BIOECOLOGY OF THE MANGO MEALYBUG, *RASTROCOCCUS ICERYOIDES* GREEN (HEMIPTERA: PSEUDOCOCCIDAE) AND ITS ASSOCIATED NATURAL ENEMIES IN KENYA AND TANZANIA

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DECLARATION

This thesis is my original work and has not been presented for a degree in any other University

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DEDICATION

This thesis is dedicated to my beloved mother, Mrs Tanga Mary Ewoh and to the memory of my late father, Mr Tanga Andrew Apeh (R.I.P) whose love, support and guidance made me what I am today. And to my lovely wife, Mrs Tanga Janice Ghemoh and daughter, Tanga Emely Febeng-Anong who has stood together amidst life’s tough hurdles. While it was difficult to work at a time you were all awake, you have filled my life with purpose and made it so meaningful that each passing day with you around me brings great joy and happiness to my heart.

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ABSTRACT

Rastrococcus iceryoides Green (Hemiptera: Pseudococcidae), an alien invasive mealybug pest of Asian origin was first detected in Tanzania in 1989. This pest rapidly spread by the mid-1990s and was soon present in Coastal Kenya and Northern Malawi, where it has been regarded and remains a major pest of mango. Because of its novelty status, there was no information on its biology, ecology and its natural enemies that could aid development of management efforts. This study, therefore, was initiated to establish the bioecology of *R. iceryoides* and its natural enemies in Kenya and Tanzania, and to explore for efficient co-evolved natural enemies in the aboriginal home of the pest in India. Based on the exploratory survey data, two correlative approaches, Desktop-GARP (Genetic Algorithm for Rule-set Prediction) and Maxent (Maximum entropy) were used to identify climatically suitable areas in Africa that are agro-meteorologically similar to the aboriginal home of the pest. The first step was to carry out a countrywide survey in Kenya and Tanzania to establish the distribution, host-plant relationship and natural enemies of this pest. The survey revealed that *R. iceryoides* infested twenty-nine plant species particularly *Mangifera indica* L. and *Cajanus cajan* (L.) Millspaugh, and the wild plants *Parkinsonia aculeata* L., *Caesalpinia sepiaria* Roxb, and *Deinbollia borbonica* Scheft. A total of six primary parasitoid species were recovered from *R. iceryoides* with *Anagyrus pseudococci* Girault (Hymenoptera: Encyrtidae) predominating. Thirty-eight species of predators belonging to 14 families were also recorded. Despite the presence of these indigenous natural enemies, their ability to regulate the population of *R. iceryoides* was inadequate. In laboratory host preference studies, *M. indica*, *Cucurbita moschata* Duchesne, *P. aculeata* and *C. cajan* were found to be the most preferred host plants in view of improving laboratory mass rearing of this pest and the parasitoid. The impact of *O. longinoda* on the biological control activities of *A. pseudococci* in the laboratory revealed that percentage parasitism of *R. iceryoides* by *A. pseudococci* was significantly higher on ant-excluded trials than on ant-attended trials. Worker ants were observed to remove mummified mealybugs, which resulted in significantly reduced percentage of adult parasitoid eclosion. *Oecophylla longinoda* showed aggressive behaviour and caused a significant mortality of *A. pseudococci* during the exposure period. The spatial and temporal population dynamics of this pest was also studied and revealed that populations of *R. iceryoides* followed an annual cycle which is synchronized with the mango fruiting season, with a peak incidence occurring during the dry season (December to February) on all plant parts. The population dynamics of *R. iceryoides* and its natural enemies were significantly and positively influenced by temperature, while it was significantly and negatively correlated with rainfall. The exploratory survey in India showed that *R. iceryoides* is widely distributed throughout the state of Tamil Nadu and infested ten cultivated and wild plant species with extremely low levels of infestation. Percentage parasitism based on the proportion of mummified *R. iceryoides* was high on all host plants. Out of eleven primary parasitoid species, *Praleurocerus viridis* Agarwal (Hymenoptera: Encyrtidae) and *Anagyrus chryos* Noyes & Hayat (Hymenoptera: Encyrtidae) were the most dominant and widely distributed species. In addition to the parasitoids, 10 predator species from 7 families were recorded. Based on the model established with data from India, it was determined that climatically suitable areas for introduction of promising parasitoids in Africa include the humid tropical coastlines of Kenya and Tanzania, as well as some restricted areas in West and Central Africa. Studies of the potential worldwide distribution of *R. iceryoides* showed that the pest might poses a serious threat on a worldwide scale as it could narrowly become established in all the mango producing countries in the continents.

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CHAPTER ONE

General Introduction

1.1 Background information

Horticulture is recognized for its potential to become one of the major sources of income generation for both small and large-scale farmers in Kenya and Tanzania; creating job opportunities and improving diet by providing essential micronutrients and vitamins (FAO, 2004; HCA, 2010). Therefore, attention on horticulture had been accorded high priority in national development plans of most of the countries in East Africa over the last 25 years. Among the horticultural products grown in Kenya and Tanzania; mango, *Mangifera indica* Linnaeus (Anacardiaceae) is among the most widely grown tropical fruit and over 80 % of the produce from East and West Africa comes from smallholders for both domestic urban (Figure 1.1) and export markets (Figure 1.2) of which the European Union (EU) is the major export destination (ICIPE, 2006). Mangoes play an integral part in rural household lives not only by being rich nutrient source but also serving as a common good that is consumed casually. In Tanzania, Kenya and Malawi mango production is consumed locally contributing to food security. In Africa, particularly in rural areas mango serves principally as a complementary food to populations during the dry-season when staple crops are not produced and food reserves have dwindled (Figure 1.3). This crop provided the most freely available fruit energy and vitamin A and C sources, especially valuable for children in a part of the world where up to 20% of infants die before the age of five (Moore, 2004). Overall, mango plays an important role in food security and nutritional quality (i.e., rich source of vitamin A & C, fibre and potassium, and provide more of the anti-oxidant beta-carotene than any other fruit), and in poverty alleviation.

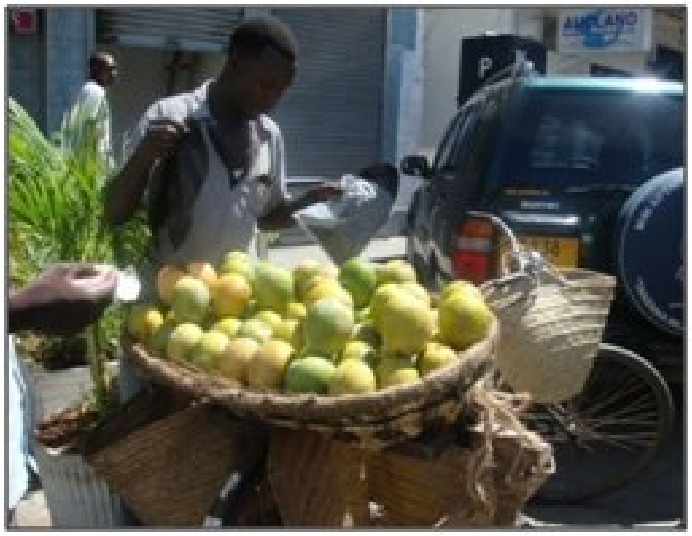


Figure 1. 1: Smallholder farmer selling mangoes in the city streets of Dar es Salaam

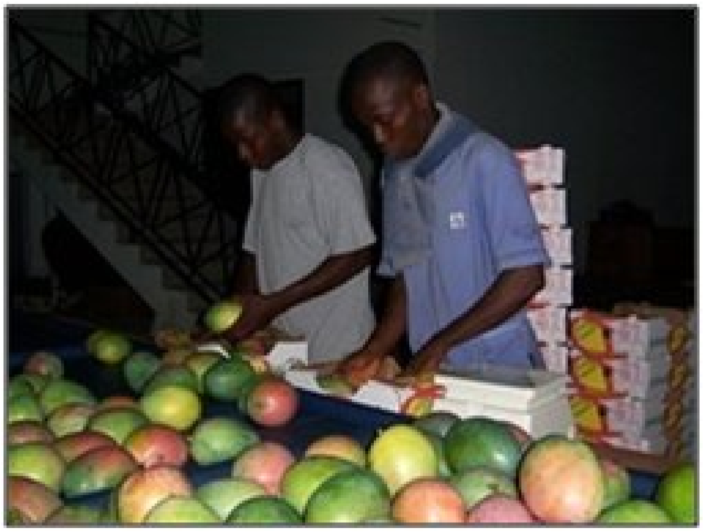


Figure 1. 2: Employees of a mango-packing plant prepare mongoes for export.

Figure 1. 3: Children in a rural village of Zimbabwe consuming mango as a complementary food during the dry-season as staple food reserves dwindle

This locally grown fruit is gaining recognition as an important source of income and foreign exchange; however, increased production in tropical sub-Saharan Africa is limited by many biotic and abiotic contraints. The major biotic constraints are limited access to markets, unavailability of planting materials, poor rural road infrastructure, inadequate air freight transport services for exports, inadequate availabilty and high cost of inputs, shortage of skilled technical expertise, unreliable electricity supplies hence effect on production and inability to comply with various international standards (Wessel, 1997; RTA, 2008). While among the biotic factors, heavy infestation by a range of pests is the most important. Mango mealybug species of the genus *Rastrococcus* are among one of the most destructive pests on mango in Africa, which in addition to other invasive devastating pests such as fruitflies hinder the mango sector from realizing its full potential in the sub-region by posing serious threat to the exploitation of foreign markets, as such helped in jeopardizing the lucrative trade in fresh fruits from the region.

Among the mealybug pest in Africa, *R. invadens* (Figure 1.4) and *R. iceryoides* (Figure 1.5) are regarded as the two most important exotic mealybug species native to Southern Asia that commonly colonize mango (Williams, 2004). *Rastrococcus invadens* Williams (Hemiptera: Pseudococcidae) was first detected in the 1980s along the coast regions of Benin and Togo (Williams, 1989), where it rapidly spread by the mid-1990s and was soon present in several other countries of West and Central Africa namely Sierra Leone, Côte d'Ivoire, Ghana, Nigeria, Cameroon, Gabon, Congo, Burkina Faso, Mali and the Democratic Republic of Congo (Moussa and Matile-Ferrero, 1988; Boussienguent and Herren, 1992; Agounkè et al., 1988). It's continued spread threatened fruit production in the neighbouring countries infesting a wide range of cultivated plants particularly mango, citrus, breadfruit [*Artocarpus altilis* (Parkinson) Fosberg], guava (*Psidium guajava* Linnaeus) and banana (*Musa* spp.), and ornamentals including Oleander (*Nerium oleander* Linnaeus), frangipani and roses (*Rosa* spp.) as well as several other wild host plants (Agounkè et al., 1988; Biassangama et al., 1991). Consequently, it represented a new major threat to Africa's huge potential for commercial horticulture necessary for both the export and domestic markets because of its devastating effects. For example, in Côte d'Ivoire, *R. invadens* appeared in 1989 at the eastern border of the country and, in less than four years became a major constraint to fruit production nation-wide (Hala et al., 2004). By 1996 the mango mealybug had reached the northern region, the main area for export mango production. It was shown that 53% of mango yield losses occurred as the result of *R. invadens* infestations in Korogho-Lataha research station. When yield losses reached 100% in some farms, farmers frequently responded by cutting down and burning of all infested trees in the orchards or sprayed with synthetic chemicals, which showed that the presence of *R. invadens* caused a degree of panic in growers (Agounké et al., 1988 and Vögele et al., 1991). On average, the infestation rates reached 82, 36 and 11% respectively in the cities, villages and orchards. Losses of mango yield attributed to *R. invadens* infestation in Côte d'Ivoire varied from 53 - 100% reduction of total production depending on the variety, the period of harvest, the site of the orchard and the region (Hala et al., 2004; Hauled, 2001). In Ghana, yield losses of up to 80% of mango yield due to *R. invadens* have been reported (Entomological Society of Nigeria, 1991).

Several authors also reported significant reduction in weight and size of fresh mango fruits in Nigeria, Togo and Benin (Ivbijaro and Udensi, 1988; Ivbijaro et al., 1991, Tobih et al., 2002). The insect affected the morphology and physiology of infested trees causing delays in flowering, fall of spikes and leaves and slowing the emission of new branches. The value of mango shipments rejected at European ports because of *R. invadens* infestations reached 200 million francs CFA in 2001 (AU, 2009). In Guinea *R. invadens* was first observed in 2000 and later confirmed by the International Institute for Tropical Agriculture (IITA). Initially localized in one region, the pest rapidly infested the entire country with heavy infestations levels causing a negative economic impact on producers and traders of this commodity. Although the rates of infestations were most important in urban areas than in orchards, the economic and social strain on farmers was greater given the importance of the revenue of mango production, trade and consumption on farmers' income and welfare.

