trinidad	Maraccas royal road, site 15			1	1

Appendix 3 Males specimens slide mounted for morphological ID.

Specimen Number	Country	Site	Host
12a	Caribbean	Dominica site 1	Coconut
12b	Caribbean	Dominica site 1	Coconut
12c	Caribbean	Dominica site 1	Coconut
12m1	Caribbean	Dominica site 1	Coconut
12m2	Caribbean	Dominica site 1	Coconut
14m1	Caribbean	Dominica site 2	Coconut
14m2	Caribbean	Dominica site 2	Coconut
14m3	Caribbean	Dominica site 2	Coconut
15m1	Caribbean	Dominica site 3	Coconut
16m1	Caribbean	Dominica site 4	Banana
16m2	Caribbean	Dominica site 4	Banana
16m3	Caribbean	Dominica site 4	Banana
21m1	Caribbean	Trinidad	Banana
23m1	Caribbean	Dominica site 4	Coconut
23m2	Caribbean	Dominica site 4	Coconut
23m3	Caribbean	Dominica site 4	Coconut
81	India	WTS4	Phoenix roebelenii
79	India	BRS	<i>Areca catechu</i>
69	India	WTS1	<i>Musa</i> spp.
79	India	BRS	<i>Areca catechu</i>
58	India	Site 20	<i>Cocos nucifera</i>
64	India	WTs1	<i>Cocos nucifera</i>
78a	India	WTS4	<i>Cocos nucifera</i>
78b	India	WTS4	<i>Cocos nucifera</i>
60m1	India	WTS3	<i>Cocos nucifera</i>
60m2	India	WTS3	<i>Cocos nucifera</i>
63m1	India	WTS3	<i>Cocos nucifera</i>

Appendix 4 Samples for molecular work

Sample Number	Collection No.	Other label	Country	Region/site	Host plant	No. RPM	FTA/Alcohol
1	37	BR1	St. Kitts		<i>Cocos nucifera</i>	1	95% ethanol
2	10	BR2	Dominica		<i>Cocos nucifera</i>	1	95% ethanol
3	24	BR3	Trinidad		<i>Cocos nucifera</i>	1	95% ethanol
4	54	BR4	Antigua		<i>Cocos nucifera</i>	1	95% ethanol
5	28	BR5	Trinidad		<i>Musa spp</i>	1	95% ethanol
6	7	BR6	Dominica		<i>Musa spp</i>	1	95% ethanol
7	29	BR7	Trinidad		<i>Musa spp</i>	1	95% ethanol
8	8	BR8	Dominica		<i>Musa spp</i>	1	95% ethanol
9	77	BR9	India	BRS (T1)	<i>Areca catechu</i>	1	95% ethanol
10	71	BR10	India	nr WTS3	<i>Areca catechu</i>	1	95% ethanol
11	71	BR11	India	nr WTS3	<i>Areca catechu</i>	1	95% ethanol
12	80	BR12	India	BRS (T3)	<i>Areca catechu</i>	1	95% ethanol
13	58	BR13	India	Site 20	<i>Cocos nucifera</i> (cv Red)	1	95% ethanol
14	81	BR14	India	WTS4	<i>Phoenix roebelenii</i>	1	95% ethanol
15	65	BR15	India	WTS1	<i>Cocos nucifera</i> (cv Red)	1	95% ethanol
16	78	BR16	India	WTS4	<i>Cocos nucifera</i> (cv Red)	1	95% ethanol
17	62	BR17	India	WTS1 (T1)	<i>Cocos nucifera</i> (cv Green)	1	95% ethanol
18	64	BR18	India	WTS1 (T2)	<i>Cocos nucifera</i> (cv Green)	1	95% ethanol
19	NA	Tr1A Tree1	Trinidad	CABI, Curepe	<i>Cocos nucifera</i>	1	FTA
20	NA	Tr1B Tree1	Trinidad	CABI, Curepe	<i>Cocos nucifera</i>	1	FTA
21	NA	In1 Coconut	India	Unknown	<i>Cocos nucifera</i>	1	FTA
22	NA	In1 Areca	India	Unknown	<i>Areca catechu</i>	1	FTA
23	71/72/75	1	India	nr WTS3	<i>Areca catechu</i>	4	95% ethanol
24	60	2	India	nrWTS3	<i>Cocos nucifera</i>		95% ethanol
25	74	3	India	BRS, TA	<i>Areca catechu</i>	4	95% ethanol
26	62	4	India	WTS1 TA	<i>Cocos nucifera</i>		95% ethanol
27	79	5	India	India, BRS TC	<i>Areca catechu</i>	4	95% ethanol
28	NA	6	India	India, Vadakkenchery	<i>Cocos nucifera</i>	4	95% ethanol
29	NA	7	India	India, Peechi	<i>Cocos nucifera</i>	4	95% ethanol

