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Natural Enemies of Red Palm Mite in India

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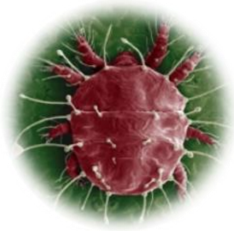

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EXECUTIVE SUMMARY

Red Palm Mite (RPM), *Raoiella indica* Hirst (Arachnida: Acari: Tenuipalpidae), is a pest of coconut, areca, date palm and many other ornamental as well as commercial palm species. The mite feeds on the underside of palm fronds. This pest, first reported in India from Coimbatore, Tamil Nadu during 1924, has gained considerable economic significance since it has been reported as an invasive species in many new-world countries.

As the name implies, the mite is bright red in colour. The body is flat with long spatulate setae, and droplets on the dorsal body setae. Females of *R. indica* are on an average 245 microns (0.01 inches) long and 182 microns (0.007 inches) wide and are larger than males and less active. The life cycle from egg to adult typically requires 23 to 28 days for females and 20 to 22 days for males. Though this species is indigenous to India, it is an invasive species to Dominican Republic, Guadeloupe, Puerto Rico, Saint Martin, Trinidad and Tobago, the US Virgin Islands, Granada, Haiti and Jamaica causing severe damage to coconut, banana and many other crops. The aim of the project was to study the population dynamics of the mite and to survey the natural enemies associated with it in its native range to develop a biocontrol strategy to tackle the problem.

The host list of RPM is extensive; however, reports indicate that *Areca catechu* and *Cocos nucifera* are the major host plants in India. They affect seedlings of both areca and coconut, especially during the summer season. Trees grown in conditions of poor drainage, irrigation and low mineral and organic matter are particularly affected compared to plants grown in well irrigated shaded nurseries. Life cycle is closely linked with monsoon and increase in population occurs after the end of rainy season.

Field and laboratory studies were carried out in order to assess the relationships between RPM, its natural enemies and other factors such as climate and host. Spatial and temporal surveys were carried out in coconut and arecanut during 2008 and 2009. Two sites were chosen for each species (Palakkad and Peechi for coconut and Kunnamkulam and Nilambur for arecanut), that are historically drier and wetter, climatically. Surveys for each treatment were conducted once in a month in order to obtain temporal data.

In each survey area, 20 trees were randomly selected within a 10km strip (only ten sites in Kunamkulam). A start point for each area was chosen and trees were selected randomly by driving for 30 seconds, stopping and selecting the nearest palm. Young palms not taller than 4 metres were selected and one lower frond was sampled. Each frond sampled was divided into 3 sections, upper (tip), middle and lower (base) and a leaflet was removed from close to the rachis giving 3 leaflets in total for each frond. When the leaflet was removed, a visual inspection for RPM and natural enemies was carried out. Natural enemies were removed using a paintbrush and placed into 70% alcohol (1st sample) then the remaining members of same species were collected alive for testing in the laboratory. The cut leaflets were labelled using the letters 'L' for the lower section, 'M' for the middle section and 'U' for the upper section. Leaflets were stored separately in cotton bags. The total number of leaflets on the frond was then estimated in order to gain an estimate of leaf area sampled. Upon reaching the laboratory, the leaflets were closely inspected under a stereomicroscope for the presence of RPM and its natural enemies.

In general, RPM populations were initially very low in November and December and high in February and March. All sites showed a significantly higher RPM population in March than in December. In most cases, the number of sites infested with RPM rose every month, with Palakkad having a larger proportion of RPM infested sites compared to the other sites. This showed that the RPM are either dispersing within the survey areas or breeding to population levels which are more detectable.

Surveys were also carried out on alternative host plants like banana, pandanus, and on many other palm varieties. Though solitary individuals were located on banana from Palakkad area, no direct evidences of colonisation were observed, indicating a lack of suitability of the host, or other potential factors limiting the RPM establishment.

Along with RPM, various predators were also recorded. The majority of predators collected during the surveys were phytoseiid mites belonging to the genus *Amblyseius.* Apart from this coccinellids were also recovered which were relatively low in number. Two different genera of coccinellids including *Stethorus keralicus* were observed*.* Other predators recorded belonged to the orders, Thysanoptera, Neuroptera, Hemiptera and Diptera.

The influence of local weather parameters and predators on RPM population were investigated. It was found that there was a significant positive effect of site temperature on RPM population. It was also found that there was a significant effect of host plant species on population levels of RPM. Even though there was no significant effect of site humidity on RPM number ($F=0.37$, $p=0.56$) there was a trend whereby higher humidity levels were related with lower RPM numbers.

Predator (phytoseiid) number was not related to site temperature, but it was slightly related with site humidity. There was, however, a very significant correlation between average phytoseiid number and rainfall of the previous month ($F=23.49$, $p<0.01$), although no correlation was seen between phytoseiid number and rainfall in the current month ($F=0.37$, $p=0.55$). These results indicates that the increase in populations of RPM is not only linked to temperature, but also to the host plant, number of predatory mites present, humidity and rainfall. Laboratory studies proved that phytoseiid feeds red palm mite, but rearing and bioassay was difficult because phytoseiids always showed escaping behaviour from the arena.

The rapid spread of RPM throughout the other parts of the world (New World) demonstrates the ability of the mite to disperse effectively between plants. However, the method of transfer between plants is unknown, along with the factors that trigger the dispersal of the mite between plants. To address this problem, wind-dispersal traps were installed in the field to study the mechanism of dispersal of RPM. Tailor made traps were set up in the field and periodic observations were made. Besides, to assess the mite density on each trap site, four coconut palms were randomly sampled each month (different trees were selected each month), from which three leaflets were collected from a lower frond and mite density was estimated. RPM was caught in April and May. RPM density was also high during that time. The study indicated that RPM dispersed through the wind current. Aerial dispersal occured when the populations were dense on the tree canopy. Results also showed that the number of solitary females found increased throughout the season. Leaf nutrient analysis revealed that Phosphorus content of the leaves and RPM numbers were related.

To summarise, the results showed that the most abundant predator associated with RPM is the phytoseiid mite. There were high numbers of phytoseiid mites during the months of December and January but there was a significant drop in numbers in the later period. Phytoseiid mites were highly correlated to rainfall of the previous month, and negatively correlated to RPM populations, even though laboratory data has shown that these mites do feed on RPM. From this information it could be postulated that the predator is indeed adapted to feeding on RPM but it is poorly synchronised. RPM on the other hand, has an abundance of suitable host plants and ideal weather conditions for population expansion.

CHAPTER I

Introduction

Red Palm Mite *Raoiella indica* Hirst (Arachnida: Acari: Tenuipalpidae) is a pest of coconut, areca, date palm and many other ornamental as well as commercial palm species. The pest was first reported in India from Coimbatore, Tamil Nadu during 1924. It feeds on the underside of palm fronds of various hosts in the orders Arecales and Zingiberales. The mite attained economic significance when it was first reported as an invasive species in the Caribbean in 2004 (Flechtmann and Etienne, 2004) and has since become widely spread in its new range.

Mites in the family Tenuipalpidae are commonly called "false spider mites" and are all plant feeders. However, only a few species of tenuipalpids in a few genera are of economic importance. The tenuipalpids have stylet-like mouth parts (a stylophore) similar to that of spider mites (Tetranychidae). The mouth parts are long, U-shaped, with whip-like chelicerae that are used for piercing plant tissues. Tenuipalpids feed by inserting their chelicerae into plant tissue and removing the cell contents. These mites are small and flat, and usually feed on the under-surface of leaves. They are slow moving and do not produce silk, as do many tetranychid (spider mite) species.

General description of the pest and life cycle

This species can be easily distinguished from other species of mites by its reddish colour, flat body, long spatulate setae, and droplets on the dorsal body setae. During all stages of life, this species is red, with adult females often showing black patches on their back after feeding. There is also a noticeable absence of the webbing associated with numerous other spider mites.

Females of *R. indica* are on an average 245 microns (0.01 inches) long and 182 microns (0.007 inches) wide, oval and reddish in colour. The dorsum is smooth, except for the presence of punctae (sculptured depressions). Females develop dark markings on the dorsum of the body after feeding. The male is smaller, but similar to the female in shape except for having a tapering of the posterior end of the body.

Males and females are sexually mature when they emerge and males actively seek out females, suggesting involvement of a sex pheromone. When a male locates a female deutonymph in the quiescent stage, it will settle close to it and wait for up to two days for the female to moult. When the female deutonymphs begin to moult, the male becomes active and moves under her, protruding its posterior end forwards to mate. Mites remain in the mating posture for about 16 minutes.

The life cycle from egg to adult typically requires 23 to 28 days for females and 20 to 22 days for males. Mated females have a 5- to 6-day pre-oviposition period after which they oviposit for 47 days under laboratory conditions. Unmated females deposit an average of 18 eggs after a 2-day pre-oviposition period, oviposit for 40 days and live for about 48 days. Males, produced by unmated females, live on an average for 22 days.

The ovoid egg is reddish, measuring 100 microns long (0.003 inches) and 80 microns wide. The freshly laid egg is attached to the leaf surface and a fine white stipe (slender hair-like structure) as long as or longer than the egg is present at one end. The tip of the stipe may be coiled and have a droplet of water clinging to it. The egg turns opaque white about 24 hours before hatching. The incubation period averages eight days for fertilized eggs and 7.3 days for unfertilized eggs. According to Nageshachandra and Channabasavanna (1984), this species has an unusual genetic system, in which all eggs laid by unmated females develop into males and mated females produce only female progeny.

The newly hatched larva is red and has three pairs of legs. A blackish tinge may develop on the posterior end of the dorsum after feeding. The larva typically feeds for three to five days and then becomes quiescent for 1.7 to 1.9 days before moulting to the protonymphal stage.

The reddish protonymph emerges with four pairs of legs and feeds for two to five days prior to becoming quiescent. The quiescent phase lasts from one to four days before deutonymphs emerge from the exoskeleton (exuvium). The female protonymph has an ovoid body with a rounded posterior but the male protonymph has a pointed posterior and a nearly triangular body. Deutonymphs are larger than protonymphs but resemble protonymphs with regard to feeding and other habits. The active phase lasts two to five days and the subsequent quiescent phase lasts from two to four days.

Origin of the Project

This species, indigenous to India, is considered an invasive species in Dominican Republic, Guadeloupe, Puerto Rico, Saint Martin, Trinidad and Tobago, the US Virgin Islands, Granada, Haiti and Jamaica (http://en.wikipedia.org/wiki/Red_palm_mite#cite_note-doacs.state.fl.us-1), causing severe damage to coconut, banana and many other crops. In this context, CABI in collaboration with KFRI conducted a joint investigation on the population dynamics and natural enemies of red palm mite, with the following objectives:

- To study the ecology and host range of the mite in Kerala, and
- Survey the natural enemies associated with the mite to explore the possibility of finding a potential natural enemy for classical biological control (CBC).

CHAPTER II

Distribution and host associates

Raoiella indica was first described in 1924, in the district of Coimbatore, India (Hirst,1924) and has since been reported in many countries across the Old World feeding on coconut palms (*Cocos nucifera*), areca palm (*Areca catechu*) and date palms (*Phoenix dactylifera*). The mite feeds on the underside of leaves and gathers in colonies of up to 1000s of individuals. In March and April-May, >1500 individual per leaf were observed in Nilambur from areca and in coconut from Palakkad area (*per. obs*). The feeding site is through the stomata and it is speculated that the mite feeds upon cells in the mesophyll tissue which causes a distinct yellowing of the leaves of the host plant (Kane *et al*., 2005).

The current global distribution is displayed in figure 2.1 (CABI, 2007). It may be noted that since the production of the distribution map, *R. indica* has also appeared in Florida, USA and Venezuela (Vásquez, *et al*., 2008). The mite owes its success to its ability to colonise many different host species mainly from the orders Arecales and Zingiberales and the apparent lack of co-evolved natural enemies in its new habitat.

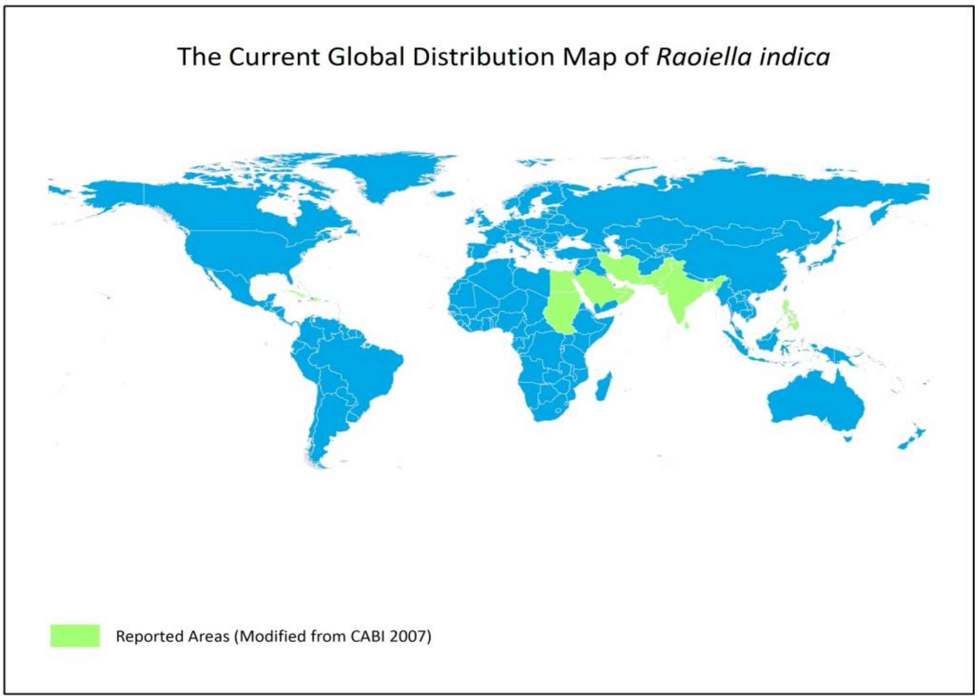


Fig. 2.1. Distribution of *Raoiella indica* in 2007 (modified from CABI, 2007)

There are no official figures on the impact of the mite on host plants, however farmers report a drop in coconut production in the Caribbean and data generated by FAO shows a sharp drop in coconut production in the Caribbean since 2004 when RPM was first reported (fig. 2.2).

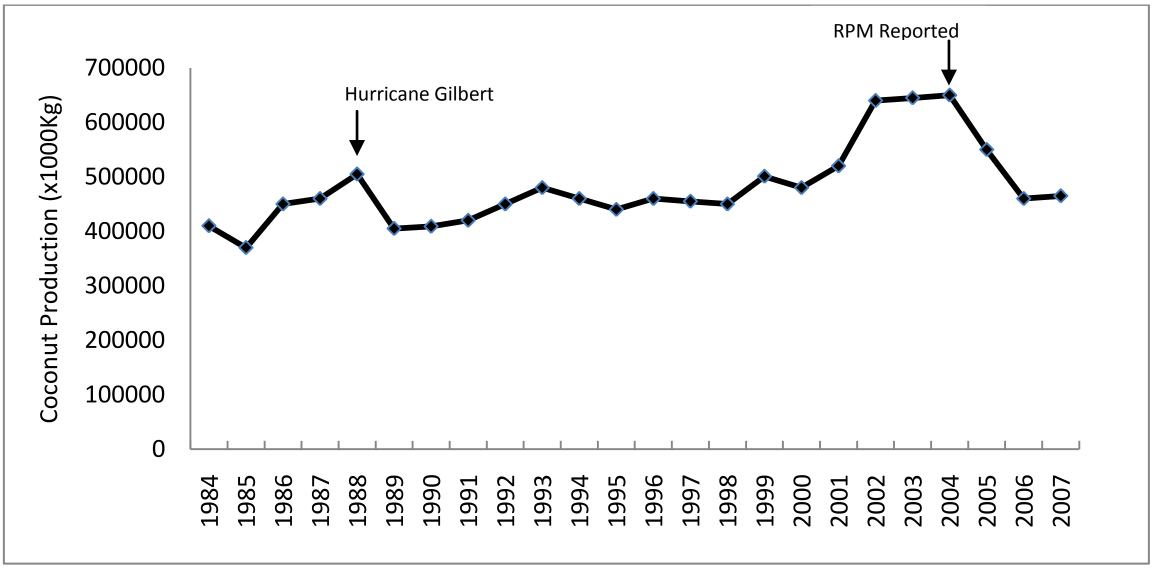


Fig. 2.2. Coconut production in the Caribbean since 1984 (figures from FAOSTAT for the Caribbean region).