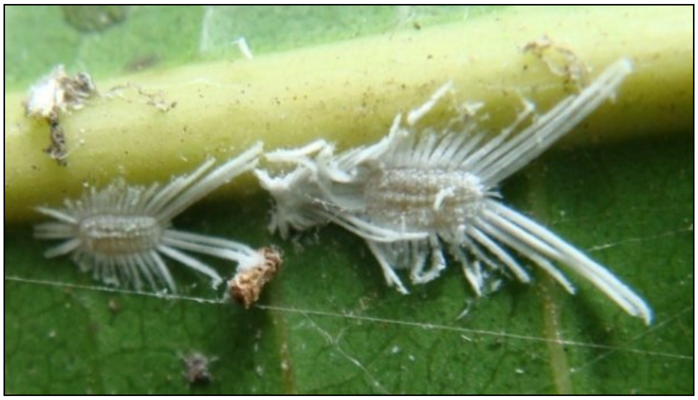


Figure 1. 4: *Rastrococcis invadens* on the abaxial surface of a leaf

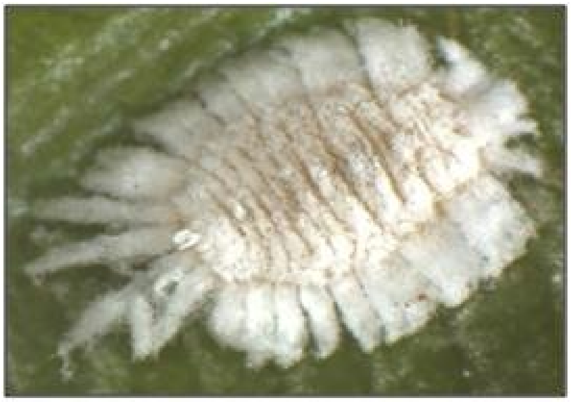


Figure 1. 5: *Rastrococcus iceryoides* on the abaxial surface of a leaf

Worldwide, mealybugs constitute one of the major threats to horticultural production, causing heavy pre-harvest and post-harvest losses and curtailing expansion of both domestic and international trade of fruits (Osman and Chettanachitara, 1989). Mealybugs are phytosanitary pests in some export markets (USA, Japan) and if found on fruit destined for these markets can result in rejection of the consignment or rerouting or sold locally with significant economic implications as this could seriously jeopardize the viability or place these important markets at risk for the future (Pieterse et al., 2010). In the tropics, the problem is aggravated by the prevailing humid warm climate, which is conducive for overlapping fruiting patterns, resulting in overlapping generations of mealybug populations and the potential for year round infestation, though at varying degree of severity. Other than *R. invadens*, which received attention because of its pest status, *R. iceryoides* is probably the best-known of the genus (Moore, 2004). Therefore, the arrival of the alien *R. iceryoides* on the continent will further aggravate this problem in East Africa where it is localized.

In Kenya and Tanzania, where *R. iceryoides* is widely distributed across several agroecological zones, it has become a major target for insecticidal sprays on mango, in addition to pruning and burning of infested plant parts (C. Tanga, unpub.; Willink and Moore, 1988). Apart from health and environmental hazards caused by chemical pesticides, pesticide applications do not generally provide adequate control for mealybugs in the long-term owing to their cryptic behavior, their typical waxy body cover, and clumped spatial distribution pattern (Franco, 2009). The eggs of *R. iceryoides* are laid into an ovisac made up of a knotted mass of long waxy filaments, which also prevents penetration of water-based sprays. It is almost impossible to have a spraying program that can bear the cost and cope with the practicalities of treating the whole range of infested plants in an affected area (Sagarra and Peterkin, 1999). Moreover, repeated insecticide use, especially broad-spectrum chemicals has been reported to adversely impacts on mealybug natural enemies (Walton and Pringle, 1999). However, extensive use of these chemicals has also favoured insecticide resistance in mealybug (Flaherty et al., 1982; Myburgh and Siebert, 1964) and has caused the use of some chemicals to be unsustainable due to heightened concerns over health and environmental impact. For example, many of these products are increasingly unacceptable because of their human toxicity and low selectivity; some are no longer available and others are targeted for reduction under national programs and regulations for sustainable use of pesticides, in light of their risk or hazard assessments (Charles et al., 2006; Franco et al., 2004b; Walton et al., 2006). Furthermore, these chemicals are expensive to horticultural farmers in Africa who mainly practice subsistence farming. These setbacks motivated the search for alternative mealybug control strategies that are more environmental benign. For example, *R. invadens* was successfully brought under control by two parasitoid wasps, *Gyranusoïdea tebygi* Noyes (Hymenoptera: Encyrtidae) and *Anagyrus mangicola* Noyes (Hymenoptera: Encyrtidae) by the end of 1990s (Bokonon-Ganta et al., 2002). The parasitoids were found in India and *G. tebigy* was first introduced in Togo in 1987 by the CAB International Institute of Biological Control in a project sponsored by the Deutsche Gesellschaft für Teschnische Zusammenarbeit (GTZ) and the Food and Agriculture Organization (FAO) (Agounkè et al., 1989). In Benin, a survey among mango producer over a large area estimated that the biological control program allowed farmers to gain on average US$ 328 annually. This amounted to an estimated net yearly gain of $US 50 million for the whole country when extrapolated to all farmers of Benin (Bokonon-Ganta et al., 2001). Unfortunately this biological control programme was discontinued while the pest is invading new areas.

Compared with *R. invadens*, very little is known with regard to the bioecology of *R. iceryoides*. The management of *R. iceryoides* will also require various sustainable methods of which biological control with natural enemies is most recommended and although several natural enemies are known to attack *R. iceryoides* in its aboriginal home in Southern Asia, none have been introduced so far into Africa. It is based on this background that the current research was developed and seeks to assess the geographical distribution, abundance, host plant-relationships and associated natural enemies of this exotic pest giving its rising economic importance, which are major precondition to effective management of the pest. Therefore, *R. iceryoides* being an alien species is an excellent candidate for classical biological control. The immediate beneficiaries of this research will be the smallholder mango growers in Africa particularly in Kenya and Tanzania. Smallholders will benefit from improved increased production and access to export markets due to high quality mango products.

1.2 Justification

Since the first report of *R. iceryoides* in East Africa (mainly Tanzania and Kenya), the mealybug has become established in the northern part of Malawi (Williams, 1989; Luhanga and Gwinner, 1993; CABI, 2000) where it has remained as a major pest of mango causing alarming yield losses and potentially poses a threat to numerous other horticultural products. Given the fact that most mealybug species have the ability to disperse over long distances, there is a great likelihood that the pest may spread further. The informal movement of plant materials between very porous African borders by many travelers and/or major agricultural projects such as development of horticulture or re-afforestation programs; and general lack of strict quarantine regulations may also facilitate the spread of this pest to new areas. Also, the regional integration of several African countries into Customs Unions allows free movement of all kinds of agricultural commodities that further increases the risk of spread.

Rastrococcus iceryoides is a pest that is new on mango in Africa and was only described in the 1989 (Williams, 1989). There is currently a paucity of information on various aspects of its bioecology that might aid in the management of the pest. As a result of *R. iceryoides*' exotic origin, polyphagy, rapid rate of reproduction and spread, biological control with natural enemies remains the most recommended and cost-effective option. This is technically possible as demonstrated in West Africa for *R. invadens*, which is a member of the same genus as *R. iceryoides*, though at tremendous cost (Agricola et al., 1989; Matokot et al., 1992; Neuenschwander et al., 1994, Boavida et al., 1995). However, the scale of distribution of *R. iceryoides* at present, poor quarantine and limited knowledge of potential cultivated and wild hosts and lack of resources in Africa suggests that suppression may be difficult, if not impossible. The most practical strategy, therefore, is the development of appropriate management measures that are suited to the local conditions in Africa and sustainable. Therefore, as part of the ongoing efforts to manage this pest, the African Fruit Fly Program of *icipe* is developing and testing a range of integrated pest management (IPM) technologies that are adaptable to the region. However, such strategies can best be utilized if the bioecology of the pest is well documented.

Therefore, this study aims at understanding the bioecology of *R. iceryoides* as part of a wider strategy to develop sustainable management strategies for the pest. The host plants range of *R. iceryoides* is documented and the suitability of different host plants to the pest as relevant to the development of mass rearing procedures and efficacy of its primary indigenous parasitoid (*Anagyrus pseudococci* Girault) has been established. Population dynamics studies have been undertaken. Considering the fact that this pest is alien to the African continent the likelihood of identifying efficient natural enemies within Africa is slim. Therefore, the study also explored for co-evolved natural enemies of *R. iceryoides* in its putative aboriginal home, India as part of a wider long-term sustainable program for the management of *R. iceryoides*. Lastly, the role of *Oecophylla longinoda* in the biological control of *R. iceryoides* by *A. pseudococci* has been determined.

1.3 Hypotheses

The following hypotheses were tested;

- *Rastrococcus iceryoides* is the most polyphagous, widespread and devastating mealybug of mango in Tanzania and Kenya, with no effective indigenous natural enemies.
- There is a huge diversity of effective natural enemy complex in the aboriginal home of the pest, India.

1.4 Objectives of the study

1.4.1 General objective

The overall objective of this study is to determine the bio-ecology of *R. iceryoides*, assess the role of indigenous natural enemies in suppressing the pest; and explore for natural enemies in its aboriginal home for introduction and release in target African countries.

1.4.2 Specific objectives

The following specific objectives were addressed;

- Assess the distribution, host plant relationships of *R. iceryoides* and its associated natural enemies in Kenya and Tanzania
- To assess the role of host plants on the development, survivorship and reproduction of *R. iceryoides*
- To assess the effects of host plants on the biological parameters of *R. iceryoides* and its key parasitoid, *A. pseudococci* under laboratory condition
- To assess the interaction between *Oecophylla longinoda*, *R. iceryoides* and *A. pseudococci* under laboratory conditions
- To establish the seasonal and annual dynamics of *R. iceryoides* and its associated natural enemies in two major mango growing areas in the Coast region of Tanzania.
- To explore for natural enemies of *R. iceryoides* in its aboriginal home and establish a climate matching to guide introduction of promising parasitoids into Africa.

CHAPTER TWO

Literature Review

2.1 MANGO

2.1.1 Origin and Distribution

Mango, *Mangifera indica L.,* one of the most celebrated of tropical fruits, is a member of the family Anacardiaceae–notorious for embracing a number of highly poisonous plants (Morton, 1987). Native to southern Asia, especially eastern India, Burma, and the Andaman Islands, mango has been cultivated, praised and even revered in its homeland since ancient times (Morton, 1987). Buddhist monks are believed to have taken the mango on voyages to Malaysia and eastern Asia in the 4th and 5th Centuries B.C. The Persians are said to have carried it to East Africa about the 10th Century A.D. (Morton, 1987). It was commonly grown in the East Indies before the earliest visits of the Portuguese who apparently introduced it to West Africa early in the 16th Century and also into Brazil (Morton, 1987). After becoming established in Brazil, the mango was carried to the West Indies, being first planted in Barbados about 1742 and later in the Dominican Republic. It reached Jamaica about 1782 and, early in the 19th Century, reached Mexico from the Philippines and the West Indies (Morton, 1987). The original wild mangoes were small fruits with scant, fibrous flesh, and it is believed that natural hybridization has taken place between *M. indica* and *M. sylvatica* (Roxb.) in Southeast Asia (Morton, 1987). Selection for higher quality has been carried on for 4,000 to 6,000 years and vegetative propagation for 400 years (Morton, 1987). Important mango varieties include Kent, Keitt, Tommy Atkins mango, Haden mango and Ataulfo mango (www.freshmangos.com).

2.1.2 Mango production and international trade

A large number of mango varieties are commercially grown in different parts of the world, some with determinate and others with indeterminate growth pattern. Within international trade, fresh mango is one of the main products. It possesses a fifth place on total fruit crop production globally (Tharanathan et al., 2006), accounting for over one-third of the worldwide production on tropical fruits (Maneepun and Yunchalad, 2004). Mangoes are grown on all continents (Galán Saúco, 2004), with at least 87 countries reported to be involved in mango production by the year 2000 (Galán Saúco, 2004; Tharanathan et al., 2006). Around 25 million tons were grown in 2000 (Galán Saúco, 2002; Galán Saúco, 2004; Maneepun and Yunchalad, 2004) of which three-quarters in Asian countries. India is by far world leader with almost half of the global mango production, however exports only a very small amount of this. Mexico, Pakistan and the Philippines are the most important exporters for fresh mangoes with 41%, 7.6% and 7.8% of the global supply respectively (Galán Saúco, 2002; Galán Saúco, 2004). International trade in mango has risen significantly by the end of the twentieth century (Galán Saúco, 2004), enabled by improved post-harvest techniques (Maneepun and Yunchalad, 2004). Over a million tons were traded in 2006 (FAOSTAT). Large markets for fresh produce are the EU, North-America and Asia (Galán Saúco, 2002; Galán Saúco, 2004).