The ecology of *Raoiella indica* (Hirst) (Acari:Tenuipalpidae) in India and Trinidad

Sample Number	Collection No.	Other label	Country	Region/site	Host plant	No. RPM	FTA/Alcohol
30	4	8	Dominica		<i>Cocos nucifera</i>	4	95% ethanol
31	28	9	Trinidad		<i>Musa</i> spp.	4	95% ethanol
32	64	Ic1	India	WTS1, TB	<i>Cocos nucifera</i>	5	95% ethanol
33	79	IA1	India	BRS	<i>Areca catechu</i>	3	95% ethanol
34	29	DB2	Dominica		<i>Musa</i> spp.	5	95% ethanol
35	9	Dc1	Dominica		<i>Cocos nucifera</i>	5	95% ethanol
36	79	IA2	India	WTS4	<i>Areca catechu</i>	4	95% ethanol
37	63	Ic3	India	WTS1, TC	<i>Cocos nucifera</i>	3	95% ethanol
38	NA	Tc1	Trinidad	Manzanilla	<i>Cocos nucifera</i>	4	95% ethanol
39	62	Ic2	India	WTS1, TA	<i>Cocos nucifera</i>	4	95% ethanol
40	?	IA3	India	Site13	<i>Areca catechu</i>	4	95% ethanol
41	6	DB1	Dominica		<i>Musa</i> spp.	5	95% ethanol
42	NA	Tc2	Trinidad	Manzanilla	<i>Cocos nucifera</i>	4	95% ethanol
43	3/10	Dc2	Dominica		<i>Cocos nucifera</i>	5	95% ethanol
44	NA	IA-FTA	India	Kerala	<i>Areca catechu</i>	4	FTA
45	NA	IC-FTA	India	Kerala	<i>Cocos nucifera</i>	3	FTA
46	NA	Ic2- FTA	India	Kerala	<i>Cocos nucifera</i>	3	FTA
47	NA	Ic3-FTA	India	Kerala	<i>Cocos nucifera</i>	3	FTA
48	NA	Tc1-FTA	Trinidad	CABI, Curepe	<i>Cocos nucifera</i>	3	FTA
49	NA	Tc2-FTA	Trinidad	CABI, Curepe	<i>Cocos nucifera</i>	3	FTA

Appendix 5 Trinidad RPM sequence

>TR1aTree1_Re_004_2014-10-25_H04.ab1

TTCCCCCTGCTAGAACAGGTAAAGAAAGTAATAATAAAAAAGAAGTAACAAGAATAGAT
AAATTAAATAAAGAAATAAAA
GAAAAAATAAAATATTTTATTTTAAATATAATAATAGTTGAGATAAAATTAATAGAACTT
AAAATTGAAGAAATTCCAGC
AATATGAAGAGAAAAAATTATTATTTTCGATAGAAAAACCAGAAAAAATATTTTTCTTG
ATCTTAAAGGAGCATAAATTG
TTCAACCTGAACCATTTAAAACCTCCTAAAATTATAGAAAGAATTATAAAGAAAAAAGAA
GGTAAAATTAATCAAAAACCTT
ATATTATTTAAACGAGGAAAAGCTATATCTAAACTATTATTTATTAATGGAACTAATAAA
TTTCCAAAACCTCCAATTAA
AGAAGGTATAACTATAAAAAAAATTATTAATATAGCATGAGAAGTAATTAAACTATTAT
AAATTATATCATTTATAATAA
AAGAACCAGATTGAATTAATTCAAATCGAATAATTACACTTAATCTTAAACCCAAAAGA
CCAGAAAAAATACTAAATAAT
AAATACATTAATCCAATATCTTTATGATTTGTTGACCAA