The host list of red palm mite is extensive; although previous reports in literature prior to the introduction of the pest in the Caribbean are only on *Areca catechu* and *Cocos nucifera* in India, Mauritius and Sri Lanka, with most records from India. Infestations on date palms (*Phoenix* sp.) have also been reported across the Middle East. In the new invasive range, hosts reported for the red palm mite include members of the order Zingiberales, which encompasses the family Musaceae and the genus *Musa* and the families Heliconiaceae, Zingiberaceae and Strelitziaceae. Numerous hosts of the family Arecaceae are also reported including those reported in the Old World.

Among the most commonly reported host plants, the origins of *Areca catechu* lie within the floristic province known as Malesia (which encompasses the Malay peninsula, Indonesia, New Guinea and The Philippines) and although the origins of coconut are widely disputed, the best hypothesis is the West Pacific currently. The hosts of the *Phoenix* sp. are likely to have originated further west than this from the Arabian Peninsula across to China. The *Musa* sp. are more complicated as many of the cultivated species are derived from crosses between two species, *Musa acuminata* and *Musa balbisiana*. The origin of the genus *Musa* is thought to be in Burma-New Guinea area, however the centre of diversity for *M. acuminata* is thought to be in Malesia in the humid tropics. For *M. balbisiana*, it is more abundant in monsoon climates with a dry season (Purseglove, 1972) and the area of origin is thought to be India or Myanamar (Rao, 1984).

Although the hosts of the RPM reported in the Caribbean are widely spread throughout South and South East Asia, the mite itself has not been widely reported on the majority of hosts in the majority of these countries. In India, *Areca catechu* was thought to have been introduced prior to the 1st century AD and *Cocos nucifera* is thought to have been present for at least 3000 years (Purseglove, 1972). This information suggests that RPM may have a native range in the Oriental/Melanesia region and thus a wide area for potential CBC investigations. The mite may have spread slowly westwards with trade and that the mite was transported to the Caribbean *via* the shipping route between the island of Reunion and the Caribbean on infested plant material. But as seen above, there are only a few reports to date further east than India. Therefore, given the scope of this project, India is a natural starting point for investigations of natural enemies. Also, the majority of research on RPM prior to the introduction into the Caribbean has also been carried out in India, where basic studies have been conducted on its dynamics and associated natural enemy fauna.

Red Palm Mite populations in the Old World

In India, *Raoiella indica* is reported as a pest particularly on seedlings (Moutia, 1958; Sathiamma, 1996) and especially during the summer season (Sathiamma, 1996). Trees grown in conditions of poor drainage, irrigation and low mineral and organic matter are particularly affected (Devasahayam and Nair, 1982; Sathiamma, 1996) and plants grown in well irrigated shaded nurseries tend to have low mite infestations (Ponnuswami, 1967). Populations of RPM have been studied on *Areca catechu* and *Cocos nucifera* by a number of authors (Moutia, 1958; Sathiamma, 1996; Yadavbabu and Manjunatha, 2007; Sarkar and Somchoudhury, 1989; Somchoudhury and Sarkar, 1987; Daniel, 1979) and an increase in population is thought to be linked with the onset of the dry season. Table 2.1 summarises the dynamics of RPM numbers in the literature and it can be seen that peaks are reached in an East to West order, perhaps following the path of the monsoon season.

Table 2.1. Dynamics of *Raoiella indica* throughout the season from India.

Reference	Places	Host-plant	Periodicity											
			Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Sathiamma (1996)	Kerala	Coconut	xx	xx	xx	xx	xx	Na	Na	Na	Na	Na	Na	Na
Ponnuswami (1967)	Kerala	Areca	Na	Na	Na	xx	xx	Na	Na	Na	Na	Na	Na	Na
Yadavbabu and Manjunatha (2007)	Karnataka	Areca	??	↑↑	xx	xx	xx	??	??	??	??	??	??	??
Daniel (1979)	Karnataka	Coconut	Na	Na	xx	xx	xx	xx	Na	Na	Na	Na	Na	Na
Somchoudhury and Sarkar (1987,1989)	West Bengal	Coconut	↑↑	↑↑	Na	Na	xx	xx	xx	xx	xx	xx	Na	↓↓

xx-peak populations
↑↑-increasing populations
↓↓-population reduction caused by rain
??-unknown reasons for population reduction
Na- no information is available

Natural enemies associated with Red Palm Mite and biological control

A number of studies have been carried out in India on the natural enemies associated with RPM. A comprehensive review of literature was made and a list of the predators and the relative host plants on which they were found is presented in Table 2.2. The main predators reported were phytoseiid mites, coccinellids and staphylinid beetles.

In West Bengal, the staphylinid beetle, *Oligota* sp. was the predominant predator throughout the season, with phytoseiid mites (*Phytoseius* sp. and *Amblyseius* sp.) found in secondary abundance. There was a correlation in population growth of *Oilgota* sp. and *R. indica*, with the lowest populations of *Oligota* sp. between November and February (Somchoudhury and Sarkar, 1987). A study in Karnataka revealed that the most prevalent predators were *Amblyseius channabasavanni* and *Stethorus keralicus* but no reference to *Oligota* sp. was made.

Historically, coccinellids and phytoseiid mites are the groups that have been of most interest for the control of mite pests in both classical and augmentation biological control. Special caution must be taken in classical biological control efforts to ensure that the introduced species will not adversely disrupt ecosystems and food webs in the introduced range. *Stethorus* sp. are widely regarded as being mite specific predators (Flint and Dreistadt, 1998) and *Stethorus keralicus* has been reported to have a high degree of specificity to the genus *Raoiella* (Daniel, 1979; Nageshchandra and ChannaBasavanna, 1983). Studies on the biology of *S. keralicus* show that the coccinellid can feed and reproduce solely on a diet of *R. indica* and can complete development from egg to adult in 12-14 days feeding on all stages of RPM (Daniel, 1976). Although various genera of coccinellids have been used before in classical biological control programmes, establishment rates have been low (Barlett *et al*., 1978; Obrycki and Kring, 1998) and there are cases where introductions of coccinellids have led to the competitive displacement of native species and non-target predation (Hoddle, 2003).

Phytoseiids have been well documented as predators of phytophagous mites and a number of species have been reported in association with RPM (see Table 2.2). The majority of phytoseiids reported in association with RPM are from the genus *Amblyseius.* According to McMurtry and Croft (1997), the Amblyseiinae are type III generalist predators and have been reported to have a higher reproductive potential than members of the Phytoseiinae and Thyphlodrominae. Studies on the bionomics of *Amblyseius channabasavanni* feeding on *R. indica* (Gupta, 2003) showed the total development time when feeding on eggs of *R. indica* was 84-113hrs (3.5-4.7 days) for adult females, with the average number of eggs eaten in total being 26.5, although all prey stages may be consumed. *Phytoseius* sp. have been reported to show a correlation to population increase of RPM in West Bengal (Somchoudhury and Sarkar, 1987), however the role of this genus in biological pest control of mites has rarely been significant (McMurtry and Croft 1997).

Of the other predators, species in the Cecidomyiidae (Diptera) may be of interest as members of this subfamily have been used in augmentative biological control programmes throughout Europe and North America, although few studies have released these dipterans as classical biological control agents.

Although attempts have been made to quantify the numbers of predators in relation to the numbers of RPM in the field and possible impact, there is still scope for further investigation, especially in order to establish a candidate predator for a classical biological control programme. Predators that would be suitable for a classical biological control programme have specific qualities that would make them advantageous. High specificity of the predator on the prey would reduce the risks of non target effects, and the ability to increase as rapidly as the prey and high voracity would enhance pest suppression. Other factors such as eco-climatic suitability and ease of culture also need to be considered. In the case of *R. indica* the wide host range means that more than one species of plant host should be investigated to get a comprehensive picture of predator-prey dynamics.

Based on a three year study, Daniel (1979) reported the status of the predators of RPM during the months, March – June when RPM was prevalent in the field. But predators in seasons of low RPM abundance were not considered, therefore important information might have been missed. The study also concentrated on *Areca catechu* which is not a common host plant in the Caribbean. McMurtry and Croft (1997) suggested that generalist predatory mites such as *Amblyseius* spp. have co-evolved more in response to the plant host than specific prey.

Table 2.2. A list of predators of *Raoiella indica* described in India in association with location and host plant

Order	**Family**	**Species**	**Location**	**Host plant**
Acari	Phytoseiidae	*Amblyseius channabassavanni* (Daniel, 1979)	Karnataka	*Areca catechu*
		Amblyseius longispinus (cited in Gupta, 2003)	?	*?*
		Amblyseius raoiellus (cited in Gupta, 2003)	Karnataka	*?*
		Phytoseius sp. (Somchoudhury and Sarkar, 1987)	West Bengal	*Cocos nucifera*
	Stigmaeidae	(Daniel, 1979)		Areca
Coleoptera	Coccinellidae	*Stethorus keralicus* (Kapur 1961; Daniel,1976; Puttaswamy and Rangaswamy 1976; Daniel 1979)	Kerala and Karnataka	*Areca catechu*
		Stethorus tetranychi (cited in Daniel,1979)	Tharikare	*Areca catechu*
		Stethorus parcepunctatus (cited in Daniel, 1979)		*Areca catechu*
		Jauravia soror (cited in Daniel,1979)		*Areca catechu*
		Spilocaria bisettata (cited in Daniel,1979)		*Areca catechu*
	Dermestidae	*Aspectes indicus* (cited in Daniel,1979)		*Areca catechu*
	Nitulidae	*Cybocephalus semiflavus* (cited in Daniel,1979)		*Areca catechu*
	Staphylinidae	*Oilgota sp.* (Somchoudhury and Sarkar, 1987)	West Bengal	*Cocos nucifera*
Diptera	Cecidomyiidae	*Feltiella sp.* (Daniel, 1979)	Karnataka	*Areca catechu*
Hemiptera	Anthocoridae	(Daniel, 1979)	Karnataka	*Areca catechu*
Neuroptera	Chrysopidae	*Chrysopa sp.* (Daniel (1979)	Karnataka	*Areca catechu*

CHAPTER III

Search for natural enemies

Field survey was carried out in order to assess the relationships between RPM, its natural enemies and other factors such as climate and host. Spatial and temporal surveys were conducted in Kerala, between November 2008 and March 2009. Preliminary laboratory studies were also conducted in the Entomology Department. Samples collected during surveys were stored for identification and further studies. Identifications were carried by the help of various experts from Project Directorate for Biological Control (PDBC) Bangalore, University of Agricultural Sciences, Bangalore and Indian Agricultural Research Institute (IARI), New Delhi.

Population data was transformed using log (x+1) before statistical analysis. Population trends were plotted using confidence limits at the 20% level (suitable for field data) and trends were acknowledged in data when the p value was at 0.2 or less. Data was analysed using data averaged from each of the sites, each month to avoid zero values in data. Analysis was carried out using ANOVA and multiple regression in the R Project 2.9.1 (R Development Core Team, 2009).

Coconut and Arecanut

Coconut (*Cocos nucifera*) and arecanut (*Areca catechu*) are widely cultivated throughout Kerala and RPM is reported seasonally in the region. Red palm mite and natural enemy surveys were conducted on coconut and arecanut. Two sites were chosen for each species that were historically drier and wetter climatically. The locations of the surveys are shown in figure 3.1. Surveys for each treatment were undertaken once a month in order to obtain temporal data for the study. Monthly surveys for *C. nucifera* commenced in November and for *A. catechu* in December 2008.

In each survey area, 20 trees were randomly selected within a 10 km strip (apart from Kunnamkulam) where 10 trees were selected due to lack of availability of host plant. A start point for each area was chosen, trees were selected randomly by driving for 30 seconds, stopping, and selecting the nearest palm.

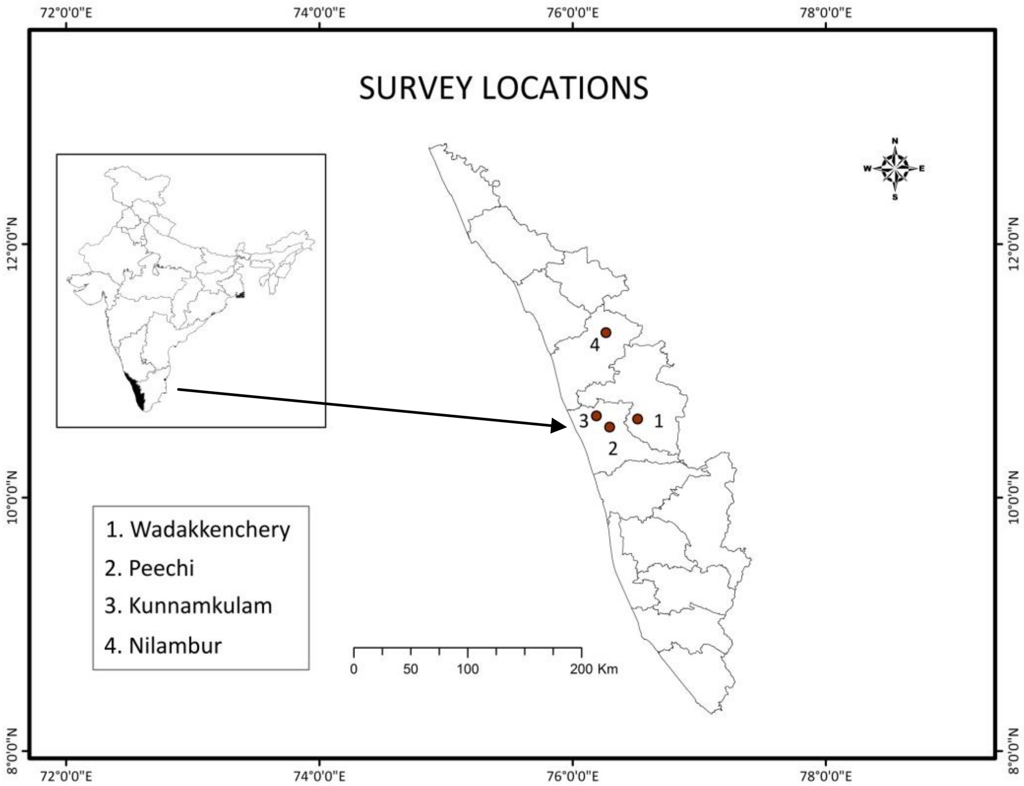


Fig. 3.1. RPM Survey locations in Kerala

Young palms not taller than 4 metres were selected and one lower frond was sampled. Each frond sampled was divided into 3 sections, upper (tip), middle and lower (base) and a leaflet from as close to the rachis as possible in each of these areas was removed (leaving 3 leaflets in total for each frond). While the leaflet was removed, a visual inspection for RPM and natural enemies was carried out. Natural enemies were picked up using a paintbrush and placed into 70% alcohol (1^{st} sample) then the remaining individuals of the same species were collected alive for testing in the laboratory. The cut leaflets were labelled using the letters 'L' for the lower section, 'M' for the middle section and 'U' for the upper section. Leaflets were stored separately in cotton bags. The total number of leaflets on the frond was then estimated in order to gain an estimate of leaf area sampled.