In Africa, Nigeria produces the largest amount of mangoes on the continent and occupies the 8th position in the world ranking of mango producing countries as at 2002, producing 730,000 metric tonnes annually (FAO stat cited by Yusuf and Salau, 2007) followed by Kenya in the 9th position but ranks second in terms of exports after South Africa. Common varieties grown in Kenya and Tanzania include Apple, Baribo, Dodo, Haden, Keitt, Kent, Van Dyke, Tommy Atkins, Ngowe, Sensation and local landraces. Apple and Ngowe varieties have high demand by the export market sub-sector. Other major mango producing countries on the continent include Sudan, Egypt, Madagascar and Tanzania (Yusuf and Salau, 2007). According to data presented at the FAO Inter-Governmental Sub-Group on tropical fruits, mango exports from Africa were estimated at 35-40,000 tons annually and worth over USD 42 million annually (ICIPE, 2006; Lux et al., 2003). The EU is the largest destination market for mangoes exported from Africa, followed by the Middle East (Lux et al., 2003). Both fresh and processed mango are been exported to the European countries with France as the major importer followed by the United States. Both account for 70% of the world mango import (Yusuf and Salau, 2007). In East and West Africa, over 80% of the produce comes from smallholders for both domestic and urban export markets (ICIPE, 2006).

Despite the substantial increase in mango exports in recent years, the share of all African suppliers to European markets remains below 20% (ICIPE, 2006) as several factors constrain mango production, among which insect pests are regarded as among the most important (Acland, 1971; Griesbach, 1992; Joubert et al., 2000; Varela, et al., 2006; ICIPE, 2006). Heavy losses are also being incurred by exporters whose mango shipments infested with these quarantine pests are intercepted and destroyed at the entry of EU markets because when insect pests are encountered on fruit, they must be identified before the fruit can be shipped. Until such time as identification can be made, the fruit is held in cold storage at a large cost to both the producer and the shipper.

If the insect dies or the specimens are damaged, no identification can be made; therefore the consignment must be destroyed or sold locally at a reduced rate. This greatly affects the revenue and reduces the profits of the smallholder grower and the traders and contributes to high mango production cost. For example, mango exports from some West African countries have even been banned from entering EU markets and the United States due to insect pest damage (Van Mele et. al., 2007) with substantial impact on producers' income reported with grave consequences on local trade, food security and export potential.

2.2 Mealybug

2.2.1 Taxonomic position, bioecology, host range and distribution of *R. iceryoides*

Rastrococcus iceryoides belongs to the class Insecta, order Hemiptera, suborder Sternorrhyncha, superfamily Coccoidea and to the family Pseudococcidae. It is the type species of the genus *Rastrococcus* and was first described by Green in 1908. Ferris (1954) gave detailed descriptions and illustrations. In his revision of the genus *Rastrococcus*, Williams (1989) notes that this species possesses major characters not found in any of the other 22 species of the genus and hence has assigned it to a separate species group. Narasimham and Chacko (1988) observed and described four main types of variation in colour and pattern of wax on the ovisac. These varieties co-exist on mangoes and retain these characters when bred on pumpkin. Williams (1989), however, notes that slide preparations of these specimens appear to be identical and, at present, there is no suggestion that they represent different species. Validation characters include the presence of an anterior ostioles; cerarii with more than 5 truncate setae; cerariion anterior thorax and head coalesced; with long dorsal setae adjacent to anal ring; antennae 9-segmented; quinquelocular pores present on venter; denticle on claw. Other species in the genus *Rastrococcus* lack anterior ostioles and have short setae near the anal ring.

Rawat and Jakhmola (1970) and Pramanik and Ghose (1991) have given an account of the bionomics of *R. iceryoides* while Dikshith (1966) studied the cytology. The adult male has one pair of wings, well-developed limbs, lacks mouthparts and lives only a few days. The female lays eggs only after fertilization. The pre-oviposition period lasts 7-8.5 days and the oviposition periods last for 5.7-7.3 days. About 450-585 eggs are laid in a white, waxy ovisac. On potato sprouts, fecundity averaged 807.8 eggs/female and the oviposition period averaged 18.2 days. However, starvation reduced the fecundity by 82.18% and the oviposition period by 58.24%. The average incubation period was 6.6 days. Female and male nymphs moult three and four times, respectively, to become adults. The average duration of post-embryonic development was 20.4-31 days in females and 18-26 days in males. On potato sprouts, the nymphal period is reported as 24.77 days in females and 25.41 days in males.

Rastrococcus iceryoides is one of the most polyphagous species of the genus *Rastrococcus*, occurring on plants belonging to diverse botanical families. Ramachandran and Ayyar (1934) have noted it on 15 host plants belonging to eight botanical families. Ali (1970) has listed over 40 plants as hosts of *R. iceryoides*. Narasimham and Sankaran (1985) note 17 host plants belonging to 10 botanical families. Williams (1989) and Ben-Dov (1994) have studied specimens of *R. iceryoides* from over 65 different host plants from 31 plant families. Both adult and immature stages are highly pestiferous and have a strong preference for mango. The most frequent host plants are: *M. indica*, *Gossypium hirsutum* L. (Bourbon cotton), Citrus, *Citrus aurantiifolia* L. (lime), Coffea (coffee), *Coffea canephora* Pierre (Congo coffee tree), *Coffea arabica* L. (coffee (arabica)), *Theobroma cacao* L. (cocoa), *Albizia lebbeck* (L.) Benth (Indian siris), *Gossypium* sp. (cotton) (Williams, 1989).

Williams (1989) noted that *R. iceryoides* is known throughout most of southern Asia and is one of the most widespread species of the genus *Rastrococcus*. It is also considered one of the most devastating pests of mango throughout its distribution range. It distribution range encompasses the whole of Indian, Bangladesh, China, Hong Kong, Singapore, Sri Lanka and Malaysia and has extended its range to Africa, mostly Tanzania, Kenya and Malawi (Figure 2.1), where it was probably introduced at the beginning of the twentieth century. The distribution map includes records based on specimens of *R. iceryoides* from the collection in the Natural History Museum (London, UK).

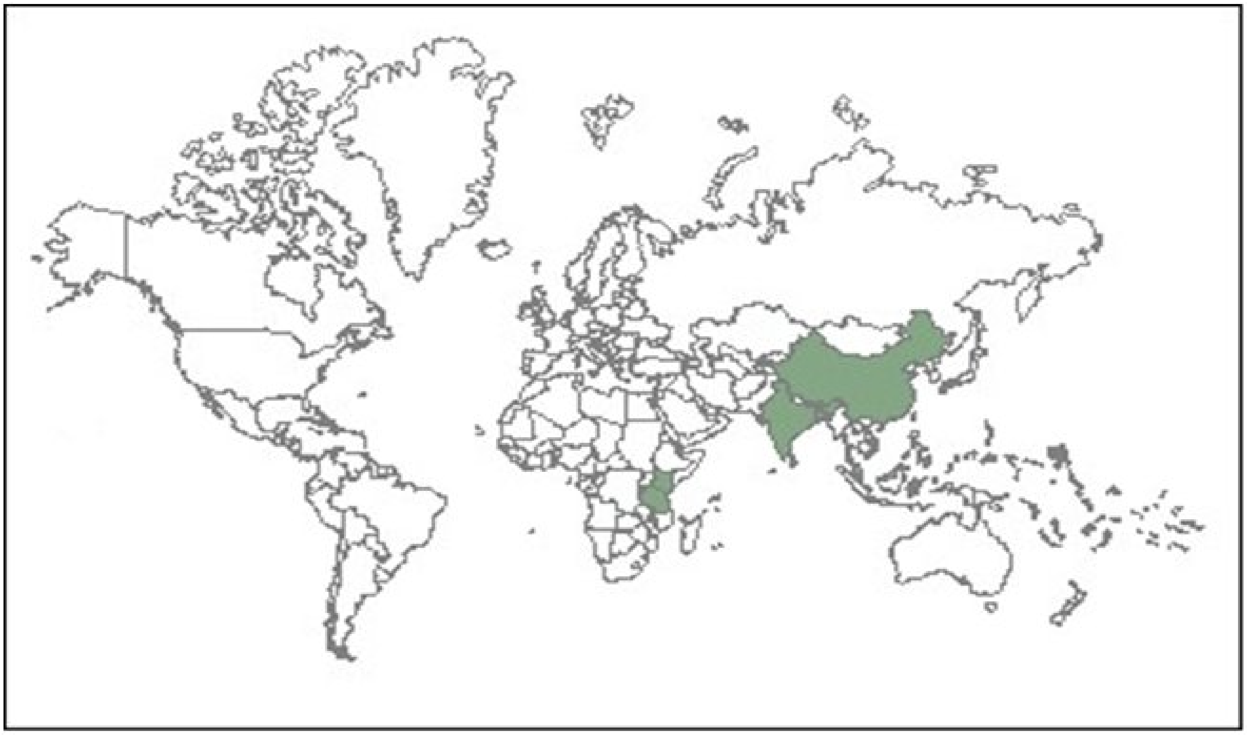


Figure 2. 1: Global distribution of *R. iceryoides* (Williams 1989).

2.2.2 The Origin of Mealybug Pest Status

Similarly to other insect pests, mealybugs have diverse origins, including endemics, immigrants, and mutants (Kim, 1993). An indigenous species may become a serious pest: when a susceptible crop species is introduced into the area; following environmental disturbance; or as a result of stress conditions. Invasive mealybug species may attain pest status as soon as they successfully colonize a new territory, and impact negatively crop yield, which may happen when they encounter a susceptible host, either local or exotic. The mango mealybug, *R. invadens*, is an introduced pest in most mango-growing areas of West and Central Africa. Infestation by *R. invadens* weakens young mango seedlings and severely affects the growth of both fruit-bearing (cultivated) and wild (non-cultivated) trees; the damage is mainly due to leaves, twigs and fruit infestation. Three major causes can lead to mealybug outbreaks:

Firstly, invasion by an exotic mealybug species, which immediately leads to severe outbreaks, is believed to be mainly driven by the combination of host susceptibility and absence of natural enemies in the invaded region (Ben-Dov 1994; Blumberg et al., 1999; Muniappan et al., 2006; Nakahira and Arakawa 2006; Roltsch et al., 2006; Williams and Granara de Willink 1992).

Secondly, the application of non-selective pesticides has been reported to disrupt the biological balance and may lead to resurgence and secondary outbreaks. For example, heavy and damaging outbreaks of *Pseudococcus longispinus* (Targ.) were reported in Israel on avocado plantations bordering cotton fields resulting from disturbances in the biological equilibrium caused by the drifting of insecticides applied from aircraft for the control of cotton pests (Swirski et al., 1980). The mechanisms involved in these two types of outbreaks were discussed by Hardin et al. (1995), and studied by Franco et al. (2004b) with regard to the mealybug pests of citrus. Although the citrus mealybug, *Planococcus citri* (Rossi) is regarded as an occasional pest, it can be extremely damaging and difficult to control. Because it infests the fruit and is often deep within the tree canopy, insecticide applications are often not very effective due to poor coverage. Applications of non-selective insecticides for mealybugs end up aggravating the problem due to the destruction of natural enemies. Among these is a particularly effective parasitoid, *Anagraphus* sp., which has been reported as the primary biological control agent responsible for the apparent "disappearance" of citrus mealybugs in the spring in groves where heavy infestations occurred the previous summer (Franco, 2009).