Appendix 6 Density statistic results for RPM individuals and mean RPM measurements from Trinidad 2012.

	Non mean data		Mean data d.f. 1,13	
	F statistic	p value	F statistic	p value
Length	8.468	0.00616 **	5.15	0.04093*
Width	3.962	0.0542 .	2.051	0.1757
c1	5.298	0.0272 *	1.799	0.2028
d1	0.6475	0.4263	0.02609	0.8742
c2	1.184	0.2837	0.4383	0.5195
d2	2.947	0.0946 .	0.7041	0.4166
e2	4.278	0.04586	1.246	0.2845
c3	0.06815	0.7955	0.06815	0.7955
d3	0.01088	0.917	0.05553	0.8174
e3	0.07334	0.7881	0.5033	0.4906
f2	2.081	0.1577	0.4135	0.5314
v2	0.0194	0.89	0.03838	0.8477
f3	1.351	0.2528	0.1412	0.7131

Appendix 7 Mean setae lengths of RPM from 4 different host plants in Kerala, India.

	<i>Areca catechu</i>	<i>Musa spp.</i>	<i>Cocos nucifera</i>	<i>Phoenix roebelenii</i>
Length	326.6±4.38a	332.9±4.30 a	315.6±4.59 a	335.8±6.02 a
Width	219.4±3.42 b	223.9±5.95 ab	209.8±3.29 a	221.1±4.10 ab
d1	22.6±0.34 a	24.7±0.25 a	23.0±0.46 a	24.2±0.54 a
d2	37.1±0.62 a	41.2±0.10 a	38.4±1.01 a	40.7±1.26 a
d3	92.8±0.87 a	95.7±5.90 a	93.1±1.53 a	100.0±2.10 a
e2	36.2±0.83a	40.0±2.10ab	37.0±1.17 a	44.4±1.35 b
e3	97.7±1.15 a	108.7±4.85 a	99.4±1.49 a	104.8±2.64 a
c1	36.6 ±0.46 b	41.9±2.30 a	38.6±0.47 a	40.4±1.00 a
c2	41.8±0.70 a	43.6±1.15 ab	41.8±0.79 a	46.5±0.96 b
c3	89.9±1.06 a	90.0±4.15 a	91.9±1.31 a	93.2±3.13 a
v2	61.7±0.82 a	68.0±0.40 abc	65.8±0.99 b	71.0±1.53 c
f2	26.0±0.69 a	30.1±0.90 a	25.9±0.77 a	27.7±0.57 a
f3	46.4±0.70 a	55.1±2.60 ab	51.3±1.51 b	54.4±1.72 bc

Appendix 8 Mean seta lengths of RPM from four islands in the Caribbean (± 1 SE).