On reaching the laboratory, the leaflets were closely examined under a stereomicroscope for the presence of RPM and natural enemies. The numbers of RPM and natural enemies were noted and one individual each of various natural enemies was preserved in alcohol. Additional materials were transferred to the culture maintained in the laboratory. The length and width at the widest part of the leaflet was measured and then converted into leaflet area measurements using a 'standard multiplier' published in

Rao and Sebastian (1994), which were 0.74 for areca and 0.63 for coconut. This protocol was repeated each month at all the four sites and the same trees were sampled on each occasion.

Majority of specimens recovered during the surveys were phytoseiid mites, most probably of the genus *Amblyseius* (long setae were observed on the dorsal terminus of the abdomen) although this is yet to be confirmed. The number of coccinellids recovered was relatively low but two different genera were observed, including *Stethorus keralicus.* Other predators found during the surveys were from the orders Thysanoptera, Neuroptera, Hemiptera and Diptera (most likely Cecidomyiidae). A list of the predators found during the survey is given in Table 3.1 and in Appendix 1 and pictures of some predators can be seen in figure 3.1.

Table 3.1. Identifications of natural enemies found during the surveys

Site	Month	Order	Genus	Species	Associated with RPM?
Palakkad 9C	November	Coccinellidae	*Jauravia* sp	Larva	No
Palakkad 18C	November	Coccinellidae	*Jauravia sp*	Larva	No
Palakkad 14UC	January	Coccinellidae	*Stethorus*	*keralicus*	Yes
Peechi 19MC	February	Thysanoptera			Yes
Peechi 7C	February	Thysanoptera			

Fig. 3.1. Phytoseiid (a) and Dipteran larvae (b) after consuming *Raoiella indica*

In general, RPM populations were very low in November and December and high in February and March. All sites showed a significantly higher RPM population in March than December, although the incremental increases between was not significant in most cases. Figure 3.2 shows the percentage of sites at which RPM were found over the months the surveys were carried out. Figures 3.3 a-d gives an overview of the RPM population dynamics at each site in comparison to phytoseiid numbers. In most cases the number of sites infested with RPM rose every month, with Palakkad (C1) having a larger proportion of sites infested with RPM than any of the other sites by March. This shows that the RPM were either dispersing within the survey areas or breeding to population levels which are more detectable. The highest average populations were recorded on areca in Kunnamkulam in March (0.34/cm^2). Over all months, the mean numbers of RPM per cm^2 were significantly higher on areca (0.106/cm^2) compared to coconut (0.04/cm^2) ($F=21.0$, $p<0.01$).

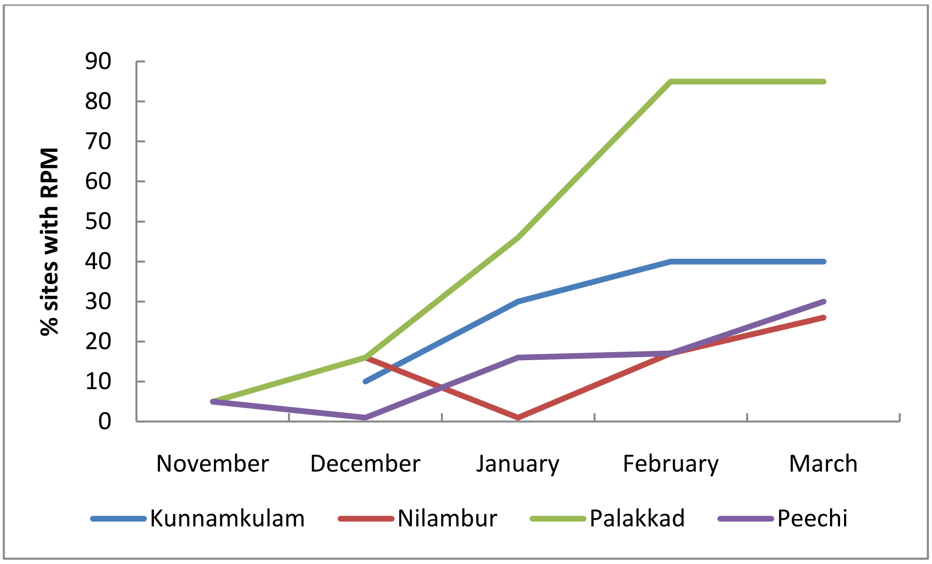
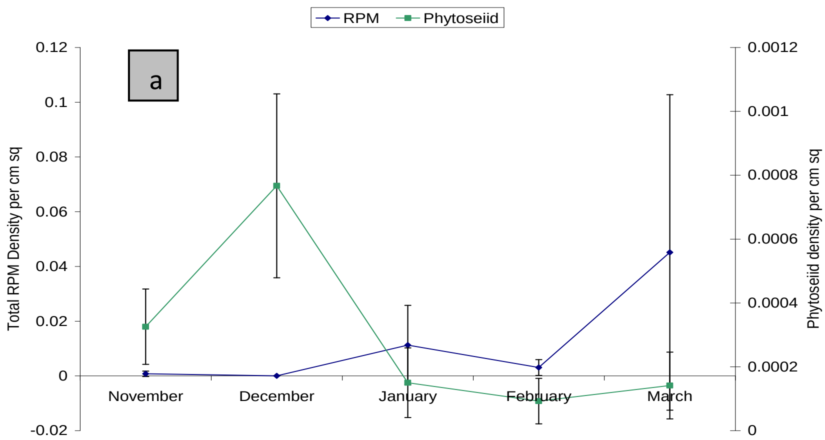
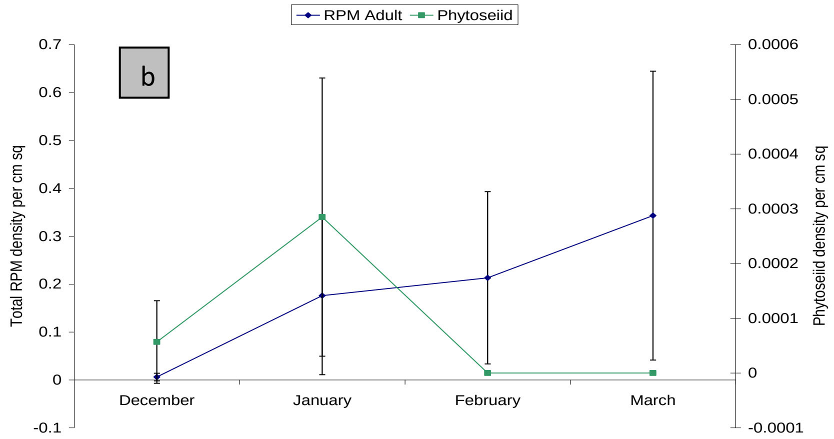


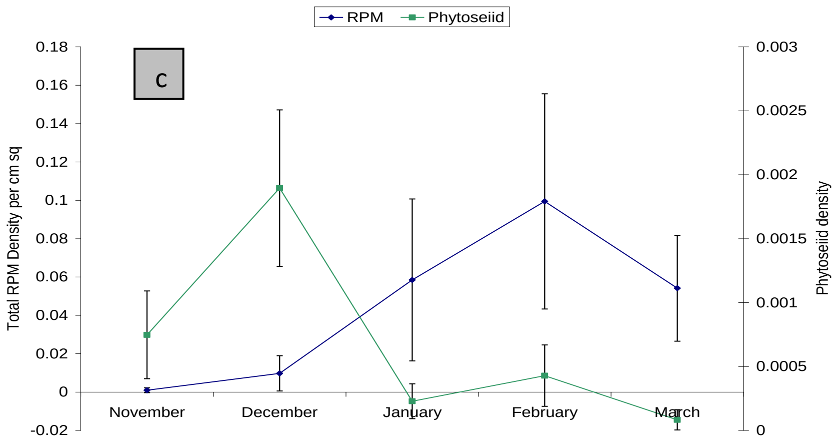
Fig. 3.2. Percentage of sites at which RPM was detected between November 2008 and March 2009.



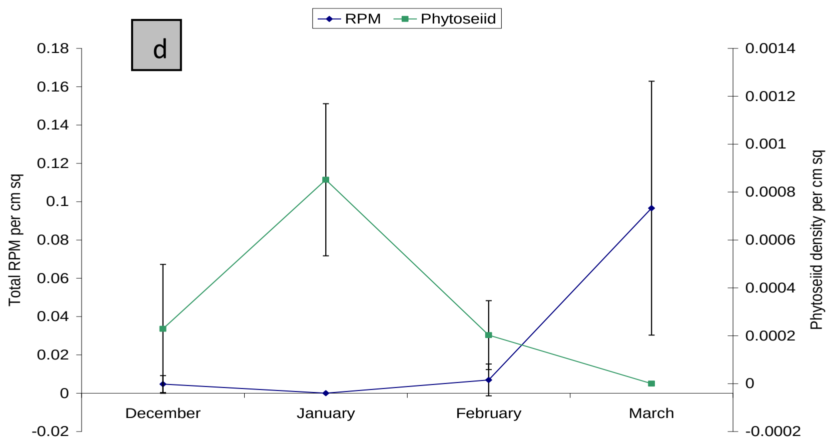
Population of RPM on coconut (Peechi)



Populations on RPM on Areca (Kunnamkulam)



Population of RPM on coconut (Palakkad)



Populations on RPM on Areca (Nilambur)

Fig. 3.3. The population dynamics of RPM and Phytoseiids between November 2008 and March 2009. A general increase in RPM numbers is observed on all sites and a general decrease in phytoseiids is observed after peaks in December and January.

Banana and coconut

Field data in the Caribbean shows that the host range of RPM is very wide encompassing many hosts from the Arecales and Zingiberales. McMurtry and Croft (1997) emphasised that phytoseiids such as *Amblyseius* may be more adapted to their preys' hosts rather than the prey themselves, emphasising the need to survey more than one host. In literature, there is no mention of the RPM on *Musa* sp. in India, although in the Caribbean, this species is reported as a major host and therefore qualitative surveys were set up to examine whether *Musa* sp*.* is a host of this pest in India.

Initially, roadside surveys were carried out to see if any colonies of RPM could be found on *Musa* sp. In these surveys RPM could not be found on majority of plants examined. Over 7 sites and roughly 68 banana plants examined (varieties *Nendran, Palayamkodan* and *Poovan*) only two specimens of RPM were detected.

Following this initial survey, a mixed plantation having coconut-banana intercropping was surveyed (Fig. 3.4) for the presence of RPM on *Musa* sp. This surveys were not quantitative, a single banana plant (sometimes more than one) were checked for RPM presence. The results showed that there were no colonies of RPM, even in a heavily infested plantation, although individuals were found on some banana plants. Since they were not observed in colonies, they probably must be surviving only as an individual without breeding.

Fig. 3.4. Coconut-banana intercropping

In order to replicate this study, further surveys were carried out in Wadakkanchery in February and March 2009. *Musa* sp. present at each of the survey sites were inspected for colonies of RPM. The colonies found on *Musa* sp. were compared to those found on coconut and presented in fig. 3.5.

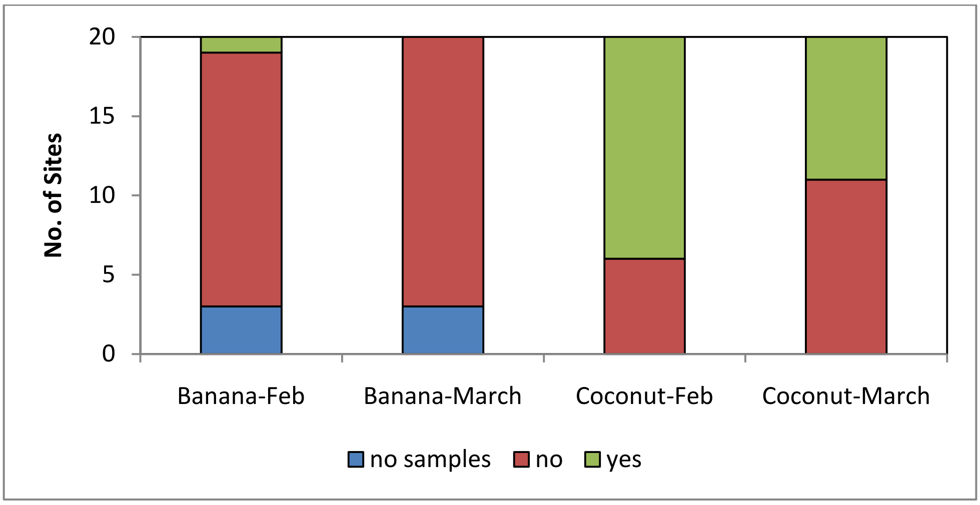
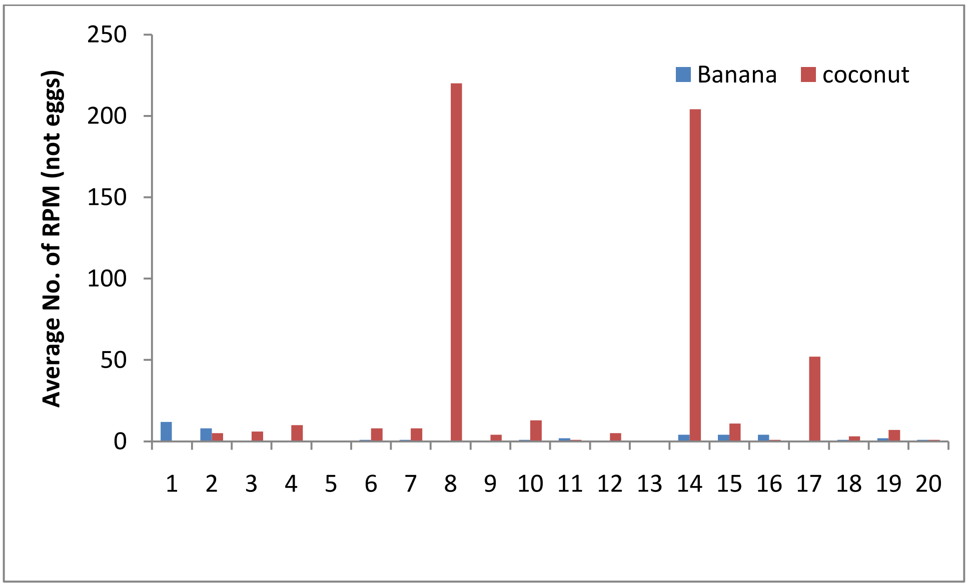


Fig. 3.5. Comparison of RPM found on coconut and banana

Only one colony was found on banana in these extensive surveys leading to the conclusion that although it is possible for RPM to reproduce on the banana plant, there are unknown factors which seem to prevent this from happening. The varieties of banana surveyed were Palalyam kodan, Poovan and Nendran, all of which are local varieties to Kerala. Further information on these varieties show that they are all crosses with *Musa acuminata* and *Musa balbisiana* (AAB) and it is suspected there may be an element of varietal resistance to RPM. Individuals of RPM were present on the banana plants indicate that reproduction is in some way not possible. Figure 3.6 shows the number of individuals found on banana plants alongside the coconut surveys in Palakkad (C1) in order to illustrate the findings.