Thirdly, the effect of environmental factors may also directly and indirectly affect the tritrophic interactions that develop between mealybugs, their host plants and their natural enemies and thereby initiate mealybug outbreaks. Several mechanisms may be involved. Host–plant characteristics may favor or be detrimental to the development, reproduction and survival of mealybugs (Boavida and Neuenschwander, 1995; Calatayud et al., 1994b; Leru and Tertuliano, 1993; Nassar, 2007; Tertuliano et al., 1993; Wysoki et al., 1977; Yang and Sadof, 1995). The resistance mechanisms of the host plant may become involved in both the fixation (antixenosis) and the development of the mealybug (antibiosis) (Tertuliano et al., 1993). Tertuliano and Leru (1992) concluded that the different levels of resistance to the cassava mealybug, *Phenococcus manhioti* (Matile-Ferrero), that were observed in the different varieties of cassava, were not associated with the concentrations of amino acids or sugars, with the ratios between these concentrations, or with the compositions of amino acids obtained from leaf extracts. The identification and assay of cyanogenic and phenolic compounds in the phloem sap of cassava and the honey-dew of the cassava mealybug were carried out by Calatayud et al. (1994a). The best correlation between antibiotic, resistance and secondary compounds analyzed was observed for the rutin contents of infested plants. Yang and Sadof (1995) showed that variegation in *Coleus blumei* (Benth.) could increase the abundance of the citrus mealybug, *P. citri*. Sadof et al. (2003) found that the life history characteristics of *P. citri* on *Coleus blumei* were not correlated with total amino acids and sucrose contents in stem exudates, and were correlated negatively with the proportions of shikimic acid precursors and positively with those of other nonessential amino acids. Host–plant characteristics have also been reported to greatly influence the performance of the natural enemies of mealybugs (Serrano and Lapointe, 2002; Souissi, 1999; Souissi and LeRu, 1997a; Yang and Sadof, 1997).

Water stressed plants has also been reported to favor population increases of mealybug (Calatayud et al., 2002; Gutierrez et al., 1993; Lunderstadt, 1998; Shrewsbury et al., 2004). Mealybug life history parameters may also be influenced by the levels of nitrogen fertilization and leaf nitrogen concentration; high nitrogen concentrations were shown to lead to enhanced performance of the citrus mealybug, *P. citri* (Hogendorp et al., 2006). The antibiotic resistance of two varieties of cassava to the cassava mealybug was observed to increase with the addition of nitrogen (Leru et al., 1994). Survival of immature sugarcane mealybug, *Saccharicoccus sacchari* (Cockerell), increased to a maximum at a soluble nitrogen concentration of 320 mg L^{-1} in sugarcane, and decreased at higher levels, whereas mealybug size increased with increasing nitrogen concentration over the whole tested range (Rae and Jones, 1992).

Weather conditions, especially temperature and relative humidity, are major ecological factors that have been found to severely affect both mealybug and their natural enemies (Chong and Oetting, 2006; Chong et al., 2004, Chong et al., 2005; Daane et al., 2004b; Gutierrez et al., 1993, Gutierrez et al., 2008a; Kontodimas et al., 2004; Nakahira and Arakawa, 2006; Walton and Pringle, 2005).

Encapsulation may adversely affect the degree of biological control exerted by mealybug parasitoids, as it may either prevent the establishment of exotic parasitoids in new regions or reduce parasitoid efficacy (Blumberg, 1997). Also the cryptic behavior of mealybugs and tending of mealybugs by ants may, respectively, originate spatial and temporal refuges from natural enemies. Several other factors can also affect mealybugs' natural enemies; which include intraguild predation and interference (Chong and Oetting, 2007a), and hyperparasitoids (Moore and Cross, 1992), although the latter factor has never been proven to significantly impair biological control of mealybugs.

2.2.3 Economic importance of mealybugs

All mealybug pests are phytophagous and often invasive species with great economic importance around the world. For example in the USA, there are 350 species of mealybug of which approximately 70% of the 66 mealybug species that are considered as pests are invasive (Miller et al., 2002). In New Zealand, most of the known 114 species of mealybugs are found only on native plants. Three cosmopolitan and invasive *Pseudococcus* species are frequently occurring pests of horticultural crops in the country, where they account for more than 99% of the mealybug fauna in orchards and vineyards (Charles, 1993). In Israel, only one among 13 mealybug pests may be considered native (Franco, 2009). In France, scale insects, including mealybugs, represent 31% (Streito and Martinez, 2005) of newly introduced species in recent years, although all mealybug pests on grapevine are native (Sforza, 2008a; Sforza, 2008b).

Rather few narrow host-range plant mealybugs are considered major pests on an international scale, as in the case of the cassava mealybug *Phenacoccus manihoti* (Matile-Ferrero, 1977; Neuenschwander, 2001; Williams and Granara de Willink, 1992; Zeddies et al., 2001). However, the most notorious species are polyphagous and have become serious pests of different crops under different environments. Many of them are cosmopolitan species belonging to the genera *Pseudococcus* and *Planococcus*; they spread between continents through international trade. Several members of the genus *Pseudococcus*, for example, *P. calceolariae* (Maskell), *P. longispinus* (Targioni-Tozzetti), and *P. viburni* (Signoret), are important pests of apple, pear and vineyards in New Zealand (Charles, 1993), whereas around the Mediterranean they are considered mainly as pests of citrus, persimmon and several other subtropical fruit trees (Franco et al., 2004b). The citriculus mealybug, *P. cryptus* Hempel is a major pest of citrus in the east Mediterranean region and it attacks coffee roots in Asia and South America (Ben-Dov, 1994; Blumberg et al., 1999; Williams and Granara de Willink, 1992). Two members of the genus *Planococcus* are among the best known pests of the family: on an international scale, the vine mealybug, *P. ficus* (Signoret) damages mainly vines (Ben-Dov, 1994; Daane et al., 2006a; Gutierrez et al., 2008a; Walton et al., 2004; Zada et al., 2008), whereas the citrus mealybug, *P. citri* (Risso), attacks mainly subtropical fruit trees under Mediterranean climate conditions and ornamental plants in interior landscapes in cooler zones (Ben-Dov, 1994; Franco et al., 2004b).

Polyphagous mealybugs pose a serious threat because of their tendency to adopt new host plants easily. The citrus mealybug has become a key pest in the mint and tarragon industry in Israel and another example that indicates the high economic importance of a polyphagous mealybug is the pink hibiscus mealybug, *Maconellicoccus hirsutus* (Green). This mealybug is indigenous to southern Asia, and actually is considered a potentially serious pest in the United States, because of its extremely broad range of economically important hosts, including citrus, ornamentals, vegetables, and native American flora. It was first reported in the Western Hemisphere in Hawaii in 1984, and later in Grenada in 1994; subsequently it has spread rapidly through the Caribbean islands and to southern California (1999) and Florida (2002). Without control, the economic impact of the hibiscus mealybug to U.S. agriculture was estimated at $750 million per year (Hall et al., 2008; Roltsch et al., 2006; Vitullo et al., 2007; Zhang et al., 2006). The solanum mealybugs, *Phenacoccus solani* Ferris and *P. solenopsis* Tinsley are examples of invasive pests of annual crops; they cause devastating damage to green pepper in Israel and cotton in the Indian sub-continent (Ben-Dov, 2005b; Hodgson et al., 2008; Nakahira and Arakawa, 2006).

Recently, mango infestation in Africa has been reported by two invasive mealybug species of Asian origin, i.e. *R. invadens* and *R. iceryoides* causing serious damage to fruit trees especially mango and citrus (Agounké et al., 1988). The former devastated mango production in West and Central Africa with yields of mango and citrus plummeted, effectively to about zero in areas with longest exposure to the insect. The accounts of yield losses are largely not quantified, but can be estimated to range between 50-80% (Neuenschwander, 2003a). Factors responsible for the lack of statistics on fruit losses caused by this insect pest can be attributed to the traditional nature of horticulture in the regions, which hampers the collection of statistical data as people's reaction towards *R. invadens* led to control measures such as trimming, and in case of desperation, felling of infested trees as result of general panic generated by the proliferation of the pest.

The damage caused by mealybugs is linked to sap uptake, honeydew secretion and associated sooty mold development, toxin injection and virus transmission, although the presence of the insects may itself lead to economic losses (Franco et al., 2000; Gullan and Martin, 2003; McKenzie, 1967; Panis, 1969). The typical injury includes: (a) leaf and fruit discoloration; (b) defoliation, flower and fruit drop; (c) reduction of fruit growth rate; (d) distortion of leaves, new shoots and fruits; (e) aborted plant shoots; (f) development of cork tissue on fruit peel; (g) soiling of fruits with mealybugs and honeydew, which encourage the development of sooty mould *Capnodium mangiferum* Cooke & Broome (Capnodiaceae) known to also raise the leaf temperature of infected mango seedlings; and (h) reduction of plant vigor (Franco et al., 2000, Franco et al., 2004b; Gullan and Martin, 2003; Hodges and Hodges, 2004; Sagarra et al., 2001). High densities or annually repeated infestations can even kill perennial plants (Hodges and Hodges, 2004; Walton et al., 2004; Walton et al., 2006). Indirect damage can result from trophic interactions between mealybugs and other insect pests that are attracted by honeydew, such as Lepidoptera (Franco et al., 2000; Mittler and Douglas, 2003; Silva and Mexia, 1999b).

Several mealybug species are vectors of viral diseases of various crops: banana (Kubiriba et al., 2001; Thomson et al., 1996; Watson and Kubiriba, 2005), black pepper (Bhat et al., 2003), cocoa (Dufour, 1991; Entwistle and Longworth, 1963; Hall, 1945), grapevine (Cid et al., 2007; Sforza et al., 2003; Tsai et al., 2008; Zorloni et al., 2006), pineapple (Sether and Hu, 2002a; Sether and Hu, 2002b; Sether et al., 2005), rice (Abo and Sy, 1998), and sugarcane (Lockhart et al., 1992). In such cases, mealybugs may be economic pests even at low densities. For example, several mealybug species are responsible for GLRaV-3 (Grapevine LeafRoll associated Virus-3) transmission to grapevine, which has been shown by the strong positive correlations between mealybug numbers and infection levels in the following season. The virus infection was predicted to spread rapidly within the vineyard within the economic impact of GLRaV-3 infection in sensitive varieties exceeding $10,000 per ha annually and profitability was sufficiently affected to justify replanting (Walker et al., 2004).

Mealybugs have also been used as beneficial insects in biological control of weeds. For example, *Hypogeococcus pungens* Granara de Willink was successfully introduced from Argentina into Queensland (Australia) for the control of harrisia cactus (*Eriocereus martinii*

Labouret) and related plants (Williams and Granara de Willink, 1992). Some mealybug species may also be manipulated as beneficial insects in conservation biological control tactics. For example, the cypress mealybug, *P. vovae* (Nasonov), which occurs on cypress trees (*Cupressus* spp.) grown in windbreaks, serves as an alternative host for natural enemies of mealybug pests in surrounding citrus orchards and cocoa plantations (Cox, 1989; Ho and Khoo, 1997; Franco et al., 2004b).

2.2.4 Feeding process

Mealybugs feed by inserting their stylets through the plant tissue to suck up sap from either phloem or mesophyll, or both. Males terminate their feeding towards the end of the second nymphal stage. Generally, stylet penetration is accomplished by secretion of solidified saliva that forms a sheath around the stylets. Similarly to other members of the suborder Sternorrhyncha, which includes scale insects, aphids, psyllids and whiteflies, mealybugs consume a diet containing mainly carbohydrates but also limited amounts of free amino acids and other nitrogen compounds (Franco et al., 2000; Gullan and Martin, 2003; Silva and Mexia, 1999a; Tonkyn and Whitcomb, 1987). Thus, except for sucrose hydrolysis, food digestion is hardly necessary. However, organic compounds in phloem sap need to be concentrated before they can be absorbed, and this occurs in the filter chamber, a specialized component of the digestive system, which enables the direct passage of water from the anterior midgut to the Malpighian tubules, thereby concentrating food in the midgut (Terra and Ferreira, 2003). The residue of ingested phloem sap, after digestion and assimilation in the insect gut, is released from the anus as a sugar-rich material, the honeydew. Up to 90% of the ingested sugars may be egested in this way (Mittler and Douglas, 2003).

2.2.5 Reproductive systems and sex determination

Most mealybug species reproduce sexually, and lay eggs (Gullan and Kosztarab, 1997; Kosztarab and Kozár, 1988). However, some, such as *Phenacoccus solani*, (Nakahira and Arakawa, 2006), *P. parvus* (Marohasy, 2003), and *Ferrisia malvastra* (McDaniel) (Ben-Dov, 2005a), reproduce parthenogenically, and others, for example, *P. longispinus* (Franco et al., 2000), and *Antonina graminis* (Maskell) (Ben-Dov, 2006), are ovoviviparous. Two different genetic systems may be found in mealybugs. The more common corresponds to a particular type of haplodiploidy, known as paternal genome elimination, in which both males and females develop from fertilized eggs; the male develops from a zygote containing one haploid genome from his mother and one haploid genome from his father, but only the maternal genome is transmitted to the offspring via the sperm, because the set of chromosomes of paternal origin becomes heterochromatic and genetically inactive (Normark, 2003; Nur, 1990). Male mealybugs are thus functionally haploid, owing to heterochromatization (parahaploidy) (Bongiorni et al., 2001). The other genetic system is thelytokous parthenogenesis, in which there are no males and therefore no mating occurs (Normark, 2003).