	<i>Musa</i> spp.	<i>Cocos nucifera</i>
Length	306.1 $\pm$ 2.75 ^a	304.9 $\pm$ 2.65 ^a
Width	205.3 $\pm$ 1.83 ^a	205.6 $\pm$ 1.50 ^a
d1	26.1 $\pm$ 0.61 ^a	26.0 $\pm$ 0.44 ^a
d2	46.3 $\pm$ 1.36 ^a	43.9 $\pm$ 0.78 ^a
d3	113.1 $\pm$ 1.77 ^a	113.8 $\pm$ 0.74 ^a
e2	46.7 $\pm$ 1.19 ^a	44.8 $\pm$ 0.81 ^a
e3	117.8 $\pm$ 1.19 ^a	118.9 $\pm$ 0.66 ^a
c1	43.0 $\pm$ 0.79 ^a	42.5 $\pm$ 0.53 ^a
c2	51.7 $\pm$ 1.22 ^a	48.4 $\pm$ 0.77 ^b
c3	114.1 $\pm$ 1.31 ^a	113.6 $\pm$ 0.65 ^a
v2	79.2 $\pm$ 1.55 ^a	77.2 $\pm$ 0.71 ^a
f2	23.7 $\pm$ 0.70 ^a	25.9 $\pm$ 0.60 ^b
f3	56.6 $\pm$ 0.91 ^a	57.1 $\pm$ 0.57 ^a

Appendix 9 Mean seta lengths (μm) of RPM collected from *C. nucifera* and *Musa* spp. in the Caribbean ($\pm 1\text{SE}$).

	CA	CD	CSK	CT
Length	326.0 $\pm$ 7.80 a	318.8 $\pm$ 3.78 a	315.8 $\pm$ 2.73 ab	299.1 $\pm$ 2.45 b
Width	219.8 $\pm$ 8.39 a	212.0 $\pm$ 3.08 a	216.9 $\pm$ 3.06 a	201.9 $\pm$ 1.16 b
d1	24.6 $\pm$ 1.27 ab	28.2 $\pm$ 0.79 a	26.4 $\pm$ 0.86 ab	25.4 $\pm$ 0.43 b
d2	47.3 $\pm$ 2.08 ab	47.7 $\pm$ 1.41 a	40.8 $\pm$ 1.03 ab	43.7 $\pm$ 0.84 b
d3	111.7 $\pm$ 3.33 a	114.3 $\pm$ 1.51 a	117.9 $\pm$ 1.84 a	113.2 $\pm$ 0.89 a
e2	50.9 $\pm$ 3.06 ab	48.6 $\pm$ 1.43 a	40.4 $\pm$ 1.42 ab	44.4 $\pm$ 0.80 b
e3	115.8 $\pm$ 3.35 a	119.7 $\pm$ 1.54 a	122.9 $\pm$ 2.28 a	118.2 $\pm$ 0.63 a
c1	41.9 $\pm$ 1.95 ab	45.0 $\pm$ 0.81 a	39.0 $\pm$ 1.54 b	42.2 $\pm$ 0.54 b
c2	51.5 $\pm$ 1.12 ab	52.6 $\pm$ 1.37 a	45.1 $\pm$ 1.00 ab	48.5 $\pm$ 0.82 b
c3	109.7 $\pm$ 3.45 a	114.3 $\pm$ 1.27 a	116.9 $\pm$ 1.80 a	113.5 $\pm$ 0.71 a
v2	77.8 $\pm$ 2.16 a	80.9 $\pm$ 1.36 a	73.4 $\pm$ 1.46 a	77.1 $\pm$ 0.82 a
f2	22.0 $\pm$ 1.81 a	27.8 $\pm$ 1.36 b	30.4 $\pm$ 1.20 b	24.3 $\pm$ 0.49 a
f3	57.2 $\pm$ 2.55 a	58.9 $\pm$ 0.90 a	59.5 $\pm$ 0.92 a	56.2 $\pm$ 0.60 a

Appendix 10 Mean setae lengths of RPM specimens from Caribbean and India.(d.f.=1,195)

	Caribbean	India	Difference
Length	305.2 ±2.07a	322.0 ±2.94b	-16.8
Width	205.5 ±1.20 a	214.6±2.17 b	-9.1
d1	26.0±0.36 a	23.1±0.27 b	+2.9
d2	44.5±0.68 a	38.3±0.57 b	+6.2
d3	113.6±0.72a	93.7±0.90 b	+19.9
e2	45.3±0.68a	37.7±0.72 b	+7.6
e3	118.6±0.58a	99.6±0.94 b	+19.0
c1	42.6±0.44 a	38.2±0.35 b	+4.4
c2	49.3±0.66a	42.4±0.50b	-6.9
c3	113.7±0.59 a	91.3±0.83b	+22.4
v2	77.7±0.66 a	65.0±0.69b	+12.7
f2	25.3±0.48 a	26.2±0.47 a	-0.9
f3	57.0±0.48 a	49.8±0.90 b	+7.2