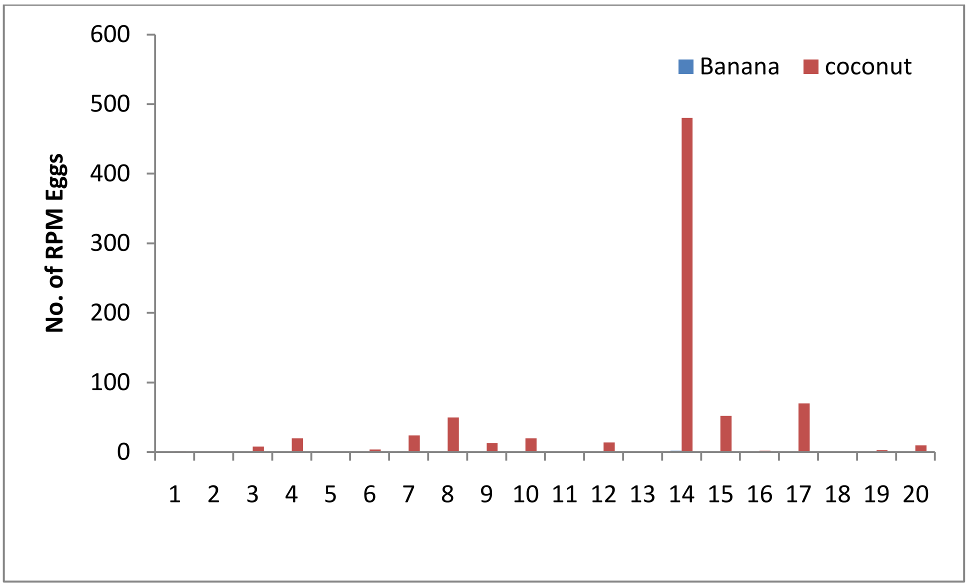


Fig. 3.6. A comparison of RPM mobile stages and eggs per leaflet on banana and coconut palms in February.

This survey was repeated in March and still no evidence of breeding colonies was found, indicating a lack of suitability of the host, or potentially other factors limiting the RPM population.

CHAPTER IV

Impact of climatic factors and phytoseiid mites on RPM

In order to obtain as much information on the factors affecting the fluctuating population dynamics of the RPM, possible influence of local weather parameters and predator populations were investigated. Weather data was collected from two weather stations, Nilambur and Peechi. Temperature and humidity were also measured at time of sampling at each site. Weather station data was averaged for temperature and humidity for each month and a cumulative total of rainfall was calculated. Analysis of data was carried out using multiple regression in order to assess any significant interactions occurring.

Factors affecting the RPM population indicated that there was a significant effect of site temperature on RPM population ($F=22.1$, $p<0.01$) (figure 4.1) and a linear regression model showed a positive relation whereby RPM number increased with temperature ($r^2=0.20$). General weather station data was also collected for the areas surveyed and it was found there was a positive relationship with maximum temperature of the previous month ($F=4.37$, $p=0.06$) and the current month ($F=2.05$, $p=0.18$). It was also found that there was also a significant effect of host plant species on population levels of RPM ($F=21.0$, $p<0.01$). Even though there was no significant effect of site humidity on RPM number ($F=0.37$, $p=0.56$) there was a trend whereby higher humidity levels were related with lower RPM numbers. By examining general humidity in the survey areas it can be seen that RPM populations are significantly related to humidity of the previous month ($F=6.65$, $p=0.02$) compared to the humidity of the current month ($F=1.79$, $p=0.21$). When the effect of rainfall was examined, there was a significant relationship between average rainfall of the previous month and RPM number ($F=2.8$, $p=0.12$) but not for the current month ($p=0.95$). Results from a multiple regression showed that there was a significant interaction between site temperature and host (figure 4.2) ($F=34.74$, $p<0.01$) with a positive relationship between temperature and RPM numbers on Areca ($r^2=0.7218$, $p=0.01$), however significant relationship was observed between RPM numbers and coconut ($r^2=0.1$,$p=0.46$).

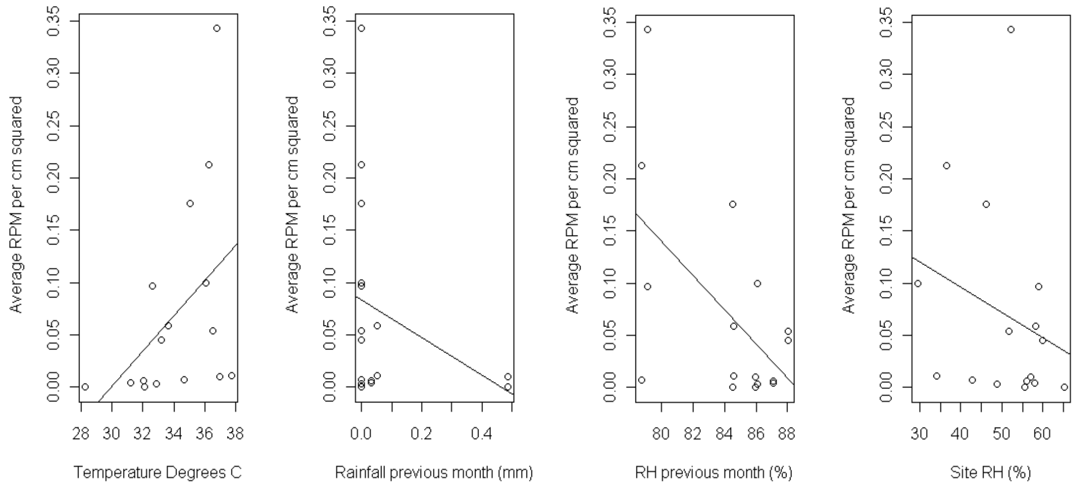


Fig. 4.1. Graphs correlating abiotic factors with RPM number. Temperature showed a positive correlation (F=8.64, p=0.01), rainfall and humidity the previous month showed a negative correlation (F=2.8, p=0.12; F=6.65, p=0.02 respectively). Site humidity also showed a negative correlation, but was not a significant as previous month data (F=0.97, p=0.34).

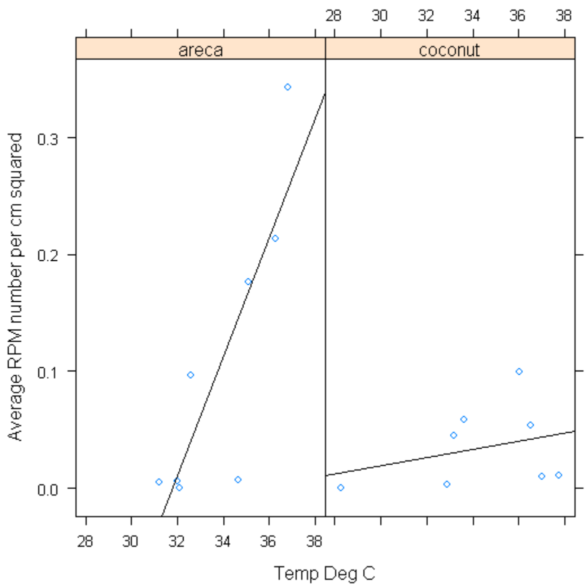


Fig. 4.2. Correlation between the average RPM number per cm^2 and temperature, on both *Areca catechu* (r^2=0.7218, p=0.01) and *Cocos nucifera* (r^2=0.1,p=0.46).

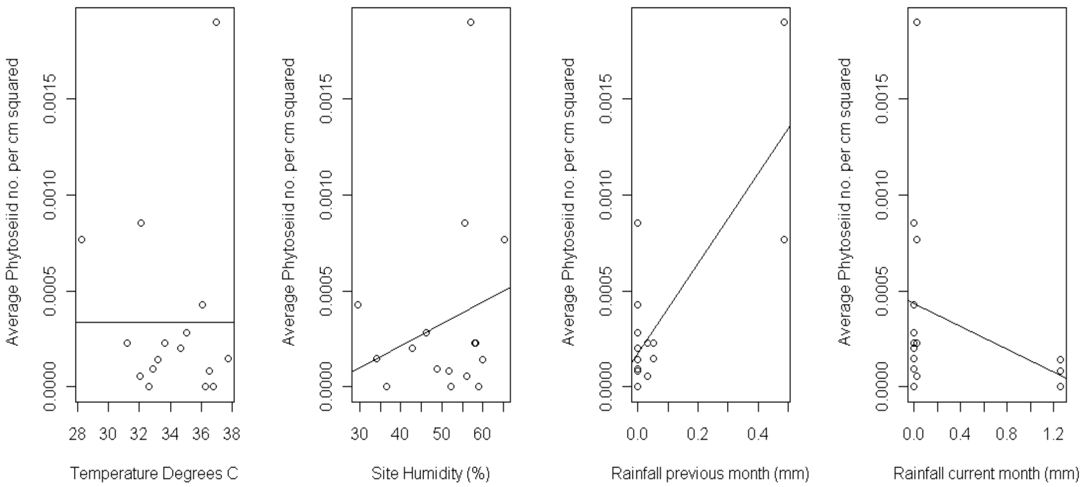


Fig. 4.3. Graphs correlating abiotic factors and phytoseiid numbers. No significant relationship was found between phytoseiid number and site temperature (r^2=<0.01,p=0.99), a slight trend was found between phytoseiid number and humidity (F=1.53,p=0.24). However, rainfall in the previous month was most significant (F=23.49, p<0.01).

The phytoseiid populations at each site are displayed in figure 3.3 a-d (Chapter III). The graphs are shown in comparison to the RPM populations and it can be seen that there is a significant peak in December for coconuts and in January for Areca at Nilambur. Phytoseiids at Kunnamkulam follow the same trend, although the peak is not significant. Following this peak, there was a significant drop in numbers on all sites and no significant changes in population numbers were seen thereafter. Average phytoseiid numbers per cm^2 were higher on coconut (0.0005/cm^2) than for Areca (0.0002/cm^2), but they were not significantly different from each other. An analysis looking into the effect of climatic conditions on the numbers of phytoseiids present showed that there was no relation between phytoseiid number per cm^2 and site temperature (r^2=<0.01,p=0.99) (figure 4.3) but there is a slight trend towards phytoseiid number (predator) being linked to site humidity (F=1.53,p=0.24) (figure 4.3). The same pattern follows for the general temperature and humidity pattern. There was however a very significant correlation between average phytoseiid number and rainfall of the previous month (F=23.49, p<0.01), although no correlation was seen between phytoseiid number and rainfall in the current month (F=0.37, p=0.55) (figure 4.3).

Figure 3.3 a-d displays temporal data collected over 4 months and shows that the peaks in phytoseiid populations and RPM populations occur at different times. In general, as the populations of RPM rise, the populations of phytoseiids decrease. A regression on

RPM and phytoseiids show that there is an inverse relationship between average phytoseiid number and average RPM number (r^2=0.126,p=0.1486) (figure 4.4a). When examined at host level, this relationship is more pronounced on the areca (r^2=0.2165, p=0.2453) than the coconut palms (r^2=0.09075, p= 0.4684) (figure4.4b).

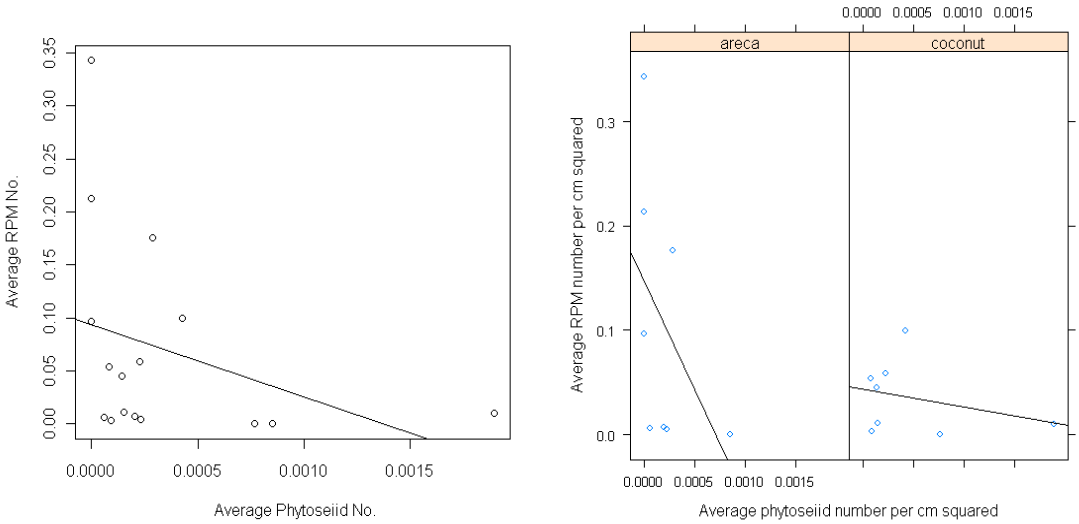


Fig. 4.4 (a) Average RPM number per cm^2 regressed on average phytoseiid number per cm^2. (b) As (a), but separated onto separate host trees.

These results help us to examine the population dynamics of RPM in association with climatic conditions and with predatory mites. The data shows that the increase in populations of RPM is not only linked to temperature, but also to the host plant, number of predatory mites present as well as humidity and rainfall of the previous month. The interaction between temperature and host plant is statistically significant on *A. catechu*. Potential interpretations for this could be that the host plant is metabolising more efficiently in hotter weather or perhaps is more readily exploited by RPM, creating ideal conditions for reproduction of RPM. Additional explanatory power could come from the negative correlation between RPM and phytoseiids on *A. catechu,* whereby when populations of RPM are high, the numbers of predatory mites are extremely low. The relations between temperature and phytoseiid population are not significant on *Cocos nucifera.*

The overall dynamics of the predatory mite are less easily interpreted. There is a trend showing a drop in phytoseiid number as humidity decreases. There is, however, a very significant relationship between rainfall of the previous month and phytoseiid numbers. The effects of a drop in humidity on *Amblyseius* sp. has been well documented for phytoseiids in literature. It has been reported that *Amblyseius andersonii* is sensitive to the relative humidity with females displaying less mortality when the humidity is higher, which correlates with drops in populations in field observations. Rainfall in the previous month will have an obvious effect on relative humidity, therefore these factors are linked.

The effect of rainfall of the previous month on the RPM and Phytoseiid mite populations appears to be very significant but with opposing effects. For RPM, it has been previously reported that humidity may affect the moulting process which may cause mortality of individuals. Alternatively, there could be some effect of rainfall on host plant quality therefore decreasing the feeding ability of the RPM. Earlier workers attributed population drops at the onset of monsoon to washing off of the mites from the leaves; however, this does not correlate with the results found here as there was no significant influence of rainfall from the current month on RPM numbers, although no surveys were conducted during monsoon rains. Other alternatives such as an increase in pathogenic fungal epizootics are also possible, although no evidence was found during our surveys. For predatory mites, the ambient conditions created by rainfall *i.e.,* higher humidity is the most likely reason for the increase in phytoseiid number, which would explain why rainfall in the previous month has an effect in the following month and not as much effect in the current month. There are no significant differences between populations found on *A. catechu* and *C. nucifera.* These results therefore indicate that the population numbers of the phytoseiid are less linked to the host plant and more limited by environmental parameters such as humidity.

One hypothesis for these results may be that during the low season, where RPM numbers are low, the phytoseiid predator may be benefitting from more suitable climatic conditions for its growth and development. When the humidity drops, the survival of predatory mites is probably lower in turn allowing the RPM to reproduce without the more intense predation pressure present when phytoseiids are more abundant.

It is not possible to draw definitive conclusions from these results as to whether host plant suitability, abiotic or biotic factors are to be attributed to populations fluctuations. The results are based on only a few months of population data but give a very important insight into how the prey and predator respond to climatic and host conditions and highlight areas for future study.

Laboratory Studies

Laboratory studies were mostly concentrated on the phytoseiids which are thought to have the most potential agent for biological control. Initial assays proved difficult as predators escaped the arenas prepared for them, however, some valuable observations were made in this study. Leaflets containing RPM were sampled from coconut palms in the Palakkad region. They were then examined under a stereomicroscope in the laboratory in order to find and remove phytoseiids. Initially, 10 RPM (mobile stages) were counted and transferred to a piece of coconut leaflet. The leaflet was then put inside a small glass tube, sealed with breathable material in order to prevent moisture build up in the tube. Predatory mites were divided into 2 groups- those with red guts and those without. There were 13 found with red guts and 6 without. Predators were left in the tubes overnight and numbers of RPM consumed were assessed the following day.