There are no sex chromosomes in mealybugs; sex is probably determined by a functional haploidy/diploidy mechanism, which seems to be dependent on the behavior of a set of chromosomes and not a single chromosome. If heterochromatization of an entire set of chromosomes takes place during the cleavage stage of embryogenesis, the embryo will develop into a male; otherwise it will develop into a female. Spermatogenesis is characterized by inverse meiosis and absence of chromosome pairing and genetic recombination (Khosla et al., 2006; Sánchez, 2008). Recently Sánchez (2008) suggested that the genome of the mother determines the heterochromatization of the inherited paternal chromosomes in mealybug embryos. According to this model, heterochromatization is controlled by a maternal factor, with the maternally derived chromosomes imprinted so that they do not suffer the fate of the male chromosome.

Sex determination in mealybugs, and consequently the sex ratio, is known to be influenced by temperature and the age of the mother (Nelson-Rees, 1960). The effect of the temperature or the age of the mother on the offspring sex ratio is attributed to a change in the ratio between the numbers of oocytes with and without the maternal factor (Sánchez, 2008).

2.2.6 Defence system

Mealybugs developed several different defense mechanisms. Many of the species tend to establish themselves in protected sites, such as cracks and crevices in bark, leaf axils, root crowns, nodes of grass stems, under fruit sepals and within fruit navels, between touching fruits or fruits and leafs, and in tunnels bored by insect larvae in roots and stems (Franco et al., 2000;

Kosztarab and Kozár, 1988). This cryptic behavior of mealybugs may originate a spatial refuge from natural enemies and harsh environmental conditions (Berlinger and Golberg, 1978; Gutierrez et al., 2008a). This type of plant colonization makes mealybugs practically invisible during the latent population phase. However, during outbreaks the population explodes from the refuge and becomes conspicuous.

The waxy secretion is the most common conspicuous trait of the mealybug family. It is a complex system that serves different functions, and which is produced by the epidermal wax glands and transported to the body surface via ducts, pores, and secretory setae of various types (Foldi, 1983; Gullan and Kosztarab, 1997). Zada et al. (2009) found that the main components of the wax of five mealybug species (*P. citri*, *P. ficus*, *P. vovae*, *P. cryptus*, and *N. viridis*) were trialkylglycerols and wax esters. The wax cover is believed to prevent water loss. The hydrophobic property of the wax enables the mealybugs to escape drowning or becoming swamped by water in their typical cryptic sites. The ovisac, which is also a wax secretion, is considered to be an adaptation that protects the offspring from both wet and dry conditions, and that may also provide an attachment to the host plant. Tubular ducts and multilocular disc pores, respectively, produce long hollow and shorter curled filaments, which make up the ovisac and the male cocoon (Cox and Pearce, 1983; Foldi, 1983). The white wax of mealybugs is strongly light reflective, and may reduce desiccation in some cases; the wax also serves to cover the honeydew droplets and to protect the mealybugs from contamination by their own honeydew and defensive exudates (Gullan and Kosztarab, 1997).

The wax cover and the secretion process are involved in mealybug defense against natural enemies. It is hypothesized that the rarity of infestation by pathogens and nematodes is related to the wax shield. Stuart et al. (1997) found varied susceptibility of *Dysmicoccus vaccinii* Miller and Polavarapu to several nematode species; they showed that removal of the waxy coating from the mealybug did not influence their susceptibility to *Heterorhabditis bacteriophora* Poinar. The lateral wax protrusions protect the mealybugs from predators and facilitate spacing of individuals within the colony. The nymphs and adult females of most mealybugs possess two pairs of dorsal ostioles, located between the head and prothorax and on the sixth abdominal segment, that discharge a globule of liquid when the insect is disturbed. This waxy liquid solidifies quickly on contact with air and is believed to have a defensive function

(Eisner and Silberglied, 1988, Gullan and Kosztarab, 1997). It was found, for example, that this discharge negatively affect *Sympherobius fallax* Navas (Neuroptera, Hemerobiidae) larvae (Gillani and Copland, 1999), green lacewings (Neuroptera, Chrysopidae), and the parasitoid *Leptomastidea abnormis* (Girault) (Hymenoptera, Encyrtidae) (Franco, 1999). Ostiolar secretions may have different functions in other mealybug species, for example, the highly developed condition of the dorsal ostioles in obligate ant-attended mealybugs suggests that the released fluid may attract the ants (Gullan and Kosztarab, 1997).

Major parasitism in mealybugs involves members of the wasp family Encyrtidae. The encyrtids are koinobiont endoparasitoids, so that the parasitized mealybug continues to live for a few days, to grow and even to reproduce to some extent. This time gap between parasitization and deterioration of the physiological condition enables the mealybug to confront the immature individual parasitoid by encapsulation. The encapsulation is a common immune defense mechanism that involves the formation of a capsule around the parasitoid egg or larva; it is usually composed of host blood cells and the pigment melanin. The capsule may kill the parasitoid and thus prevent successful parasitism (Blumberg, 1997). Various levels of encapsulation have been shown to occur in different mealybug species, in response to parasitism by encyrtids (Blumberg, 1997; Blumberg et al., 1995; Blumberg et al., 2001; Blumberg and van Driesche, 2001; Chong and Oetting, 2007b; Giordanengo and Nenon, 1990; Sagarra et al., 2000). Conversely, encyrtid parasitoids may use superparasitism as a strategy to overcome the immune response of unsuitable hosts (Blumberg et al., 2001). Besides superparasitism, other factors also affect the frequency of parasitoid encapsulation including: (a) host and parasitoid species; (b) the host's physiological age and condition; (c) the host and parasitoid origins (or strains); (d) the rearing and/or ambient temperature; and (e) the host plant species and stress conditions (Blumberg, 1997; Blumberg et al., 2001; Calatayud et al., 2002).

2.2.7 Host plants

Mealybugs feed on a variety of herbaceous and woody plants, including the angiosperm, gymnosperm and fern families. However, most of the species with known hosts develop on herbaceous plants, especially grasses (Poaceae) and composites (Asteraceae) (Ben-Dov, 2006; Kosztarab and Kozár, 1988).

As expected, information on the host ranges of mealybugs is mainly derived from observations of species of economic importance. Most species are oligophagous or stenophagous (or monophagous) while others are polyphagous (Ben-Dov, 2006; Kosztarab and Kozár, 1988). However, such a characterization is problematic as most of the economically important species are known to be associated with long lists of hosts, and their performance varies widely, ranging from development of high population density, which eventually would kill the host plant, to poor development that renders the survival of the population for several generations questionable. Plant growth conditions may strongly affect the success of the population: under irrigation and fertilization plant species become favorable hosts of mealybugs, whereas in different environments the performance is usually poor. During laboratory studies many of the mealybug pest species easily could be reared on alternative hosts, such as potato sprouts or squashes, which are not colonized by mealybugs in the field. For example, the citrus mealybug has been found on plants from 70 botanical families, 60% of which are characterized as non-woody plants, whereas on the international scale this mealybug is a pest of subtropical and tropical crops, such as citrus (*Citrus* spp.), persimmon (*Diospyros kaki* Thunberg), banana (*Musa paradisiacal* L.), and custard apple (*Annona* spp.), or it damages various types of plant species in interior landscapes, greenhouses in particular. Another example of the apparent contradiction between the long lists of host plants and the narrow ranges of damaged crops is the case of the citriculus mealybug, *P. cryptus*; although this mealybug is known from 35 host plant families (Ben-Dov, 2006), in Israel it causes damage only to citrus trees. Under low pressure of natural enemies, for example, when they spread in new environments, mealybugs are observed on relatively large numbers of host plants, in contrast with the situation when there is effective biological control.

2.2.8 Dispersal

Adult males and newly emerged first-instar nymphs, or crawlers, of most mealybug species display dispersal actively. Other nymphal stages and adult females may also move limited distances (Kosztarab and Kozár, 1988) but, similarly to most scale insects, crawlers are the mealybugs' main dispersal agents. There is evidence that this developmental stage of scale insects is dispersed passively by the wind, and may be carried for distances of a few meters to several kilometers, or even more, from the natal plant–host, although mortality is very high (Gullan and Kosztarab, 1997). In contrast, Williams and Granara de Willink (1992) reported that mealybugs were believed to be distributed by air currents over only short distances. As well as wind, water, bed-soil, humans, and domestic and wild animals may aid the passive dispersal of mealybugs (Kosztarab and Kozár, 1988). Among arthropods, ants have also been reported to disperse some mealybug species (Gullan and Kosztarab, 1997; Malsch et al., 2001; Ranjan, 2006). Nevertheless, if conditions are favorable, crawlers usually settle on the natal host plant, often close to their mother, which leads to an aggregative distribution (Gullan and Kosztarab, 1997; Nestel et al., 1995).

Many species of mealybugs have been widely distributed by commercial traffic, mostly carried on imported plant material (Williams and Granara de Willink, 1992). Because of their cryptic habits and small size, mealybugs are difficult to detect at borders during quarantine inspections, especially if their population density on plants is low (Gullan and Martin, 2003).

2.2.9 Mealybug relationships with ants

Ants are often associated with mealybugs as honeydew consumers. Hemiptera-tending ants are mostly species of the subfamilies Myrmicinae, Dolichoderinae, and Formicinae (Degen and Gersani, 1989; Mittler and Douglas, 2003). The tended mealybugs benefit from the protection against natural enemies, and the removal of honeydew prevents contamination, which may be especially detrimental to first-instar nymphs (Cudjoe et al., 1993; Daane et al., 2006b, Daane et al., 2007; Gullan and Kosztarab, 1997; Moreno et al., 1987). Some ant species actively construct shelters for mealybugs which provide some protection from unfavorable environments and natural enemies (Franco et al., 2000; Helms and Vinson, 2002; McLeod et al., 2002) leading to frequent mealybug outbreaks (Daane et al., 2006b, Daane et al., 2007; Silverman and Brightwell, 2008).

Several studies have documented that ant attendance reduces the parasitism of honey-dew excreting Hemiptera through attacks and disturbances against parasitoid foraging activities or oviposition attempts (Martinez-Ferrer et al., 2003; Itioka and Inoue, 1996; Stechmann et al., 1996; Vinson and Scarborough, 1991; Novak, 1994). Whitehead (1957) and Myburgh (1986) reported that ant foraging on plant canopies reduces natural enemy activity and promotes mealybug infestation and therefore, biological control of the insect pest is compromised. For example, Flanders (1945) documented an increase in the yellow scale, *Aonidiella citrina* Coquillet (Hemiptera: Diaspididae), and a decrease in parasitism by endoparasitoid *Comperiella bifasciata* Howard (Hymenoptera: Encyrtidae) in the presence of Argentine ants, *Linepithema humile* (Mayr) (Compere, 1940). Flander (1943) and Compere (1940) reported that in South Africa *Metaphycus helvolus* (Compere), a parasitoid of black scale was found to be effective only in the absence of *L. humile*. In California, Daane et al. (2007) found that *L. humile* promoted populations of obscure mealybug *Pseudococcus viburni* (Signoret) while lowering populations of its parasitoids *Pseudaphycus maritimus* (Erhorn) accompanied by a serious reduction in its parasitoid populations. The common pugnacious ant, *Anoplolepis custodiens* (Smith) has also been reported to incidentally disturb the parasitoids of California red scale, *Aonidiella aurantii* (Maskell), while tending soft brown scale in citrus orchards in South Africa (Samways and Tate, 1984; Steyn, 1954). The cocktail ant *Crematogaster peringueyi* Emery is disruptive to natural enemies of soft brown scale *Coccus hesperidum* L., and vine mealybugs *Planococcus ficus* (Signoret) (Kriegler and Whitehead, 1962). The Argentine ant, *L. humile* was found to be disruptive to the black scale, *Saissetia oleae* Olivier, parasitoid *Coccophagus scutellaris* (Dalman) in California (Horton, 1918).

In Australia, Buckley and Gullan (1991) found that the incidence of coccid parasitization was correlated with the relative inoffensiveness of the attendant ant species in a field study. Buckley and Gullan (1991) reported a very low parasitism rates (< 10%) of coccids in the presence of *Oecophylla* and *Solenopsis* species and > 15% in the presence of the more aggressive *Tapinoma* and *Iridomyrmex* species. In California, *L. humile* reduced parasitism and host mutilation of the California red scale by the parasitoid *Comperiella bifasciata* (Howard) (59.1%) and *Aphytis melinus* DeBach (79.5%) in a laboratory trial (Martinez-Ferrer et al., 2003). Itioka and Inoue (1996) in a comparative field investigation found a 94% decrease of the mealybug *P. citriculus* populations in a Satsuma orange orchard by natural enemies in the absence of the attendant ant, *Lasius niger* Linnaeus (Hymenoptera: Formicidae). In the absence of Argentine ant, populations of the citrus mealybug, *Planococcus citri* Risso (Hemiptera: Pseudococcidae), and the wooly whitefly, *Aleurothrixus floccossus* Maskell (Homoptera: Aleyrodidae), were effectively reduced by their respective natural enemies (Moreno et al., 1987).