Appendix 11 Molecular results

Sample No.	Collection No.	Other label	Country	Region/site	Host plant	Qubit Template concentration (ng/μl)	result: DNA
23	71/72/75	1	India	nr WTS3	<i>Areca</i> <i>catcehu</i>	0.0788	
24	60	2	India	nrWTS3	<i>Cocos</i> <i>nucifera</i>	0.0264	
25	74	3	India	BRS, TA	<i>Areca</i> <i>catcehu</i>	0.0726	
26	62	4	India	WTS1 TA	<i>Cocos</i> <i>nucifera</i>	0.0268	
27	79	5	India	India, BRS TC	<i>Areca</i> <i>catcehu</i>	0.0510	
28	NA	6	India	India, Vadakkenchery	<i>Cocos</i> <i>nucifera</i>	0.0682	
29	NA	7	India	India, Peechi	<i>Cocos</i> <i>nucifera</i>	0.0374	
30	4	8	Dominica		<i>Cocos</i> <i>nucifera</i>	0.0576	
31	28	9	Trinidad		<i>Musa</i> spp.	0.0516	
32	64	10-Ic1	India	WTS1, TB	<i>Cocos</i> <i>nucifera</i>	0.0830	
33	79	11-IA1	India	BRS	<i>Areca</i> <i>catechu</i>	0.216	
34	29	12-DB2	Dominica		<i>Musa</i> spp.	0.0966	
35	9	13-Dc1	Dominica		<i>Cocos</i> <i>nucifera</i>	0.110	
36	79	14-IA2	India	WTS4	<i>Areca</i> <i>catechu</i>	0.146	
37	63	15-Ic3	India	WTS1, TC	<i>Cocos</i> <i>nucifera</i>	0.0490	
38	NA	16-Tc1	Trinidad	Manzanilla	<i>Cocos</i> <i>nucifera</i>	0.123	
39	62	17-Ic2	India	WTS1, TA	<i>Cocos</i> <i>nucifera</i>	0.0886	
40	?	18-IA3	India	Site13	<i>Areca</i> <i>catechu</i>	0.0698	
41	6	19-DB1	Dominica		<i>Musa</i> spp.	0.0278	
42	NA	20-Tc2	Trinidad	Manzanilla	<i>Cocos</i> <i>nucifera</i>	0.138	

Sample No.	Collection No.	Other label	Country	Region/site	Host plant	Qubit Template concentration (ng/μl)	result: DNA
43	3/10	21-Dc2	Dominica		<i>Cocos nucifera</i>	0.136	

Appendix 12 Morphological features measured on *Amblyseius largoensis* specimens from India and Trinidad.

Measurements

Length dorsal shield

Width dorsal shield at s4

Ventrianal length

Ventrianal width at ZV2

Ventrianal width at anus

j1

j3

j4

j5

j6

J2

J5

z2

z4

z5

Z1

Z4

Z5

s4

S2

S4

S5

r3

R1

Measurements

Length spermathaeca

Sge IV length

Sti IV length

Length JV2

Length JV2

Length JV3

Chelicera length

Chelicera plus base length

Hypostome

palps length

St IV length

Peritreme length

Appendix 13 A preliminary study was set up to assess the egg consumption of *Amblyseius*