Red Gut Phytoseiids

Of the 13 tubes, 6 had phytoseiids missing the following day, so were eliminated from the results. The average number of RPM consumed in the time period were 3.6 RPM, with the highest number consumed being 7 RPM and the lowest being 1.

White Gut Phytoseiids

Of the 6 white gutted predators, none of the predators were recovered; however, there was evidence of RPM consumption (remnants of RPM), which on average was 1.2 RPM per phytoseiid, although in one tube 4 RPM were consumed. Of the red gutted mites which were recovered from the initial laboratory study, 4 were transferred to arenas for further observations along with other red gutted phytoseiids collected from the field. Arenas were set up where 10, 20 or 30 RPM were placed on small piece of coconut leaf disc, 2 replicate were set up of each. The coconut material was placed on sponge in 140mm petri dishes which was covered with water and kept in a loosely sealed plastic box to stop the entry of ants and evaporation of water (see figure 4.5). Observations were made daily and the number of RPM consumed was noted. Results can be seen in Table 4.1.

Fig. 4.5. Arenas for predatory mite assays- (a) arenas in humid boxes (b) Individual arena.

Table 4.1. Results from preliminary lab investigations

Number of RPM added	Day 1	Day 2	Day 3	Day 4	Average RPM consumed per day
10	2	1	1*	+	1.3
10	5	3	2	1*	2.75
10++	0	3	-	-	1.5
10	0	2	-	-	1
20	4*	-	-	-	4
20	8	14**	-	-	11
30	8	4*	-	-	6
30	3	-*	-	-	3
30	-	6	1*+	-	2.3
30++	6	*	-	-	6

(+ phytoseiid egg found, ++ assay started from egg stage, * Phytoseiid missing, **Phytoseiid put into alcohol for ID).

The difficulty in rearing the mites was apparent. After consumption of RPM the predatory mite was extremely active upon the leaf discs and on most occasions the predatory mite could not be found on inspection of the dish at a later point. The results gathered show that the predator will freely feed upon red palm mite and is able to produce viable eggs, however more in depth studies would need to be carried out before any conclusions could be drawn from the data.

CHAPTER V

Wind traps: Population dynamics, dispersal and host plant nutrition

Occurrence of individual red palm mites was observed on alternative host plants present near or under taller coconut palms. This indicates the possibility of the mites dropping down from a host plant to find a new host. This behaviour has been observed in Eryophyoidea (Acari) and it has been hypothesised that the reason for leaving the host plant to disperse on air currents is because the mite is relatively small and immobile; thus crawling from one plant to the next is only an option if the plants happen to be close and in contact with each other (Jeppson *et al*., 1975). In addition to this, it has been demonstrated that certain species of eryophyids actively travel at certain times of the year (Jeppson *et al*., 1975). The rapid spread of RPM throughout the other parts of the world (New World) demonstrates the ability of the mite to disperse effectively between plants; however, the method of transfer between plants is unknown, along with what triggers the dispersal of the mite between plants. Is it a question of passive dispersal on strong wind currents, or passive phoretic dispersal on animals or humans? Alternatively, is the mite 'raining down' from tall coconut palms onto understory vegetation? In addition, is there an effect of crowding on the colonies that induces behavioural changes or do cues come as a result of reduction in host plant quality or a combination of the two? By investigating the method of dispersal it is hoped that these questions will be answered and the drivers behind the rapid spread in the New World discovered.

An understanding of the population dynamics of RPM may throw light on the drivers of its dispersal. The mite populations tend to build up at the end of the rainy season in November/December and rapidly increase in size throughout the hot dry months. With the onset of the monsoon rains in May-June, populations crash back to almost zero and this cycle repeats annually. This phenomenon widens the possible interaction of multiple factors such as changes in host plant quality throughout the season, difference in predator pressure and changes in RPM population density.

Results (Chapter III) showed that the percentage of sites where RPM was detected increased throughout the season, peaking in March/April and declining rapidly once more to zero in July. Wind speed data gathered from KFRI weather station showed that wind speed was generally higher in January and February, which could be hypothesised to be related to the increase in prevalence of RPM throughout the 20 plots studied. Wind speeds of up to 16 km/h have been recorded in this area, although from March-October wind speed remain below 5 km/h.

Several authors have investigated the drivers for dispersal of mites. Li and Margolies (1993) investigated mite age, density and host plant quality using *Tetranychus urticae* and found that newly emerged adult females were more likely to display dispersal behaviour when the quality of leaves changed and or density increased. Smitley and Kennedy (1988) observed that aerial dispersal of the mites was greatest in predator-suppressed field plots, under dry weather conditions. Using our data on the population dynamics of RPM, it can be hypothesised that RPM will disperse when populations are dense, and host plant quality is low (towards the end of the dry season). It could, therefore, be hypothesised that this is the time of year when RPM disperse onto new host plants. In some instances as in *Tetranychus* sp., adult females showed dispersal behaviour (Li and Margolies, 1993) and therefore investigating the male:female ratio would also provide important information on the dispersal of RPM. The presence of solitary females on a host plant may imply that this female had arrived from its natal site and colony to find out a new area to lay eggs. However, the mode of dispersal is not yet to be understood clearly; females may be crawling down or dispersing on air currents from the natal site. Therefore traps to catch aerial dispersers would provide information on when the mites initiate inter-plant dispersal.

Various methodologies have been employed by researchers to study the dispersal of mites on wind currents. Tixier *et al.* (2000) used aerial traps made with funnels (31 cm diameter) filled with water and a wetting agent approximately 50 cm above the host plant. Duffner et al. (2001) and Gamliel-Atinsky *et al*. (2009) used a freely rotatable wind chamber (20cm diameter) attached to a pole which tracked the direction of the wind using a weather vane. The design by Gamliel-Atinsky *et al*. (2009) was made up of two sections; firstly a narrow tube (20cm diameter) which opened into a larger tube, with the aim to slow air velocity and therefore deposit mites onto Vaseline coated slides at the bottom of the chamber. Lawson *et al.* (1996) used cylindrical sticky traps at 2/3 the height of the tree and Smitley and Kennedy (1988) used Tack Trap coated microscope slides pointing in the four cardinal directions of the compass (N,S,E,W).

The aim of this study was to sample the presence/absence of aerial dispersal by RPM in the Palakkad area in relation to density, sex ratio, host plant quality and predation pressure/competition.

Study sites

Nine sites in total were chosen in the Palakkad area to study the population dynamics and dispersal of RPM. From our surveys, the area was known to have a ubiquitous spread of RPM infestations on coconut palms, with a seasonal population cycle whereby populations build up progressed from December onwards throughout the hot season and crashed by June, July with the onset of the monsoon (Taylor *et al.*, 2011). Pilot wind traps were deployed in 5 sites in December 2009 and 4 sites in March 2010 when mite densities increased (Fig. 5.1). Leaflets were sampled monthly from each site after wind trap was deployed. Sites I,II,III,IV and V were part of the pilot study and sites, NI, NII, NIII, NIV were established during March 2010 in addition to the original sites. Sites I and NIV had two wind traps. The data on various factors were collected from the trap locations as detailed below:

Site Characterisation

At each survey site, the characteristics of the site were recorded *i.e.* sunny/shady, wet/dry (Table 5.1). The influence of these factors, if any, on population dynamics of the mite was examined.

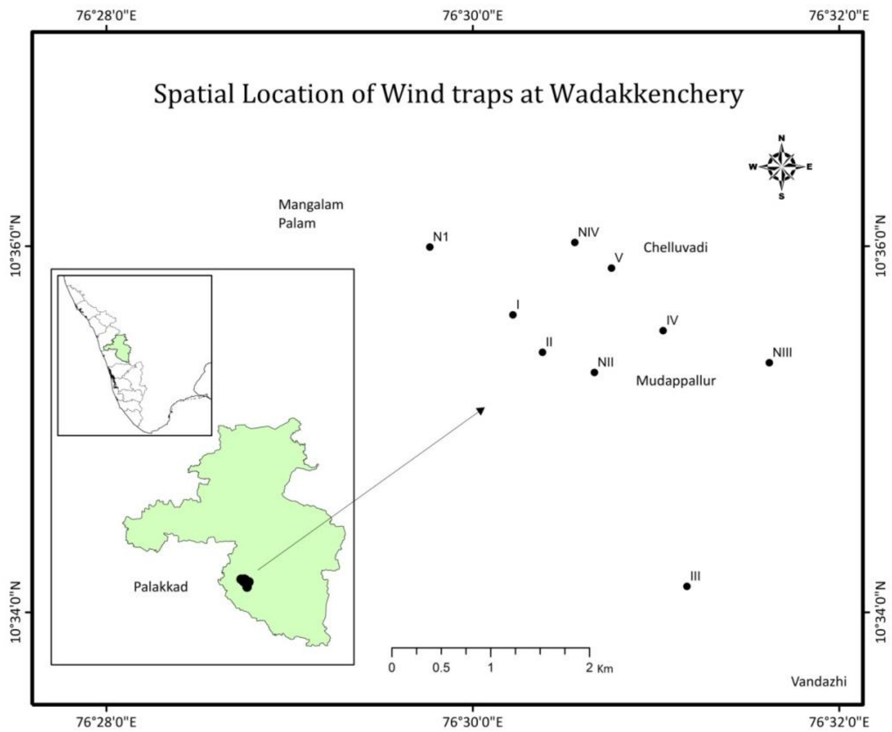


Fig. 5.1. Locations of the 9 wind trap sites

Table 5.1. Characterisation of wind trap and leaflet sampling sites

Site No.	No. Coconuts Red	Green	Bananas	Wet/Dry	Sun/Shade
I	Na	Na	Yes	Dry	Sunny
II	12	6	Yes	Wet	Shady
III	11	3	Yes	Dry	Sunny
IV	15	11	Yes	Dry (irrigated occasionally)	Shady
V	0	9	Yes	Dry	Shady
NI	~6	~54	Yes	Wet (irrigation/next to paddy field)	Sunny
NII	0	12	No	Wet? (2m above paddy)	Sunny
NIII	4	9	Yes	Dry	Sunny
NIV	0	130	Yes	Wet (Irrigated)	Sunny

Leaflet sampling

To assess the mite density on each trap site, four coconut palms were selected at random each month (different trees were selected each month), from which three leaflets were collected from a lower frond. The lower frond was chosen to standardise the leaflet collection methodology; in addition populations have been shown to be significantly higher on lower fronds (data not shown). Leaflets were chosen from the base, middle and tip of the frond and were placed in separate linen bags and returned to the lab. Each leaflet was washed carefully using approximately 5ml of 80% alcohol and mites or insects present if any were collected. The samples were scored counting the number of RPM present including the sex ratio; the number of predators and number of phytophagous insects/mites present using a stereo microscope. This gave an accurate estimate of the density of all the organisms present on the leaflets. Samples were taken monthly from each site.

Leaflet nutrient analysis

After collecting the insect and mite, the leaflets from a frond were pooled and dried in an oven at 60 °C. The dried leaf material was stored in paper covers and later used for nutrient analysis. Prior to nutrient analysis leaflets were once again dried at 60°C and ground to fine powder.

Wind Trap Design

A freely rotatable design of wind trap was chosen due to the small nature of the mites to be sampled. There were concerns over using sticky traps to sample the mites for two reasons. Firstly, sticky traps would destroy the sample and deem it unidentifiable and secondly, there were concerns about the aerodynamics of mites movement in the slipstream surrounding the sticky trap and not getting caught. Two publications as a guide for designing the freely rotatable wind trap (Duffner *et al.*, 2001; Gamliel-Atinsky *et al.*, 2009). The wind trap has 5 component parts (Figure 5.2A and B.). Part A is the wind dispersal trap itself. The smaller tube (5cm diameter) is designed to face upwind constantly to allow air to flow through the small aperture; this is attached to a 1.5 litre drinks bottle with the top cut off. The small tube opens up into the larger rear chamber (constructed from a plastic drinks bottle, diameter 9cm), where air flowing through should slow down and deposit any small particles such as mites onto an acetate slide at the bottom of the chamber. The wind trap itself, is held in place by Part B: the wind trap holder. This part is designed to enable the trap to stay facing upwind by being able to turn. The grey tube of the wind dispersal trap (part A) is fitted into the hoops, which held the trap firmly in place. Part C is the cycle pedal which act as the pivot, allowing part B to turn. The lower portion of the part C is joined with a metal connector and the attached assembled wind trap is inserted into a metal post (of 2m tall), part E. The post is then placed centrally in the plot, so mites from surrounding trees may be picked up if they are dispersing aerially. The metal post is approximately 2m tall or 2/3 height of the trees in the plot. One trap per post is mounted. In the rear of the trap an acetate tray on which a fine layer of Vaseline is spread acted to trap the mites which are deposited on the tray.

The trays were initially changed weekly as it was not clear how many mites were going to be collected per week and whether the slides would get dirty/unreadable from dust blowing in the air. After the first month, however it was deemed sufficient to do this every 2 weeks as slides were readable. The first prototype used an aluminium tray with glass microscope slides attached to it, however this was found to be too heavy and impeded the movement of the trap with the wind, thus an acetate sheet was cut to size and replaced this, improving the movement of the trap.

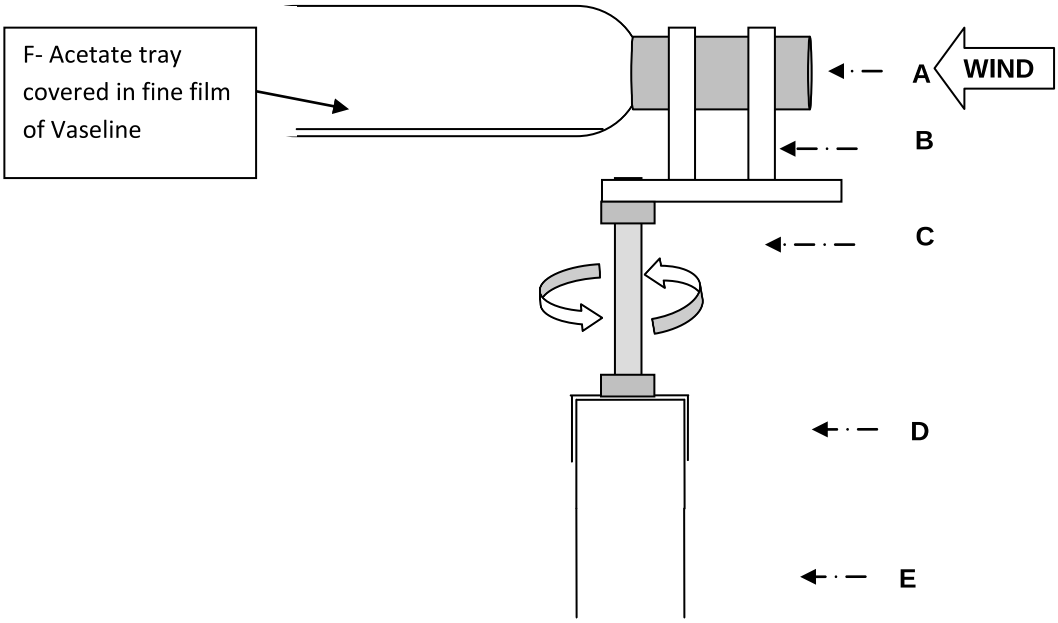


Fig. 5.2A. Wind-trap-Technical diagram-graphical

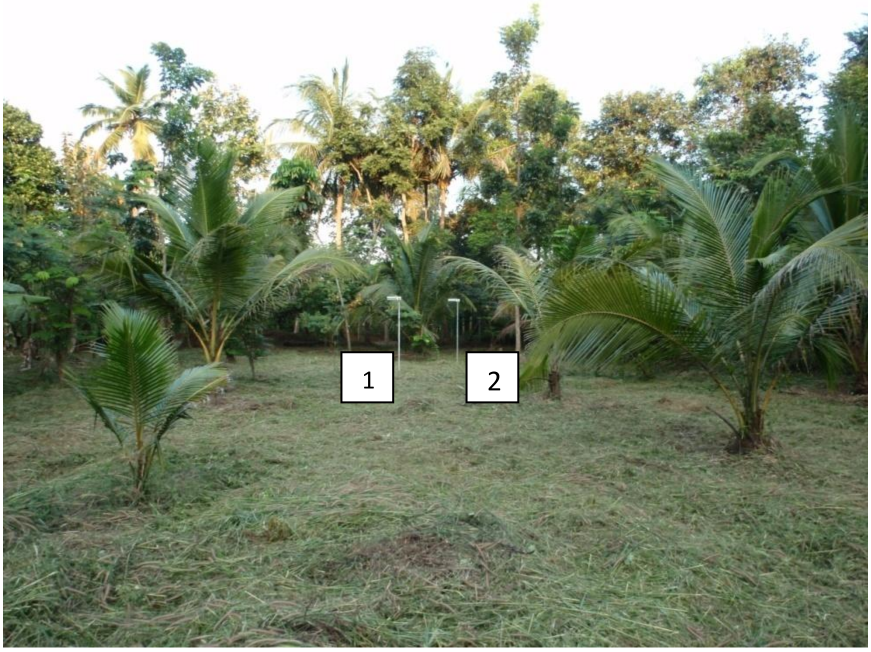


Fig. 5.2B. Wind-traps in field (1 and 2) was erect on a 2m GI pipe.