2.2.10 Management of mealybugs

2.2.10.1 Biological Control

2.2.10.1.1 Natural enemies

Mealybugs have many natural enemies, including parasitic wasps, arthropod predators and entomopathogenic fungi. However, parasitoid encyrtids (Hymenoptera, Encyrtidae) and predatory lady beetles (Coleoptera, Coccinellidae) are the most common natural enemies of mealybugs. Mealybug-parasitizing encyrtids are primary endoparasitoids; most of them undergo solitary development. Their host specificity is not a clear issue. Franco et al. (2000) compiled, from published literature, about 70 encyrtid parasitoid species that were reared from the citrus mealybug, whereas only four species were considered to be principle parasitoids of this mealybug. *Coccidoxenoides, Gyranusoidea, Leptomastidea, Leptomastix, Pseudaphycus*, and *Tetracnemoidea* are examples of encyrtid genera of mealybug parasitoids (Charles, 1993; Franco et al., 2000; Noyes and Hayat, 1994; Rosen, 1981). The most up-to-date assessment of parasitoids attacking *R. iceryoides* in its native home is provided by Noyes and Hyat (1994). The most important parasitoids of *R. iceryoides* in India are *Anagyrus chryos*, *A. sabas, An. agraensis, A. mirzai, Leptomastrix nigrocoxalis* and *Praleurocerus viridis*.

Coccinellids accept a wide range of food, but they complete larval development and produce viable progeny only if they consume their ‘essential food’. Four genera of Chilocorinae (*Brumus*, *Aspidimerus*, *Stictobura* and *Orcus*) and six of Scymninae (*Diomus*, *Nephus*, *Sidis*, *Parasidis*, *Cryptolaemus* and *Pseudoscymnus*) prey preferentially on mealybugs (Iperti, 1999). Other important groups of predators are brown lacewings (Neuroptera; Hemerobiidae) and predatory gallmidges (Diptera; Cecidomyiidae). The most important predators of *R. iceryoides* in India include: *Aponephus lentiformis* gen. et sp. nov., *Cryptolaemus montrouzieri* Mulsant, *Scymnus coccivora* Ayyar and *Spalgis epeus* Westwood. The lycaenid, *S. epius*, clears complete infestations of *R. iceryoides* when it feeds (Vasundhara et al., 1990).

As sap feeders, mealybugs are not likely to be exposed to viral or bacterial infections (Moore, 1988) and only a few species of entomopathogenic fungi were reported to be associated with mealybugs and confirmed to be pathogenic; they include *Aspergillus parasiticus* Speare, *Cladosporium oxysporum* Berk. and M.A. Curtis, *Hirsutella sphaerospora* H.C. Evans and Samson, and *Neozygites fumosa* (Speare) Remaudière and Keller (Browning, 1994; Delalibera et al., 1997; Leru, 1986; Moore, 1988; Samways and Grech, 1986).

It is apparent that given natural enemies may flourish in some situations and perform poorly in others. There are numerous cases at hand showing limited abilities of natural enemies to attack respective hosts in one region contrasted to their capabilities against the same hosts in some other regions. An excellent example of climatic limitation is that of the citrus black fly parasitoid, *Eretmocerus serius* Silvestri (Insect: Hymenoptera). When this parasitoid was introduced from Malaysia into Cuba, Trinidad, Brazil and Costa Rica, it became well established and soon provided very effective control of the pest. However, when it was introduced into western Mexico, establishment occurred but only in scattered areas, and little control was realized. The parasite clearly was not well adapted to the severe winters and arid summers prevailing in many regions in Mexico (Clausen, 1958; Messenger and Bosch, 1971). A more recent case is the introduction of natural enemies against the cassava green mite in Africa.

Populations of *Neoseiulus idaeus* (Demmark and Muma) and *Typhlodromalus manihoti* (Moraes) were imported from Columbia to Africa but none of them ever became established in the wide range of agronomic and ecological conditions tested, apparently because of inadequate alternative food sources and extended periods of low relative humidity. However, populations of the same species introduced from Northeastern Brazil were released and have since established and spread into various African countries (Yaninek et al., 1993). Natural enemies may therefore be soughted in the native home of the pest and in areas with climate similar to that of the destination (Legner and Bellows, 1999). In classical biological control attempts, particular attention should be paid in searching and introducing ecologically compatible natural enemies to increase their probability of establishment.

2.2.10.1.2 Classical biological control

Biological control of mealybugs has been practiced for many years; it involves three main tactics, that is, classical biological control, augmentative releases, and conservation biological control. However, since the major mealybug pests are invasive species, classical biological control has been the major control tactic. Moore (1988) reviewed the natural enemies used against mealybugs in biological control programs worldwide. According to him, more than 70 species of parasitoids have been introduced against mealybugs, and at least 16% of the introductions were considered to cause substantial or complete control. Most of the introduced parasitoid species were encyrtids, but species of Aphelinidae and Platygasteridae proved to be successful on several occasions. Often a single parasitoid was considered to be responsible for the success, even when more than one was introduced. Noyes and Hayat (1994) reviewed the use of encyrtids for biological control of pest mealybugs, and found that out of a total of 385 importations of encyrtids, targeting 22 mealybug species, about 24 and 7% were considered to give partial or successful control in the field and in greenhouses, respectively.

With regard to predators, Moore (1988) analyzed the use of *C. montrouzieri* separately from that of other mealybug predators. This lady beetle has been used many times against at least 10 different species of mealybugs and was considered to give substantial or partial control in about 19% of the introductions; on some occasions it has been regarded as an outstanding biological control success. Of the other 46 predator species – mostly coccinellids, but also cecidomyiids, chrysopids, hemerobiids and lycanids – used in biological control of mealybugs, only the cecidomyiid *Kalodiplosis pseudococci* Felt was regarded as having given significant control, when used against *Dysmicoccus brevipes* (Cockerell) in Hawaii in conjunction with two parasitoids.

Stiling (1993) in his analysis of why in biological control campaigns, some introduced enemies fail to reduce pest populations substantially, showed that the major reason given for failure is related to climate (34.5%). Moore (1988) analyzed the reasons for the failures of both parasitoids and predators of mealybugs to become established in biological control programs. In the case of parasitoids he cites the following documented reasons: (i) incorrect identification of the target mealybug species; (ii) the target was a native species; (iii) hyperparasitism; (iv) failure of the parasitoid to adapt to unfavorable climates; and (v) other reasons, such as interference with ants, use of pesticides, and small numbers of individuals released. With regard to predators, Moore (1988) listed six main reasons for failure: (i) no adaptation of the released species to climate; (ii) effect of pesticides; (iii) density of the prey; (iv) effect of the host plant; (v) inability to reach the prey; and (vi) effects of other organisms.

The lack of adequate food resources for natural enemies within or near to agroecosystems may limit the performance of biological control agents against mealybugs. For example, Davies et al. (2004) observed that the survival and reproduction of *Coccidoxenoides perminutus* Girault, a parasitoid of the citrus mealybug, *P. citri*, was significantly influenced by the nature of the nectar on which the parasitoid was fed. In light of these results, it was suggested that habitat management, for example, by providing suitable nectar sources for adult parasitoids, might be a means to conserve and enhance *C. perminutus* activity in the field.

In recent years successful classical biological control programs against mealybugs have targeted the cassava mealybug, *Phenacoccus manihoti*, in Africa (Neuenschwander, 2001), the mango mealybug, *R. invadens* in West Africa (Bokonon-Ganta et al., 2002), the pink hibiscus mealybug, *Maconellicoccus hirsutus* (Green) in the Caribbean and California (Roltsch et al., 2006), and the papaya mealybug, *Paracoccus marginatus* Williams and Granara de Willink, in Palau (Muniappan et al., 2006). It is important to note that successes were mostly achieved in tropical regions where the target area for classical biological control and the area of origin of the introduced parasitoids displayed similar climatic conditions.

In a few cases modeling has been used as a tool to analyze actual systems and to identify major constraints, in order to improve biological control of mealybugs. For example, the model developed by Gutierrez et al. (2008a) predicted that the parasitoid *A. pseudococci* would have a larger impact on the vine mealybug, *P. ficus* than either *L. abnormis* or *C. montrouzieri*, and that biological control of the mealybug in California would require additional species of natural enemies and/or could be achieved by reducing the size of the spatial-temporal refuge. In another use of a modeling approach, Gutierrez et al. (2008b) concluded that biological control of the vine mealybug might be adversely affected by climate change. Gutierrez et al. (1993) developed a tritrophic model of the cassava system, and used it to explore the basis for the successful control of cassava mealybug, *P. manihoti* in Africa by the exotic parasitoid *Epidinocarsis lopezi*

(DeSantis), and also to examine the causes for the failure of the related parasitoid *E. diversicornis* (Howard) to establish itself.

2.2.10.1.3 Augmentative control tactics

When the mealybug population is low, the population densities of its specific natural enemies, especially the predators, are also low. Parasitoids, which are better fitted to survive at low mealybug densities, may find it difficult to reach their hosts in their most appropriate refugia, and these small colonies may also be well protected by ants. However, inoculative or inundating releases of parasitoids may compensate for their low survival. Augmentation of the parasitoid population, when mealybugs leave their typical refugia for new colonization sites on the host plant, may improve the mealybug/parasitoid ratio (Mendel et al., 1999). If the population density during a particular season is low, released parasitoids tend to disperse over a rather large area in their search for mealybug colonies (Mendel et al., 1999). The kairomonal response of the parasitoids to the mealybug sex pheromone can be utilized to keep the released individuals in the targeted area. The parasitoids search for mealybugs in the vicinity of the pheromone release points (Franco et al., 2008c), therefore, the intensity of parasitization may be increased in treated plots. Another tactic that may be considered involves measurement of the population of natural enemies in the managed area. Advance acquisition of information should be considered, in order to plan augmentation of natural enemies in the coming growing season. It is expected that if there was considerable mealybug mortality in a particular area, it might be attributed to the activity of parasitoids and predators that had survived in these areas, and not because of migration of natural enemies from a long distance. Therefore, information about the natural enemy density late in the season may be achieved by setting up traps baited with mealybug colonies, with or without the sex pheromone (with respect to each individual case).

The first known case of an augmentative biological control program dates back to before 1917 and was aimed at controlling the citrophilus mealybug, *P. calceolariae*, a pest of citrus in Southern California, by using the coccinellid predator *C. montrouzieri* (Luck and Forster, 2003; van Lenteren, 2006). Since then, this Australian ladybird beetle has been commonly used in various countries on diverse crops (Copland et al., 1985; Franco et al., 2004b), and is actually one of the few species of natural enemies commercially available for biological control of mealybugs by means of augmentative tactics. Augmentative releases of *L. dactylopii* and *C. montrouzieri* against *P. citri* have been reported to be effective in several Mediterranean countries, and in other citrus-growing areas, such as Australia and California. However, Mendel et al. (1999) released 5,000–10,000 individuals of *L. dactylopii* or 10,000–50,000 of *A. pseudococci* per hectare and obtained no significant impact on either the mealybug infestation or on fruit damage.

2.2.10.1.4 Pheromone-based management tactics

Sex pheromones of insects, including mealybugs, are natural compounds emitted by virgin females in order to attract conspecific males for mating. The sex pheromones are effective in extremely small quantities; they are non-toxic and can be applied in various ways. Unlike pesticides, these chemicals are species-specific and do not affect beneficial insects. The behavioral impacts of the semiochemicals are limited to the target pest organisms. The potential of mealybug sex pheromones as an alternative and ecologically friendly means for monitoring and control is important and promising. Sex pheromones are used in lures for monitoring, detection of outbreaks, and for population management. Monitoring systems provide vital information for the timing of insecticide applications. Population levels can be reduced or controlled by mass trapping, mating disruption, or lure and kill. The success of these methods depends on the availability of the pheromone, and on an appropriate formulation and deployment.

Because of the worldwide economic importance of the pest, there is a need to improve the efficiency of pheromone synthesis and to make the pheromone available for control application. A series of analogs of this pheromone was prepared, in order to find a less expensive attractant (Liu et al., 1995; Dunkelblum et al., 1987), but most of them were insufficiently attractive, except for a homolog in which a cyclobutaneethanol moiety replaced the cyclobutanemethanol moiety in the natural pheromone. The advantage of the homolog is that its synthesis is easier and less expensive than that of the pheromone.