***largoensis* between Trinidad and India.**

Several attempts were made to conduct this study using leaf discs on cotton wool/foam etc, however it was not possible to contain the *A. largoensis* specimens and they often ran off and drowned or went missing overnight. To negate these issues, five *A. largoensis* adult females were placed in small 2ml glass tubes sealed with micro-pore tape, to which a counted number of RPM eggs were added. The numbers of eggs consumed were assessed at regular intervals (between 20 and 36h) and a mean hourly rate of egg consumption was calculated and then converted to a 24h rate (it was not possible to take reading at precisely 24h due to differing lengths of times in assessments and counting of eggs etc). New eggs were counted and added to the tube over a period of three days and a mean egg consumption rate was calculated. Tubes in India were maintained at ambient conditions (approx. 31°C during the day), however results for tubes in Trinidad had to be discounted as air-conditioning cooled the labs over the three day period. This study was therefore not written up in full.

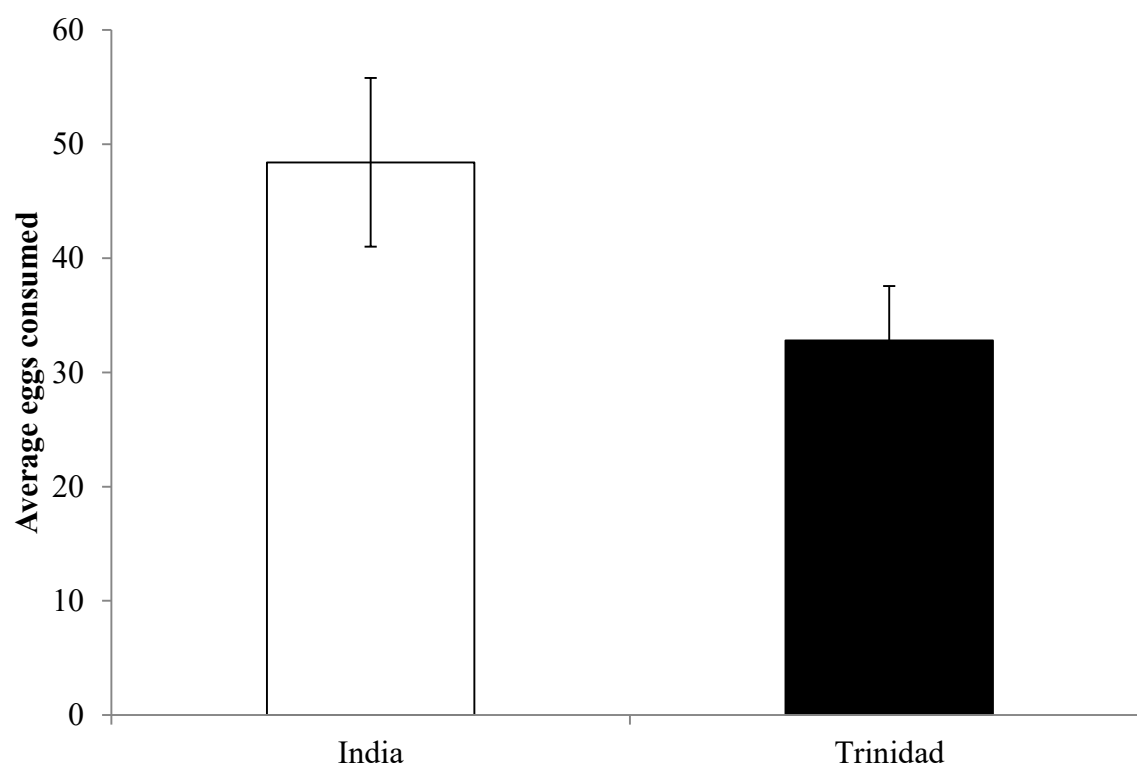


Figure 9.1 Mean number of eggs consumed by *Amblyseius largoensis* in India and Trinidad. $n=5$ for India, $n=5$ for Trinidad. All predators were assessed over a 3-day period and an average calculated. These averages were then averaged and displayed as above (± 1 SE).