Wind trap inspection

Initially, Vaseline coated slides were collected weekly as there was concern that debris on the slides would impede the inspection; however, after 1 month the sampling schedule was reduced to every 2 weeks. Various methodologies for inspecting the slides for mites were investigated including washing with detergent and filtering and inspection under the microscope. It was found that the best methodology involved inspection under a stereo microscope for RPM specimens. When any mites/insects were found on the slide they were collected using a fine paintbrush and stored in 80% alcohol for further inspection. These specimens were measured to obtain the size range of specimens collected to ensure that the traps were catching the desired particle sizes comparable to that of the RPM. The numbers of RPM caught were noted, in addition to other wingless arthropods. They were categorised as predators or phytophagous arthropods. RPM and other wingless arthropods were slide mounted using Heinze media and identified. Species level identification was only carried out for RPM; other groups were identified only genus level.

Data Analyses

The number of RPM per leaflet was used to calculate an average number of RPM per leaflet per tree. Four trees per site were sampled per month. However, these were not considered to be pseudoreplicates each month, as different trees were sampled monthly. This allowed an average figure for each site to be calculated. Changes in RPM and predator density throughout the season were calculated using log (x+1) transformed counts from each tree and analysed using an ANOVA. When there was a significant difference in mite numbers new factor levels were constructed to show significant differences between different time points. Linear regressions were carried out with nutrient against log (x+1) transformed population densities of RPM.

Population dynamics of RPM and predators on leaflets

RPM numbers remained low during December, January and February but increased signifcantly between February and March ($p<0.05$, Tukey HSD; Figure 5.3.). RPM numbers were combined for December, January and February ('early') and March, April, May ('late'). There were siginficant differences in RPM numbers between these two time periods ($F=65.1$, $n=140$, $p < 0.001$). Densities were variable between sites (Figure 5.4); with significantly higher RPM densities on site I and V in March, sites IV and NII in April and III in May. Numbers of predators sampled varied significantly throughout the season ($F= 4.3715$, $p <0.001$), with significantly fewer predators in March than December ($p=0.03$) and May ($p=0.001$) (Tukey HSD post hoc). Predators sampled included Phytoseiid mites (including *Amblyseius* spp.), Coccinellids (including *Stethorus* spp.), Dipteran larvae (most probably Cecidomyiidae) and spiders. The majority of predators found were phytoseiid mites (65 in total throughout the season) compared to 6 coccinellids, 2 dipteran larvae and 4 spiders. The relationship between RPM density and predators on leaflets are shown in Figure 5.5.

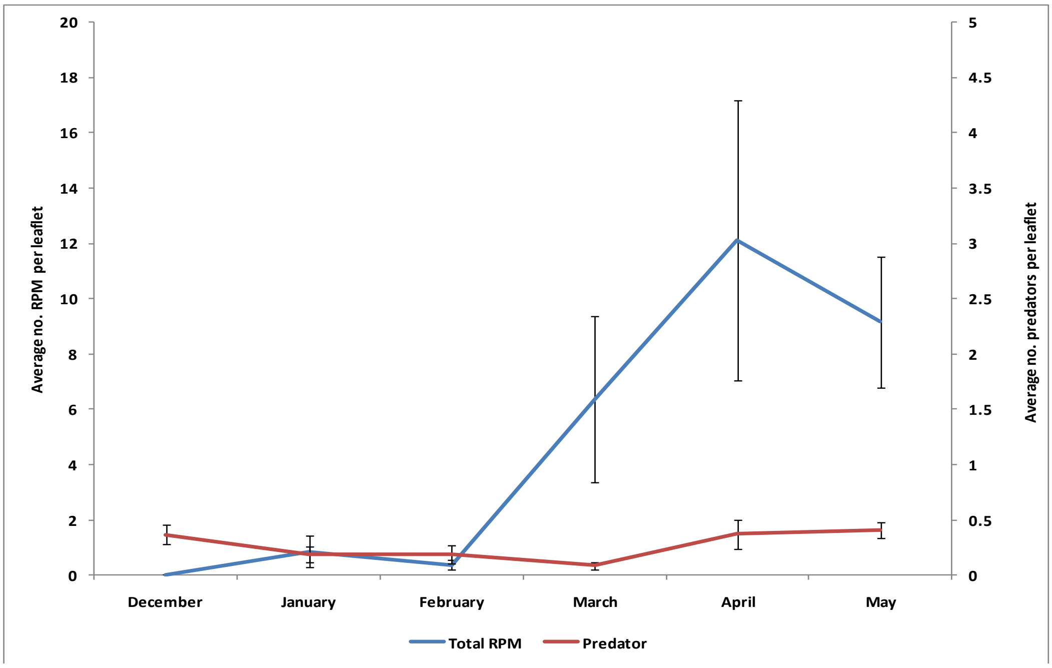


Fig. 5.3. Average no. RPM and predators per leaflet/tree (n=20 trees December, January, February; n=36 trees March, n=18 trees April, n=32 trees May). Per leaflet estimation were taken from a subsample of 3 leaflets per tree from 1 frond. Figures shown +/- 1SE. Letters indicate significant differences between RPM number. Predator density is marked on the secondary Y axis.

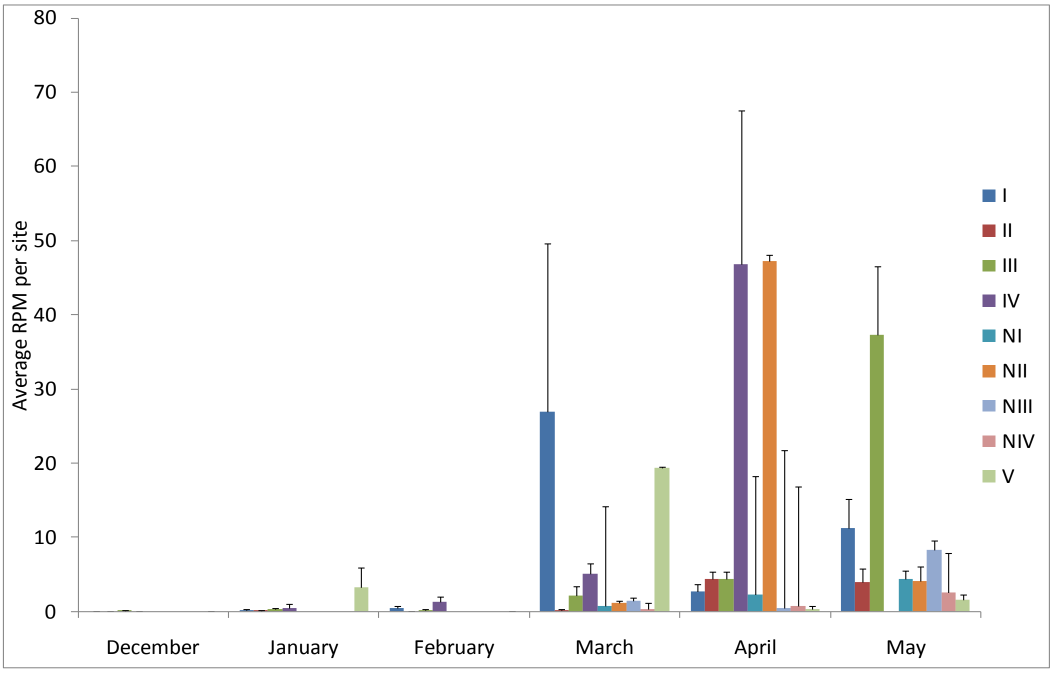


Fig. 5.4. Average RPM number per site throughout the season. 3 leaflets on 4 trees were sampled per site. An average of 3 leaflets was taken for the value per tree, which was used to construct the overall average per site. Error bars shown are ±1SE.

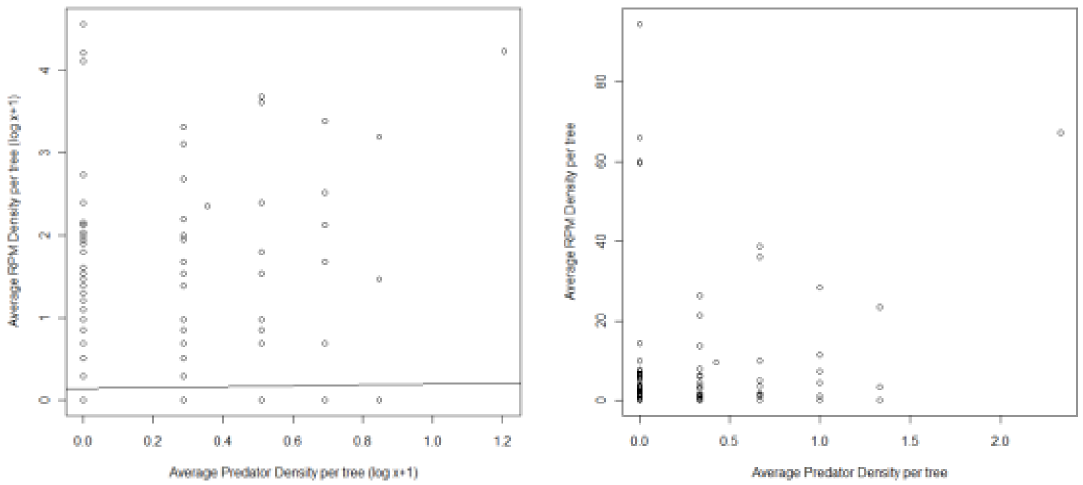


Fig. 5.5. Relationship between predator density and RPM density. A linear regrssion investigating the relationship found the two were significantly correlated (Multiple R-squared: 0.05413, Adjusted R-squared: 0.04756, F-statistic: 8.24 on 1 and 144 DF, p-value: 0.004715) ??

Sex Ratio of RPM on leaflets

Out of the 438 sampling points throughout the season, RPM were observed on 199 occasions and of those occasions, males were observed on 104 different leaflets and females were observed on 163 different leaflets (Figure 5.6) shows these data as a percentage of RPM presence throughout the sites surveyed). In December, only one RPM was observed from all of the sites and this was a solitary female on one leaflet. Throughout the season, there were 72 occasions where female RPM were observed on leaflets with no males and 13 occasions when males RPM were observed with no females, and 91 occasions where both sexes were present out of a possible 177 counts; thus on 40.7% of occasions females were observed without males compared to 7.3% of occasions where males were observed without females and 51.4% of the time when both sexes were observed. Solitary females were observed on leaflet 46 times during the survey (*i.e.* no more than one individual collected) compared to 9 solitary males collected, this figure was broken down per month and displayed in Figure 5.7. The graph shows that the percentage of occasions where solitary RPM females were observed increased steadily throughout the season when all sampling occasions were taken into account; however Figure 5.8 shows the percentage of occasions solitary RPM males and females were sampled expressed as a percentage of the number of occasions RPM were found per month. Figure 5.9 shows the average sex ratio per month when both males and females were present on leaflets.

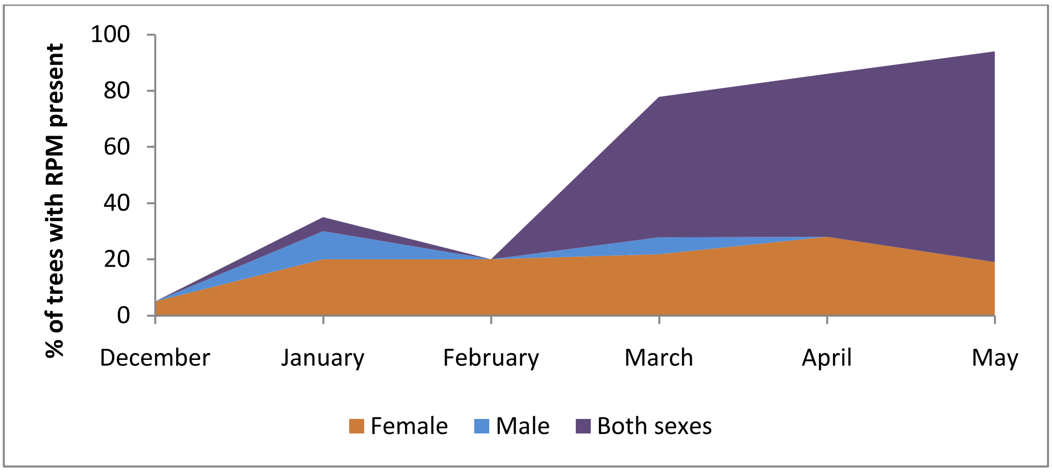


Fig. 5.6. The percentage of trees with RPM present throughout the season. The total area represents the total number of RPM present expressed as a percentage and the blue, green and red filled areas represent the subset apportioned to either male only, female only or both sexes being present.

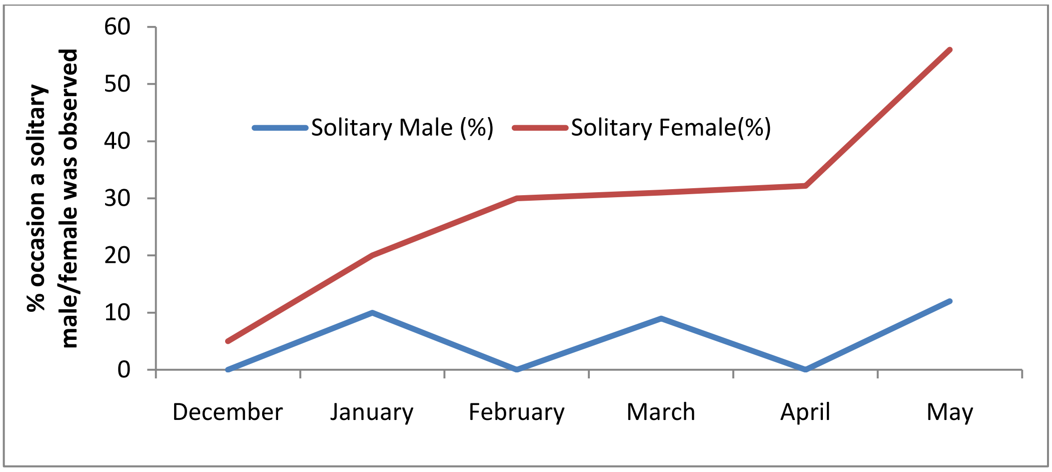


Fig. 5.7. Percentage of occasions a solitary male or female were observed throughout the study

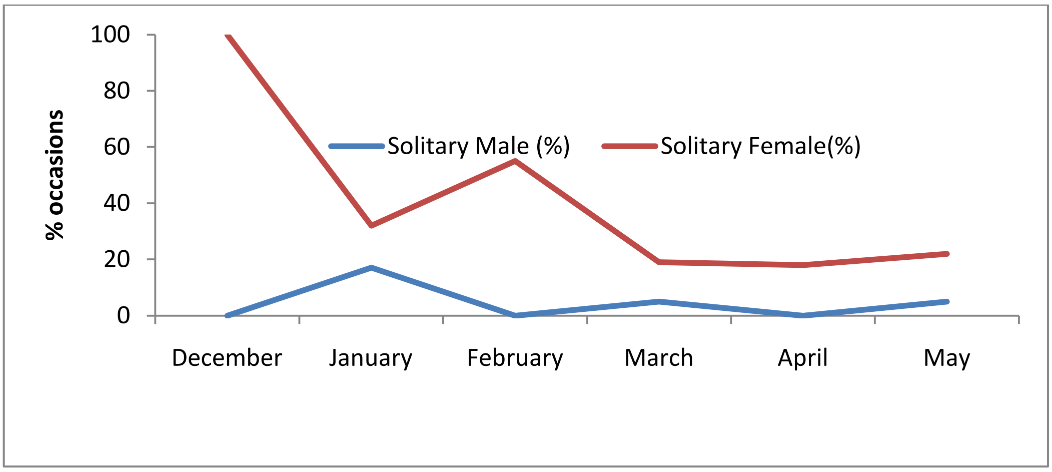


Fig. 5.8. Percentage of occasions solitary RPM males and females were sampled expressed as a percentage of the number of occasions RPM were found per month.