2.2.10.1.5 Kairomonal response

The sex pheromone of mealybugs may be used by their natural enemies as a kairomonal cue in host or prey selection. Millar et al. (2002) suggested that *A. pseudococci* (most likely *Anagyrus* spec. nov. near *pseudococci*) in California vineyards was attracted to the pheromone of *P. ficus*. Franco et al. (2008c) showed, in light of both field and olfactometer experiments, that the females of the encyrtid *Anagyrus* spec. nov. near *pseudococci*, a major parasitoid of both the vine mealybug, *P. ficus* and the citrus mealybug, *P. citri* were attracted to the sex pheromone of *P. ficus* but not to that of *P. citri*, and that this kairomonal response was an innate behavior trait. This host–parasitoid relationship has been further investigated, and preliminary data from field trials in which sentinel mealybugs on sprouted potatoes were exposed in citrus orchards, suggested that the presence of *P. ficus* sex pheromone significantly increases the parasitization rate of *P. citri* colonies by *Anagyrus* spec. nov. near *pseudococci* (Franco et al., 2008b). Similar kairomonal responses have been suggested in a few other parasitoid species of mealybugs. Rotundo and Tremblay (1975) reported that traps baited with virgin females of *P. calceolariae* captured significant numbers of the encyrtid *Tetracnemoidea peregrina* (Compere) (=*Arhopoideus peregrinus*). A kairomonal response of the encyrtid *Pseudaphycus maculipennis* Mercet to the sex pheromone of the obscure mealybug, *P. viburni* was also observed in field experiments with pheromone traps (Bell et al., 2008; Bell et al., 2006). Two species of mealybug parasitoids were caught in traps baited with the sex pheromone of *P. cryptus* in a citrus orchard in Japan (Arai, 2002).

The kairomonal response of a parasitoid to the sex pheromone of its host mealybug could impair its practical use for mealybug pest management by mass trapping and lure-and-kill tactics (Franco et al., 2008c). This side effect may be avoided by using pheromone analogs that lack kairomonal activity but that still preserve the pheromonal attractiveness to the males, as was successfully accomplished for other scale insects, for example, *Matsucoccus* spp. (Mendel et al., 2003). Optimizing the devices used in these tactics, for example, with regard to design and color, could also minimize the negative impact on natural enemies of the pests (Franco et al., 2008c).

The results obtained by Walton et al. (2006) with pheromone-based mating disruption of vine mealybug indicated that the treatment had no negative effect on the level of parasitization of *P. ficus* by *A. pseudococci*. Franco et al. (2008c) suggested that the kairomonal response of

Anagyrus spec. nov. near *pseudococci* could be explored in connection with biological control tactics, by enhancing para- sitization of *P. citri* as a component of integrated pest management strategies, by means of a similar approach to that used against aphid pests (Powell and Pickett, 2003). The use of semiochemicals for enhancing the effectiveness of biological control tactics against pest mealybugs offers a potential novel approach that needs further investigation and exploration.

2.2.10.2 Chemical control

There are great similarities among the insecticide arsenals used to control mealybug species on different crops. An insecticide arsenal that is both suitable for organic farming and able to cope effectively with mealybug pests does not exist in practice. Since the growers will need to treat small hot spots of the mealybug, it is expected that some soft insecticides will be used and that more than one application may be needed, to selectively eliminate such hot spots. When these hot spots are treated several points should be taken into account: (i) hot spots are expected to be in areas that are practically free of problematic mealybug populations; they actually constitute oases for parasitoids and predators; therefore, the ratio of mealybug to natural enemy populations in the hot spots should be considered before initiation of any control operation; (ii) an insecticide will be applied when augmentation with predators is not useful or cannot be implemented; (iii) a low-residue short-life insecticide is the most appropriate; (iv) augmentation of natural enemies will be needed if the hot spots are too numerous.

In principle, three main modes of insecticide application are adopted: (i) foliage cover spraying for management of above-ground populations; (ii) application of insecticide solution to the soil to enable it to penetrate to the root zone, so as to combat subterranean colonies; and (iii) chemigation by application of systemic compounds via the irrigation system, for example, drip irrigation. Systemic insecticides are also used against mealybugs by smearing them on the stem or main branches. Two other, less common, techniques are fumigation, usually applied for eradication, for example, with methyl bromide, or slow-release strips to prevent colonization. Organophosphates – such as chlorpyrifos, acephate, dichlorvos and diazinon – and, to a lesser extent, carbamates – such as aminocarb, carbaryl, thiodicarb or methomyl – are broad-spectrum nerve insecticides which have been used against mealybugs that colonize the plant canopy since the early 1960s (e.g., de Souza et al., 2007; Gonzalez et al., 2001; Shafqat et al., 2007). These insecticides when applied in high volume could successfully overcome the obstacles that make mealybugs hard to kill: (i) their hydrophobic wax cover, which repels hydrophilic insecticides; (ii) their tendency to feed in hidden and protected parts of the plant; (iii) their typically dense colonies; and (iv) the frequent overlapping of generations. Effective control is achieved when most of the mealybug population is in the dispersive crawler stage or the young nymphal instars, and when the host plant does not provide effective shelter. However, satisfactory control is often difficult to achieve over an extended period. These chemicals have detrimental effects on the environment as a whole and on natural enemies in particular (Anand and Ayub, 2000; Babu and Ramanamurthy, 1998; Meyerdirk et al., 1982). The multivoltinous character of pest mealybugs and the frequent application of inefficient control measures accelerate the development of insecticide resistance (Flaherty et al., 1982). Systemic organophosphates such as dimethoate could overcome some of these obstacles (Grout and Stephen, 2005; Meyerdirk et al., 1982; Prasad et al., 1998). Pyrethrins and rotenone replaced these compounds in organic agriculture with limited effectiveness. Chlorpyrifos-impregnated strips are applied to protect banana bunches from mealybug infestation or as stem barriers for the control of ants (Addison, 2002; Gross et al., 2001).

Oils have long been used for the control of scale insects but they have been ineffective against mealybugs. However, integration of narrow refined oils with other insecticides was suggested as a means to dissolve the insect’s wax covering and thereby improve the insecticide efficacy (Cranshaw et al., 2000; Morishita, 2005). Insect growth regulators (IGRs), such as buprofezin, a chitin-synthesis inhibitor, or kinoprene, which mimics juvenile hormone, were sought as replacements for organophosphates and carbamates in controlling mealybugs; they have been considered a suitable alternative because they exhibit low human toxicity, they are more selective to many beneficial species, and they are specifically targeted at processes involved in particular stages of mealybug development. However, many of the IGRs are toxic to ladybeetles (James, 2004; Cloyd and Dickinson, 2006). Buprofezin is a commonly applied IGR against mealybugs (Muthukrishnan et al., 2005); however, its effectiveness is mainly limited to eggs and young stages, so that adult females may escape the consequences of the treatment. Buprofezin also suffers from the same limitations as other foliarly sprayed compounds. More recently, an effective group of compounds has been found which combine toxicity to mealybugs with safety to other non-targeted organisms; they are the neonicotinoids. These compounds act on the central nervous system, and easily replace carbamates, organophosphates or pyrethroids, since there are no records of cross-resistance associated with them. These systemic compounds show high effectiveness against mealybugs. Examples include: dinotefuran applied to the canopy; acetamiprid applied by smearing on the stem or the branches (Gross et al., 2000; Larrain, 1999); and imidacloprid and thiamethoxam that are introduced by watering the soil (Daane et al., 2006a; Daane et al., 2006b; Fu Castillo et al., 2004; Grout and Stephen, 2005; Martin and Workman, 1999; Sazo et al., 2006). In organic agriculture, azadirachtin, an IGR chitin inhibitor derived from the Indian neem tree, may be used in similar modes (Irulandi et al., 2001).

2.2.10.3 Cultural control

Cultural control has long been observed to slow the spread of mealybug infestations in orchards. The following tactics can be applied in mealybug infested mango orchards:

1. Work in mealybug infested orchards should be scheduled such that once pruners or harvesters are done with work in the orchards; they are finished for the day. Alternatively, workers should be asked to shower and change their clothes before entering an uninfested orchard. Showering and change of clothings are necessary because all life stages of mealybug (particularly, crawlers) can be carried on the workers' skin, clothing and hair. These mealybug life stages can survive for eight to 24 hours and therefore can lead to new infestaions in uninfested orchards, if the cleaning protocol is not followed.
2. The prunings from an infested orchards can be treated in one of the following ways:
   - Prunings of infested plant parts should be taken out of the orchards and burnt immediately.
   - The prunings should be shredded and mulched in the middle of the row away from the mango stem. Although, shredding makes very small pieces out of the prunings, mealybug crawlers can still survive on pieces of sticks about one to two inches in length. Therefore, shreddings should be bagged in heavy-duty construction disposal bags, and the sealed bag taken away from the zone of the mango stem shortly after pruning. The

bagged prunings can then be allowed to rot or decompose for two to three weeks in the orchards.

3. The equipment used for pruning or harvesting in an infested orchard should be steam cleaned. Care should be taken to make sure all plant materials are removed.
4. For harvest targeted for the market, once the fruits are bagged and boxed, the truck bed on which the boxes are loaded should be cleaned of any plant debris before leaving the orchard. The truck should go directly to the storage facility to unload. The truck should also be steam cleaned at the storage facility before returning to any part of the orchard.
5. For fruits transported in a truck, the truck should be covered with a plastic tarp to avoid infested plant material or crawlers from being blown out of the vehicle during transport. All vehicles and tarps should then be steam cleaned as well before picking up more fruits from the orchards.
6. Removal of alternative host is also strongly advised.

2.2.10.4 Pathogens

Only the entomopathogenic fungi are recorded among the Pseudococcidae and these records are sparse and confused. The fungi that have been confirmed as pathogenic against mealybugs include: *Neozygites fumosa* (Speare), *Hirsutella cryptosclerotium* (Fern.-García, H. C. Evans & Samson), *Cephalosporium sp*. (probably *Verticillium lecanii* Zimmerman), *Aspergillus parasiticus* and possibly *Cladosporium oxysporum* (Berk & Curtis). However, practical development of these requires much research.

2.2.10.5 Integrated pest management

Worldwide, the most recommended control strategy for reducing yield loss by mealybugs that enables growers to comply with stringent quality production in export market is integrated pest management (IPM). With integrated control, chemical, biological and cultural control methods are used in conjunction with each other. Each method contributes to the total control strategy. In an integrated programme various aspects are considered to ensure an environmentally friendly, yet effective end result.

Prevention is better than cure. This principle is highly applicable in the management of mealybugs, which are hard to kill pests on several crop plants. This is because mealybugs form colonies in protected areas of the plants and all the life stages are covered with waxy coating, therefore, making it difficult to control with conventional insecticides. Cultural, mechanical, biological and chemical methods of control have to be adapted throughout to contain the mealybug population thus preventing the loss caused by the mealybugs. Integrated pest management of mealybugs will therefore include the following steps:

- The infested plant portions with mealybug colonies should be prunned during the dry season to expose eggs or nymphs to natural enemies and sun heat and/or destroyed. Fallen infested leaves under the tree canopy should be collected and burnt to avoid further spread of the pest.
- Infested fruits on the trees at the time of harvesting should be collected and destroyed.
- All the pruned materials from mealybug infested orchards should be collected and destroyed.
- To combat the menance caused by mealybugs in orchards, regular monitoring and early detection of infestation are essential. Mango orchards and neighbouring areas should be free from other suitability host plant capable of harbouring mealybug populations in and around the orchards throughout the year.
- Ant colonies associated with mealybugs in orchards should be located and destroyed with drenching of chlorpyriphos 20 EC @ 2.5 mL/L or apply malathion 5% dust @ 10 kg/ac, since ants are known to be associated with the build-up of mealybug populations.
- In orchards where mealybug incidence is widespread, periodic prunning of affected portions coupled with spray of dichlorvas 76 WSC 0.2% (@ 2.5ml/lit) or methyl parathion 50 EC 0.05% (@1ml/lit) or dimethoate 30 EC 0.05% (1.75ml/lit) in combination with fish oil resin soap @ 20g per litre of water should be used to reduce mealybug incidence.
- The same insecticides should not be repeated in subsequent rounds as rotation of insecticides would be desirable to counteract the tendency of the mealybugs to develop field resistance.