Appendix 14 *Amblyseius largoensis* method of attack

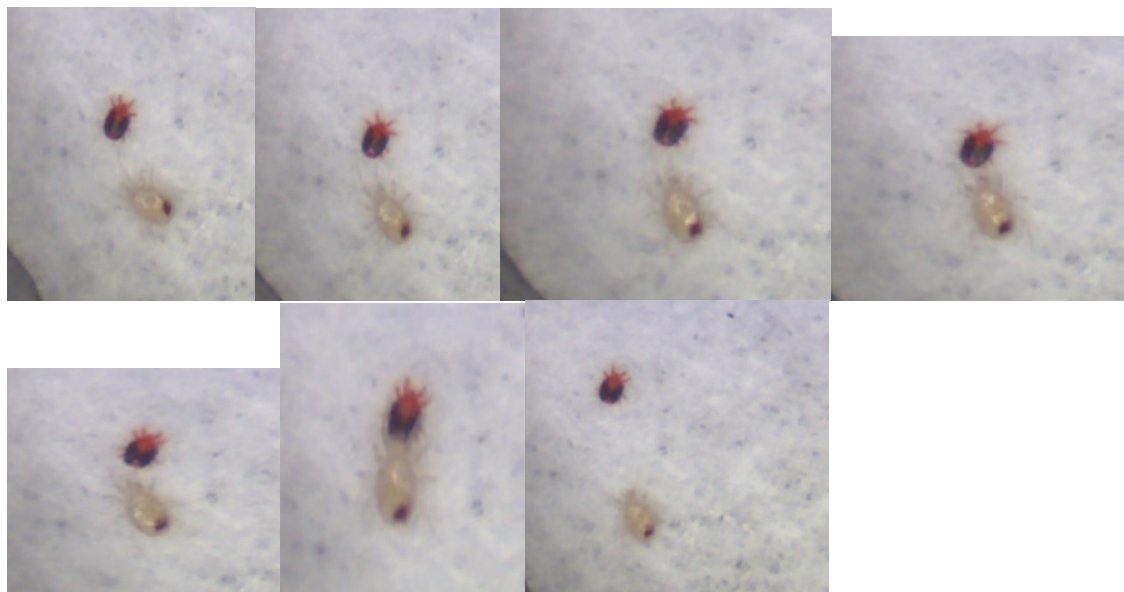


Figure A

Aborted attack

In Figure A, *A. largoensis* approaches the RPM from the rear, the predator places the front right leg on the RPM and then both legs and attempts to grab hold of the RPM with its mouthparts, however, the predator immediately shrinks back. The predator in this example has a second attempt, from the front RHS, but again immediately shrinks back when contacting the setae.

Successful attack

During a successful attack attempt the predator approached the RPM and attempted to seize it on multiple occasions. Analysing the video, it can be seen that the predator tried to position itself to feed on 16 occasions moving around the RPM in an attempt to find a suitable feeding site. Initially, the predator touched the RPM setae with front left leg then moved laterally around RPM feeling setae. The predator then lunged at the RPM using both front legs first, then mounted the RPM attacking with chelicerae. The predator re-orientated keeping mouthpart's in same location, and shrank back slightly, whilst still holding RPM, it retracted momentarily and lunged again. It did this several times, re-orientating around the RPM body- as if trying to find a suitable location for eating. After 1 minute, the predator started feeding, however it only fed for about 10 seconds. The predator then pulled the RPM backwards and flipped it onto its dorsal surface so all of the setae were pointing down. The predator was then able to feed on a further 3 occasions without being repelled by setae over the space of 7mins. After 9mins the predator pulled the RPM so it was positioned vertically and attempted to attack from

the dorsal surface (with setae) on 3 occasions, but it was repelled each time. The RPM then lay flat again on the dorsal surface with setae facing down after being dragged by the predator legs (not deliberate) and the predator was able to feed once more without being repelled. Figures B – H give a detailed log of the attacks.