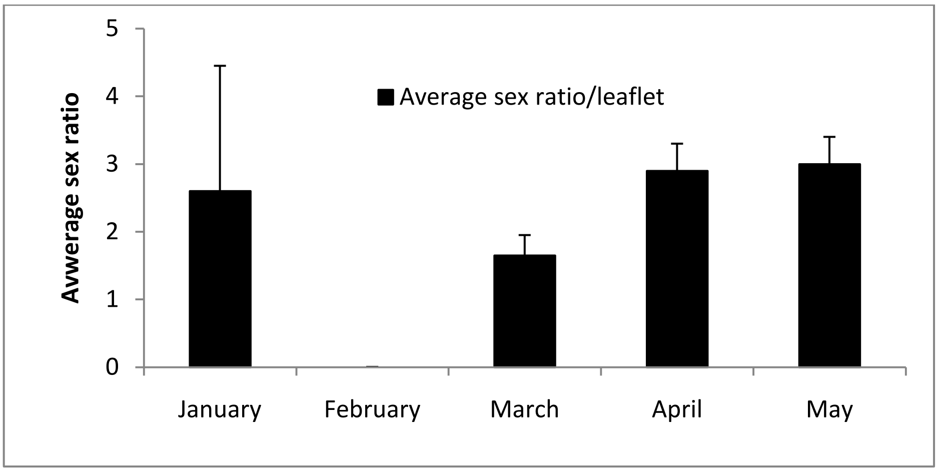


Fig. 5.9. Average sex ratio per leaflet per month. Data is taken from those data which had both males and females present to give a leaflet level sex ratio. In December and February, only females were found.

Host plant quality and RPM density

A pilot study was carried out using leaflets collected from site III to see if there was any relation between host plant nutrition and density of RPM. The results showed there to be a significant relationship between average RPM per leaflet/tree and the nutrient content of leaflets. Average nutrient content of leaflet was analysed from 3 leaflets of the same frond (pooled together) and averaged to give average nutrient per tree. RPM numbers were counted per leaflet and an average was calculated per tree. The result showed that there was a significant relationship between Phosphorus content of the coconut tree leaflets and RPM number (Multiple R-squared: 0.2493, Adjusted R-squared: 0.2117; F-statistic: 6.641 on 1 and 20 DF, p-value: 0.01800). No significant relationship was found between mite number and other nutirents (Figure 5.10). The nutrition of the coconut leaflets was investiged throughout the season and it was found that there was a signficant difference in the levels of Phosphorus in coconut leaflets throughout the season ($F=16.92$, $p<0.001$; Figure 5.11). Phosphorus levels from December and January were combined to make a new factor level 'Early' and the remaining months were combined to make a factor level 'Late'. There was a significant difference in the levels of phosphorus between these two time periods ($F=61.64$, $p<0.001$) indicating there to be a significant drop in Phhosphorus levels from February onwards (Figure 5.11). Analysis also showed that there was a significant drop in Nitrogen levels of coconut leaflets between December and January ($F=3.8915$, $p=0.02$; Tukey HSD $p=0.03$; Figure 5.11). No significant differences were observed for Calcium or Potassium throughout the season.

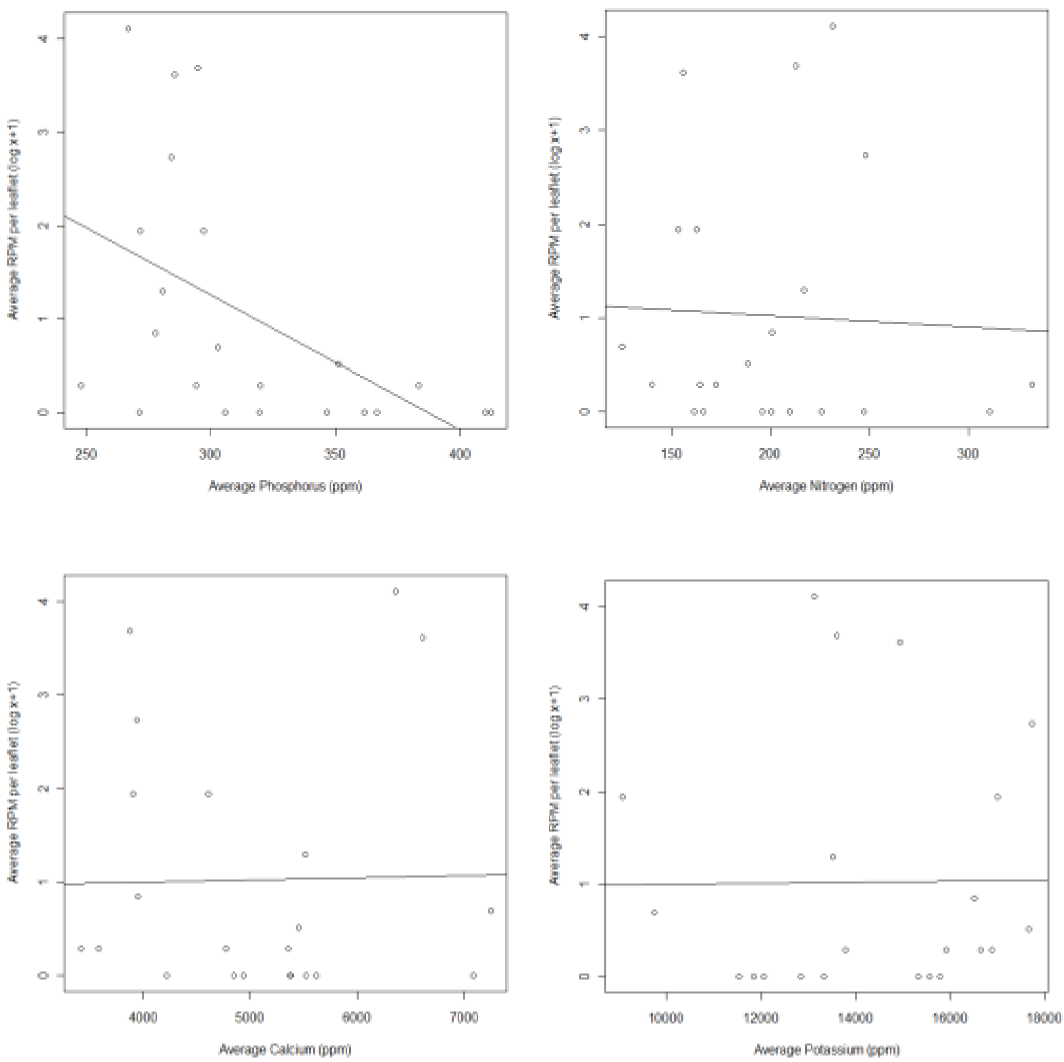


Fig. 5.10. The relationship between the levels of 4 nutrients in coconut leaflets in relation to RPM populationd (log x+1).

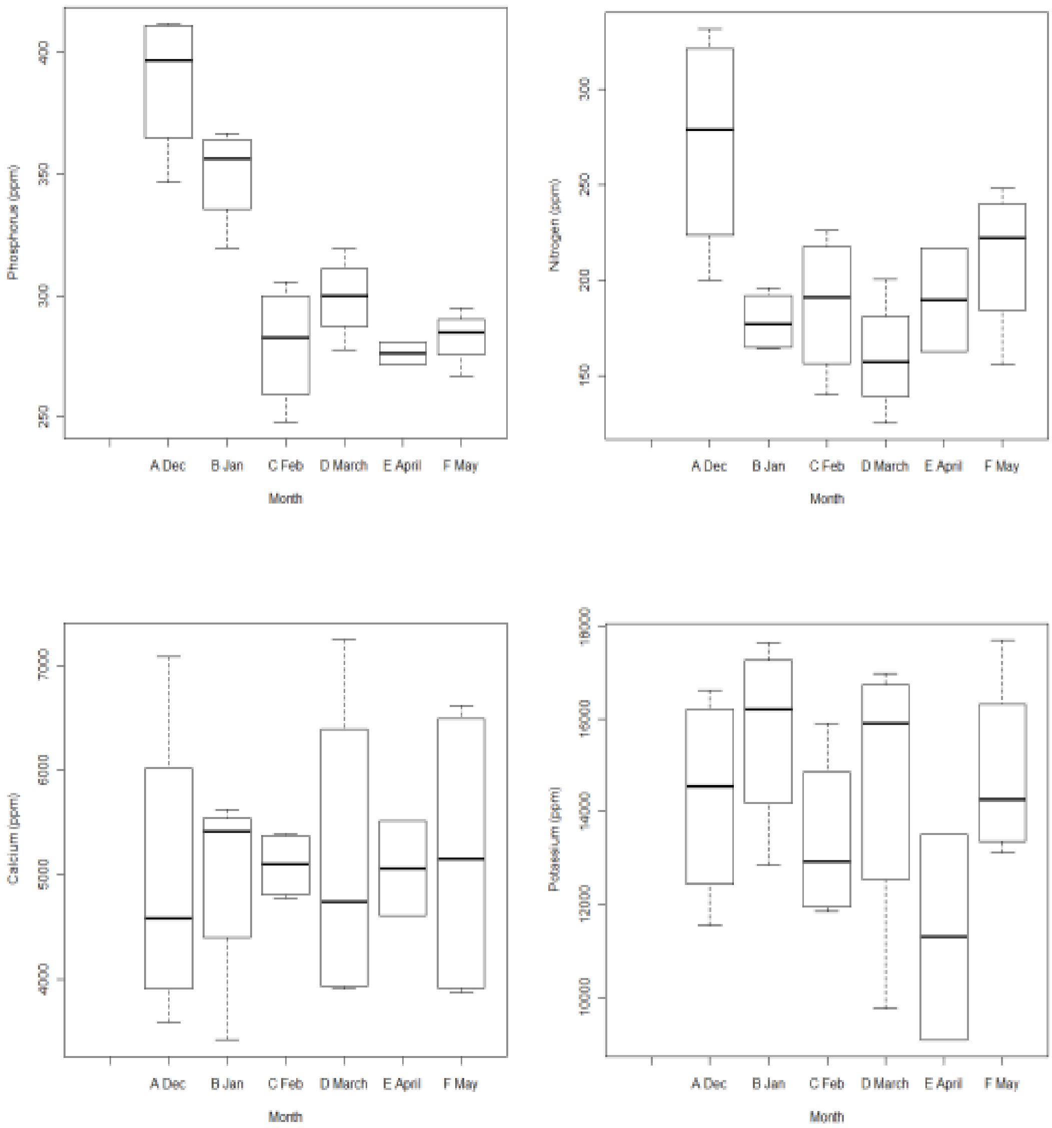


Fig. 5.11. Variation in the nutrition of coconut leaflets from December 2009-May 2010.

Validation of the wind traps

When wind traps were first deployed, they were inspected after 2 days in the field. Table 5.2 gives the details. All traps were set facing south when they were deployed and movement of the traps was observed when they were inspected after two days. Movement of the traps was also observed and adjustments made if they were not moving freely. Small particles of the size of mites were observed on the slides after two days indicating that the efficiency of the traps to catch particles of the intended size; however no mites were observed in the traps at this point in time. At the end of the study, the size range of insects and mites caught by the wind trap was analysed to ensure once again that the correct size range was being sampled (Figure 5.12). The range observed was approximately between 100-500μm. Winged arthropods were discounted as these were able to actively land in the wind trap. Adult female RPM are approximately 300μm so the range was ideal for the collection of dispersing mites. One trap (trap 1) was placed close to a coconut in site I and another one (trap 2) placed central to all palms. On 23 occasions trap 1 (Middle) caught arthropods, compared to (19 occasions where the trap next to the tree (1T) caught arthropods, therefore it was deemed the location of the trap was still as efficient when not located directly adjacent to the palm. Indeed, by placing centrally this avoided bias. Figure 5.13 shows the number of trap catches post March and there was a fairly even, if low trap catch between all sites. Traps NIV and NV were located close to each other and picked up over twice as many arthropods in general than the other sites.

Table 5.2. Wind trap inspection after 2 days in the field.

Trap Number	Trap direction when deployed	Trap direction after 2 days	Coconut tree height on site	Notes after 2 days
I M (middle of plot)	South	South-South-East	approx 4m tall	Small mite sized particles observed
I T (next to palm=1m from frond)	South	South-South-East	approx 4m tall	Small mite sized particles observed
II	South	South-South-East	approx 4-5m tall	Not many particles on slide
III	South	North-North-East	approx 3-4m tall	Possible RPM seen-later validated as red earth particles (roughly same size as RPM)
IV	South	North-North-East		No mites found

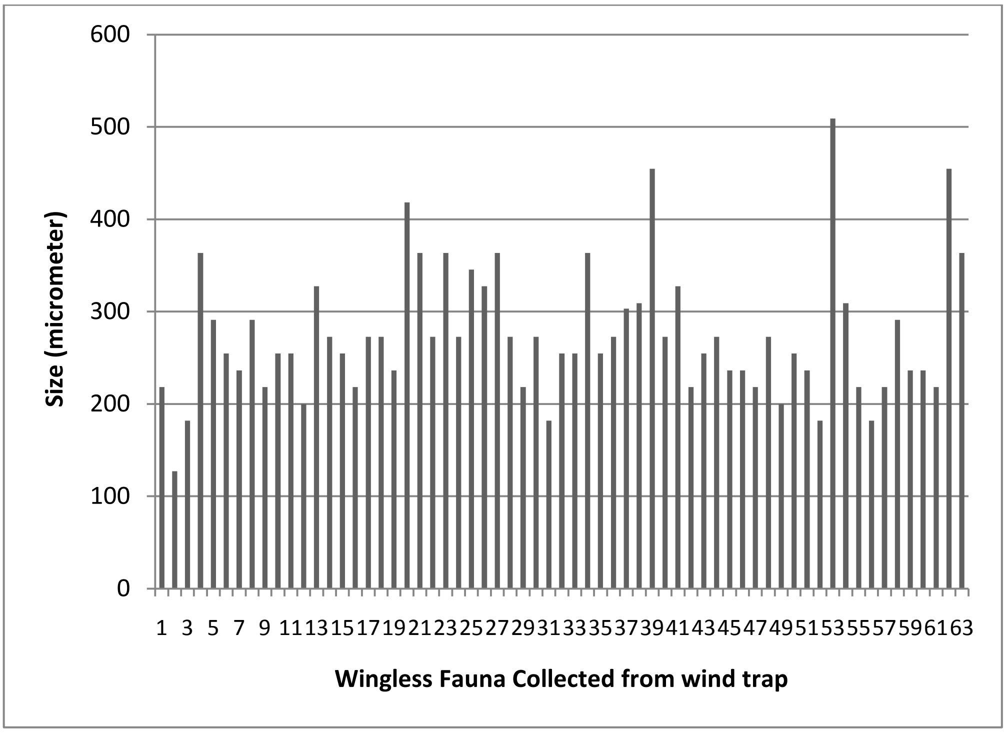


Fig. 5.12. Size range of the wingless fauna caught in the windtraps.