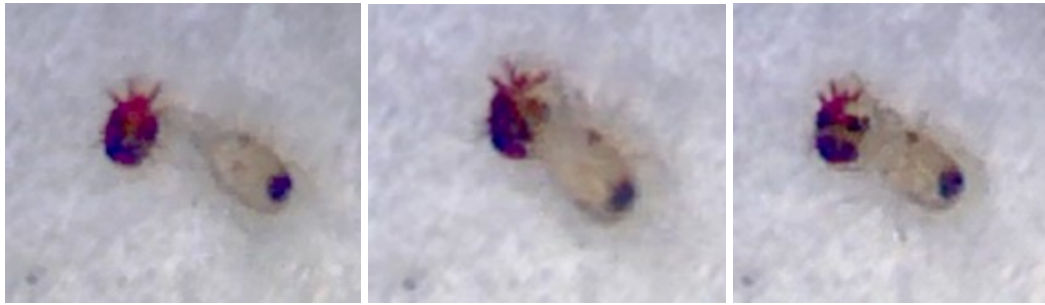


Figure B Initial attack on RPM. The predator lifts RPM with front legs and tries to eat from the dorsal side but is repelled when trying to insert mouthparts. It approaches again from the other side and lifts with front legs and is repelled once more.



Figure C Predator moves positions around RPM to try and find a location where it can approach to feed

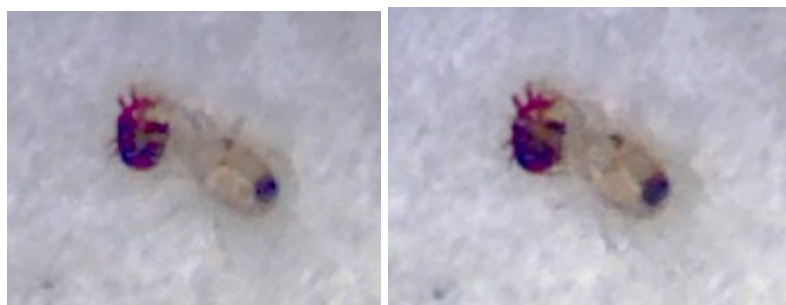


Figure D The predator lifts its front legs and holds the RPM then lunges forward with the chelirae/hypostome.



Figure E the successful approach whereby in the last picture the predator starts feeding



Figure F The predator has flipped the RPM so it is dorsal side down (thus setae down) and is able to feed without being repelled (it feed with ease on 3 occasions). The palps and front legs are used to secure the RPM during feeding. During eating the palps are raised up and flat on the RPM body surface and it is the chelicerae and hypostome which are flush with the mite cuticle

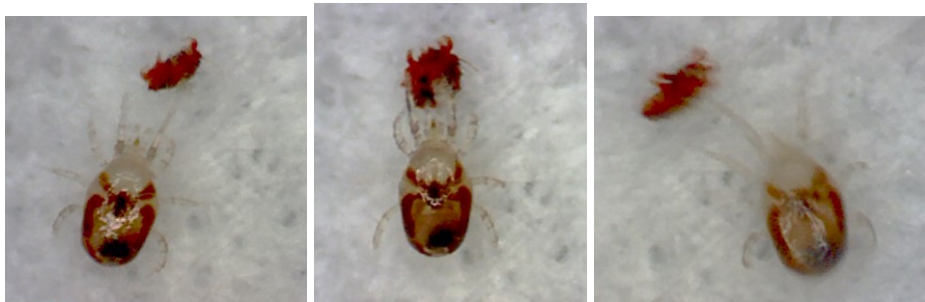


Figure G The front leg got caught on one of the setae and the RPM stuck to it, this caused the predator to drag the RPM so it was vertical. When the predator approached from the dorsal side, it was immediately repelled (see right hand picture). The predator had 3 attack attempts where it is repelled by the setae



Figure H Eventually the predator drags the RPM back onto its dorsal surface and it is able to resume feeding without being repelled.

Amblyseius largoensis consuming a dead RPM specimen *in-situ* on leaflet from anterior attack angle



Appendix 15 Predatory Neuropteran consuming RPM mobile stages on leaflet in Trinidad.