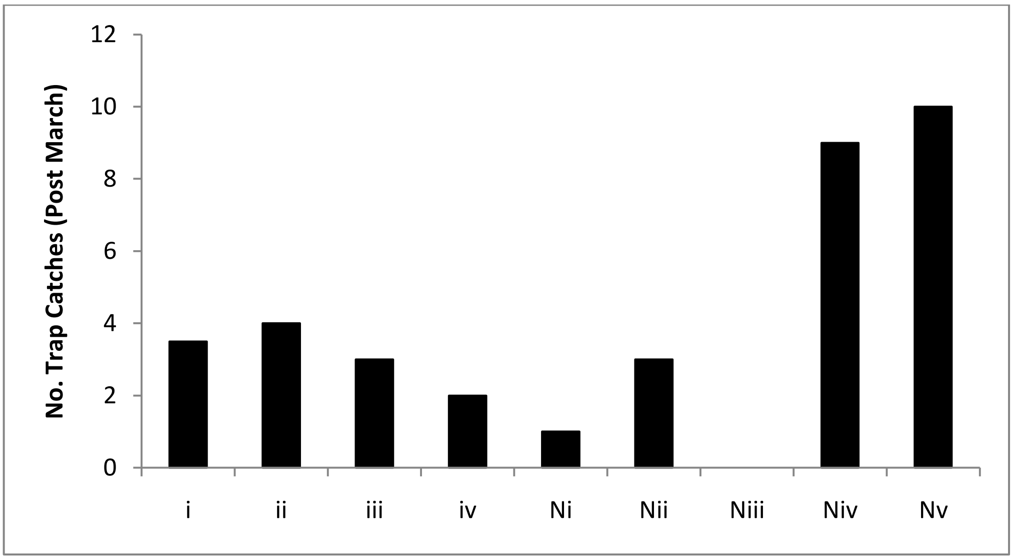


Fig. 5.13. Number of trap catches per site (post March when all traps were up and running) (i) is the average between trap next to palm (IT) and trap centrally located (IM).

The majority of mites caught in the wind trap were Tetranychids, caught in January 2010. These mites were suspected to be Identification *Oligonychus* spp, but the correct identity could not be made as the sample did not include males. Wind traps specimens were categorised into four groups: Phytoseiidae, Tetranychidae, Tenuipalpidae (RPM) and Other. Figure 5.14 shows the results of the different groups caught in the wind traps monthly. It can be seen the RPM was only caught in April and May. These correlate with when the RPM densities were at their highest on leaflets Figure 5.15. No figures on wind speed were available due to equipment failure at the local weather station.

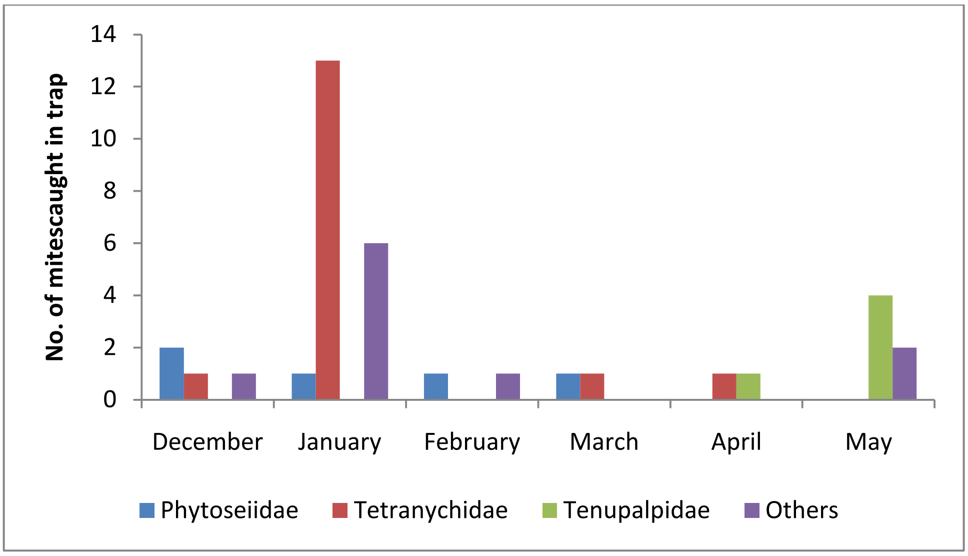


Fig. 5.14. Total number of mites caught in wind traps broken down by family, per month. The red bar represents the number of RPM caught in the trap.

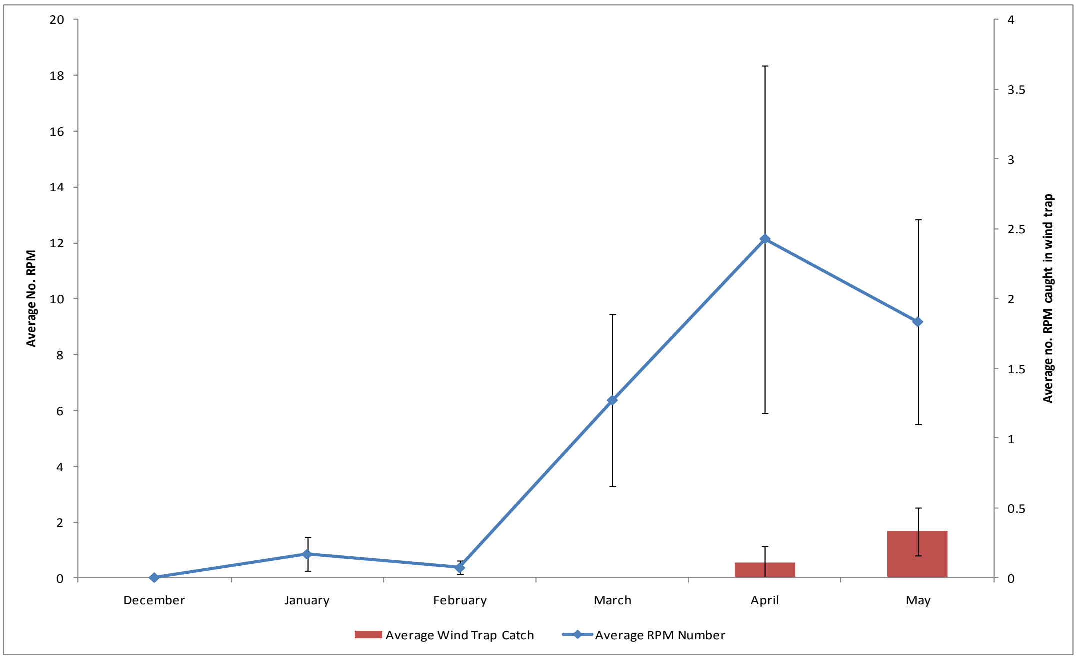


Fig. 5.15. Average no of RPM per site throughout the season from leaflet sampling and wind trap catches. The left hand axis represents the number f RPM per leaflet, the right hand axis represents the average number of RPM caught in the wind trap. (n=5 for December, January, February; n=9 for March, April, May). Standard errors shown are SE between average numbers.

The data generated from the trap have showed that RPM dispersed through the wind current. Aerial dispersal occured when the populations were dense on the tree canopy. An alternative interpretation may be that the increase in trap catches occured as RPM were more prevalent in the area therefore the chances of detecting an aerially dispersing mite is high. The data on sex ratios however can be used to interpret the dispersal phenomenon further. The presence of solitary females in a much higher abundance than solitary males indicated that females are most likely the dispersal stage. Results have also shown that the number of solitary females increased throughout the season. A possibility could be that early on in the season, the females disperse within the tree canopy and later disperse aerially as competition for space increases. This could be investigated by looking at RPM numbers throughout the canopy at different time points throughout the season. This study concentrated on sampling RPM from lower fronds. Our study showed that there were significantly more RPM found on the lower fronds of coconut palms in the same area studied in both April 2009 and May-June 2009, indicating that later on in the season, populations are mainly found on the older, lower fronds of the coconut palm.

An interesting relationship with Phosphorus content of coconut leaflets and RPM numbers was observed; whether this relationship is merely a correlation or whether the nutrient has an effect on RPM numbers is yet to be established. But reports on *Tetranychus urticae* shows the opposite effect where populations flourish with high levels of P (Wermelinger et al. 1991). High numbers of Tetranychids were found to disperse in January and it could be hypothesised that this was due to a significant drop in Phosphorous.

CHAPTER VI

Implications of the findings for classical biological control

Important factors to take into account when embarking on a classical biological control programme are the biology and ecology of both predator and prey species. This study has thrown light on the most abundant predator species and its interaction with the pest (RPM) and the abiotic factors. All these factors are of great importance when assessing the best course of action for control of an invasive pest.

Indian situation was a natural starting point for exploration for natural enemies of RPM as the mite is widely reported in the hot summer months on both coconut and areca nut. The results from the surveys showed that the most abundant predators found in association with RPM populations were the phytoseiid mites. There were high numbers of phytoseiid mites during the months of December and January but there was a significant drop in numbers after this. It was also found that the presence of the phytoseiid mites were highly correlated to rainfall the month before, and negatively correlated to RPM populations. However, laboratory data has shown that these mites do feed upon RPM. From this information it could be postulated that the predator is indeed adapted to feeding on RPM but it is not adapted to the ecozone in India and poorly synchronised. RPM on the other hand, has an abundance of suitable host plants and at times, ideal weather conditions for population expansion. It must be noted that RPM is likely to have been introduced into India along with its host plants. Although coconut and areca are both naturalised and widely grown across India, it is believed they were introduced with trade. *Areca catechu* was thought to have been transported to India prior to the 1st century AD and coconut is thought to have been present in India for at least 3000 years (Purseglove, 1972), but both are non native. Other predators were also found during our surveys such as *Stethorus keralicus*, *Jauravia* sp., cecidomyiid larvae and thrips, which however were of a more patchy distribution than the phytoseiid mites.

From a literature review, it was evident that RPM was in low abundance at this time and this was evident from the number of sites and the number of RPM recovered during the early stages of our survey. Numbers of RPM gradually increased at a number of sites throughout the survey period and population numbers are expected to rise until the onset of the monsoon, where numbers of RPM are expected to crash. The number of RPM reported between the monsoon (June) and November/December are expected to be very low, and now sites have been identified with current high levels of RPM, it would be a natural progression to continue to monitor these sites during this period. The value of these extended surveys would help us understand a) why populations crash b) where the RPM go and c) what keeps the populations levels down. We were unable to gain this level of detail on commencement of surveys in 2008 as there were no known sites with populations of RPM. The value of ongoing surveys would also allow our findings to be replicated by continuing observations into another season. Further continued observation on the population dynamics of RPM in comparison to the phytoseiid mite will give us a greater understanding as to what controls RPM populations.

From this study it appears that a combination of biotic (predators) and abiotic factors combined keep populations of RPM at lower level in general. The most promising predator for further study as part of a CBC programme appears to be the phytoseiid mites. Suggested areas of future study are listed below, as although the results of this study give a good indication of the most appropriate natural enemies to pursue, it also raises more questions that need to be addressed as part of a CBC programme.

1) Study the population dynamics of both pest and predator species throughout the monsoon season-whether the epizootics occurs when humidity is higher?, Whether predators are more abundant during the wet season?, Do RPM get washed off from leaves or move to different location on palm?
2) Population dynamics of Phytoseiid mites (the most abundant predator) are not climatically matched to RPM populations. This needs to be further investigated.
3) Carry out extended laboratory investigations to study the feeding behaviour of phytoseiid mite.
4) Further investigations look into the abiotic factors affecting the population dynamics of RPM.

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Appendix 1. List of other natural enemies collected during the study

Sl. No.	Leaf ID	Location/Date	Family/Suborder	Species	Remark
1	9b	Nilambur/Jan 2009	Phtytoseiidae	*Amblyseius* sp. 1	*A.largoensis* species group
2	14a	Nilambur/Jan 2009	Phtytoseiidae	*Amblyseius* sp. 1	*A.largoensis* species group
3	20a	Nilambur/Feb-09	Phtytoseiidae	*Amblyseius* sp. 2	Near *A.largoensis*
4	18c	Palakkad/Nov 2009	Phtytoseiidae	*Amblyseius* sp. 2	Near *A.largoensis*
5	13c	Peechi/Jan 2009	Phtytoseiidae	*Euseius ovalis*	Associated with spider mite
6	6b	Nilambur/Jan 2009	Phtytoseiidae	*Amblyseius* sp. 1	A.largoensis species group
7	14c	Palakkad/Jan 2009	Phtytoseiidae	*Amblyseius* sp. 2	Near *A.largoensis*
8	14c	Palakkad/Jan 2009	Phtytoseiidae	*Amblyseius* sp. 2	Near *A.largoensis*
9	14c	Palakkad/Jan 2009	Phtytoseiidae	*Amblyseius* sp. 2	Near *A.largoensis*
10	9a	Nilambur/Dec 2008	Oribatida		
11	14c	Palakkad/Jan 2009	Phtytoseiidae	*Amblyseius* sp. 2	Near *A.largoensis*
12	17c	Palakkad/Jan 2009	Phtytoseiidae	*Amblyseius* sp. 2	Near *A.largoensis*
13	14c	Peechi/Jan 2009	Phtytoseiidae	*Euseius ovalis*	
14	14c	Peechi/Jan 2009	Phtytoseiidae	*Euseius ovalis*	
15	12c	Peechi/Jan 2009	Phtytoseiidae	*Euseius ovalis*	
16	12c	Peechi/Jan 2009	Phtytoseiidae	*Euseius ovalis*	
17	12c	Palakkad/Jan 2009	Phtytoseiidae	*Amblyseius* sp. 1	
18	12c	Peechi/Jan 2009	Phtytoseiidae	*Euseius ovalis*	
19	5c	Palakkad/Jan 2009	Phtytoseiidae	*Amblyseius* sp. 2	Near *A.largoensis*
20	20c	Palakkad/Jan 2009	Phtytoseiidae	*Amblyseius* sp. 3	Near A.largoensis
21	4a	Kunnamkulam/Jan 2009	Phtytoseiidae	*Euseius ovalis*	
22	20a	Nilambur/Dec 2008	Phtytoseiidae	*Typhlodromis* sp.	
23	5a	Kunnamkulam/Jan 2009	Phtytoseiidae	*Euseius ovalis*	
24	5a	Nilambur/Jan 2009	Phtytoseiidae	*Amblyseius* sp. 1	*A.largoensis* species group
25	7a	Nilambur/Jan 2010	Phtytoseiidae	*Amblyseius* sp. 1	*A.largoensis* species group
26	1c	Nilambur/Dec 2008	Phtytoseiidae	*Amblyseius* sp. 1	*A.largoensis* species group
27	1c	Nilambur/Dec 2008	Phtytoseiidae	*Amblyseius* sp. 1	*A.largoensis* species group
28	9c	Palakkad/Jan 2009	Phtytoseiidae	*Amblyseius* sp. 3	*A.largoensis* species group
29	12c	Palakkad/Jan 2009	Phtytoseiidae	*Amblyseius* sp.	
30	20c	Palakkad/Nov 2008	Oribatida		
31	15a	Nilambur/Jan 2009	Oribatida		
32	15c	Palakkad/Nov 2008	Tetranychidae		
33	15c	Palakkad/Nov 2008	Oribatida		