- Insecticidal spraying in infested orchards should be carried out in such a way as to cover the entire lower surface of leaves, twigs and branches where all the meaybug life stages are known to occur in large numbers. It is important for insecticide spraying to be carried out in the evening hours to avoid toxicity to non target insects like pollinators.
- The use of broad spectrum insecticide should be avoided to encourage the conservation of natural enemies like parasitoids and coccinelids, which are known to be efficient parasites and predators, respectively, on eggs and nymphal stages of mealybugs. For example, the release of the Australian ladybird beetle (*Cryptolaemus montrouzieri* Mulsant) @ 5000/ha or 10 beetles/plant has been shown to clear the mealybug population present in infested orchards.
- The use of neem seed extract (4%) or garlic oil (1%) on tree trunk below the alkathene band has been shown to kill congregated crawlers.

CHAPTER THREE

Distribution, Host-Plant Relationships and Natural Enemies of *Rastrococcus iceryoides* Green (Hemiptera: Pseudococcidae) in Kenya and Tanzania

ABSTRACT

Rastrococcus iceryoides Green (Hemiptera: Pseudococcidae) is an invasive mango mealybug pest in Kenya and Tanzania. It is believed to be native to Southern Asia. A survey was carried out in Kenya and Tanzania from February 2008-July 2009 to study the geographical distribution of the pest, its host plant relationships and associated natural enemies. In both countries, our results showed that *R. iceryoides* is widely distributed across the coastal belt. Heavy infestations occurred on *M. indica* and *Parkinsonia aculeata* L. in Matuga and Kinango (Kenya); and Morogoro, Kinondoni, Tanga, Kibaha and Mkuranga (Tanzania). *Rastrococcus iceryoides* was recorded from 29 cultivated and non-cultivated host plants from 16 families. Twenty-one of these host plants are new records. Among the cultivated host plants, *M. indica* and *Cajanus cajan* (L.) Millspaugh recorded the highest levels of infestation. *Parkinsonia aculeata, Caesalpinia sepiaria* Roxb, and *Deinbollia borbonica* Scheft were found to be the most infested non-cultivated plants. Infestation levels across the different plant parts were generally significantly higher on the twigs compared to the leaves and fruits with a maximum of 8153 mealybugs/20 twigs and 6054 mealybugs/80 leaves of *M. indica* in Kibaha, Tanzania. A total of six parasitoid species were recovered from *R. iceryoides* with *Anagyrus pseudococci* Girault (Hymenoptera: Encyrtidae) predominating (21% parasitism on *M. indica* in Tanzania; 20% on *P. aculeata* in Kenya). Despite this level of parasitism, the ability of the parasitoid to regulate the population of *R. iceryoides* was inadequate. In addition, nineteen species of hyperparasitoids from six families and thirty-eight species of predators from fourteen families were recorded. Despite the diversity of these natural enemies, *R. iceryoides* has remained one of the most damaging pests of its preferred host (mango) in Kenya and Tanzania. Therefore, there is the need for foreign exploration and introduction of efficient coevolved natural enemies from its aboriginal home of Southern Asia to minimize its impact on horticulture in Africa.

Key-words: *Rastrococcus iceryoides*, distribution, host plants, biological control

3.1 Introduction

Mealybugs (Hemiptera: Pseudococcidae) are important group of phytophagous insects that cause significant damage on a variety of horticultural crops worldwide (Miller et al., 2002). In Africa, *Rastrococcus invadens* Williams and *Rastrococcus iceryoides* Green are regarded as two important exotic mealybug species native to Southern Asia that commonly infest mango, *Mangifera indica* Linnaeus (Anacardiaceae). The former devastated mango production in West and Central Africa but was brought under biological control through introduction of an exotic parasitoid *Gyranusoidea tebygi* Noyes from India (Noyes, 1988; Bokonon-Ganta and Neuenschwander, 1995). Based on its economic importance and the ease with which it colonised major part of West and Central Africa, *R. invadens* has been the subject of many studies, both descriptive and experimental, and its geographical distribution and host-plant relationships is well documented (Williams, 1986; Agounké et al., 1988; Willink and Moore, 1988; Bokonon-Ganta et al., 1995; Tobih et al., 2002). *Rastrococcus iceryoides* on the other hand is restricted to East Africa (mainly Tanzania and coastal Kenya) and northern Malawi where it has remained a major pest of mango (Williams, 1989; Luhanga and Gwinner, 1993; CABI, 2000). Compared with *R. invadens*, very little is known with regard to the ecology of *R. iceryoides* and no detailed studies have been conducted on the geographic distribution and abundance of this pest in Kenya and Tanzania.

As with other mealybug species, *R. iceryoides* sucks sap from leaves, young shoots, inflorescences and fruits and sometimes results in shedding of mango fruit-lets. It also excretes sugary honeydew on which sooty moulds develop thereby reducing fruit marketability. As a result of sooty mould, export opportunities are often impaired due to quarantine regulations (CPC, 2002). Sooty mould that fouls the leaves reduces photosynthetic efficiency and can cause leaf drop. In village homesteads, heavy infestation usually renders the trees unsuitable for shading. In Kenya, Tanzania and Malawi, damage can range from 30% to complete crop failure in unmanaged orchards (CABI, 2000; Tanga, unpublished data). In Tanzania, the pest has become the major target for majority of insecticidal sprays on mango, in addition to pruning and burning of infested plant's parts (Willink and Moore, 1988; Tanga, unpublished data), which is not an affordable solution. Unfortunately, insecticides do not generally provide adequate control of mealybugs owing to their waxy coating. Some growers have even resorted to cutting down mango trees as a result of *R. iceryoides* destruction while others have abandoned mango cultivation. It is speculated that the intensity of damage by *R. iceryoides* may have been due to the expansion of mango production and introduction of hybrid cultivars, which are highly susceptible to attack by the pest (Boussienguet and Mouloungou, 1993).

In Southern Asia, the putative aboriginal home of *R. iceryoides*, the pest is believed to be highly polyphagous and has been reported from over 65 host plants from 35 families (Williams, 1989; Ben-Dov, 1994). However, in Africa, there's still no comprehensive knowledge on the host plants of *R. iceryoides* apart from the damage observed on mango crop. To be able to make an informed decision to manage the pest effectively, with regard to trap placement within orchards, sanitation and mixed-cropping practices, the growers must be aware of the host-plant relationships of *R. iceryoides*.

Natural enemies play an important role in regulating the populations of mealybugs and globally there are several success stories of biological control of different species of mealybug including Africa (Neuenschwander, 2001; Bokonon-Ganta and Neuenschwander, 1995; Kairo et al., 2000; Meyerdirk et al., 2004). Despite the importance of natural enemies in suppressing population of mealybugs; and since the introduction of *R. iceryoides* into the continent in the late twentieth century (CABI, 2000), no information exists in literature on the natural enemy compositions of the pest in Africa. However, in India, a diversity of parasitoids and predators has been reported to regulate the populations of *R. iceryoides* (Tandon and Lal, 1978; CABI, 2000). To guide future management interventions, the indigenous natural enemies associated with *R. iceryoides* must be quantified. Information on the distribution, host range, abundance and associated natural enemies of *R. iceryoides* can provide basic information for developing reliable and cost-effective management method for the pest. As part of an ongoing larger project on integrated pest management (IPM) of major mango pests, the objectives of this study were to assess the geographic distribution of *R. iceryoides* in the coastal regions of Kenya and Tanzania, establish its host-plant relationships and document the natural enemies associated with the pest in these countries.

3.2 Materials and Methods

3.2.1 Field surveys

3.2.1.1 Sampling sites

Field surveys were conducted in twenty-two localities across the Coastal and Rift Valley Provinces of Kenya and twelve localities in five different Regions of Tanzania (Table 3.1, Figure 3.1) between February and June 2008. The sampling sites in both countries were chosen based on previous knowledge of horticultural production and especially mango in the various localities. These provinces and regions are regarded as the major mango production areas of Kenya and Tanzania (Greisbach, 2003; Nyambo and Verschoor, 2005). In both countries, sampling was carried out in cultivated fields, backyard gardens, woodlands, roadside, forested areas and protected reserves. At each location, the position of each sampled site (approximate latitude, longitude and altitude) was taken using a Global Positioning System (GPS) device (Table 3.1).

3.2.2 Plant collection, handling and assessment of infestation

Plants were sampled using the destructive sampling technique. At each location 80 leaves and, 20 twigs (≈10 cm length) were plucked or excised at random from different host plants records from literature. When available, 5 fruits were also randomly picked from target host plants. Plant parts were individually transferred to paper bags and transported to the laboratory in cool boxes. In the laboratory, tally counters were used to quantify the total number of *R. iceryoides* per sampled plants parts using a head lens and or stereomicroscope. Severity of mealybug infestation for each locality and host plant was scored from the sampled foliage, twigs, and fruits following the scale developed by Tobih et al. (2002) for *R. invadens* with slight modification (see Table 3.2). Infestation by *R. iceryoides* was also expressed as the total number of mealybugs of all developmental stages per plant part sampled for each locality.

From the field collected mealybug, three to five adult mealybug samples were randomly selected and slide-mounted using the methodology of Watson and Kubiriba (2005) at the *icipe* Biosystematics Unit to confirm their identity. Reference samples of the mealybugs were maintained at the Unit. Samples of leaf and or twig and fruit (for small fruit) from unknown plant species were collected, pressed and bagged. The collected plant samples were identified using the keys of Kenya trees, shrubs and lianas (Beenjte, 1994). Photographs were also taken of each plant and or fruit sampled to aid in plant identification and voucher specimens of all collections of the plant species are maintained at *icipe*. The plant nomenclature system used conforms to the International Plant Names Index database (IPNI, 2005) and the Missouri Botanical Garden database W^3 TROPICOS (MBOT, 2006).

3.2.3 Parasitoid, predator and ant species associated with *R. iceryoides*

After the census of mealybugs on infested plant parts, live and mummified specimens were transferred into plastic paper bags with well-ventilated tiny openings made using entomological pins # 000 (length 38 mm, 0.25 mm diameter) or transparent plastic rearing containers (22.5 cm height x 20 cm top diameter x 15 cm bottom diameter). An opening (10 cm diameter) was made on the front side of the cage to which a sleeve, made from very fine organza material (about 0.1 mm mesh size) was fixed. The same material was fixed to the opposite opening (10 cm diameter) of the cage to allow for ventilation. A third opening (13 cm diameter) was made on the roof of the cage, which was also screened with the same material. Streaks of undiluted honey were applied to the roof of the cages and maintained in the laboratory at 25 ± 2^{o}C, 70 ± 10% RH, photoperiod of 12:12 (L: D) h and ambient temperatures (26-28^{0}C) until parasitoid emergence. Mummies with emergence holes were discarded after counting. Mummified mealybugs from each infested host plant species and locality were maintained separately. Parasitoids that emerged from the mealybug cultures were collected daily and counted. All parasitoids that emerged were initially identified at Annamalai University, India and later confirmed at the National collection of Insects, PPRI-Agricultural Research Council (ARC), Pretoria, South Africa.

At each sampling date and site, predators of *R. iceryoides* were sampled by beating 10 randomly selected branches of each host plants over a 1 – m^2 cloth screen using a 60 cm long stick. The sampling was done during the early hours of the morning of 8:30-9:30 am. The predators that were dislodged onto the cloth were then recorded and preserved in 70 % ethyl alcohol. Immature stages of predators were reared on mealybugs in transparent plastic rearing containers (22 cm length x 15 cm width x 15 cm height) with an opening (10 cm diameter) made on the front side of the plastic container to which a sleeve, made of organza material was fixed. The set up was maintained at 26-28˚C, 60 - 80% relative humidity (RH), under a photoperiod of

12L: 12D in the laboratory at the National Biological Control Programme (NBCP), Kibaha, until they developed to the adult stage and later counted.

For ant sampling, surveys were carried out weekly during the dry season (December to March) in a 10 hectres mango orchard grown according to standard agronomic practices with no pesticides application in Kibaha. The orchard was selected on the basis of availability and accessibility of major ant species observed. The interactions between ant and mealybug populations in the orchard were randomly assessed by means of visual inspection. Thereafter, two ant-infested plants with mealybugs were randomly selected on each survey date for the incidence of mummified mealybugs, as affected by the presence of ant species. On each plant, 2 twigs (≈20 cm length) having ants tending mealybugs were cut and placed individually in plastic bags, and taken to the laboratory for examination. All mealybugs (life stages and mummified mealybugs) and ants found on each twig was counted and recorded. Mummified mealybugs from each sampled twig were kept in closed polyethylene containers (2.5 cm diameter x 6 cm height) with perforated lids for ventilation. Samples were maintained under laboratory conditions of 26 ± 2°C, 60–80% RH, and 12:12 (L:D) h for possible emergence of parasitoids. During the survey, care was taken to make sure that no tree was sampled twice within the same month. Ants were identified by Dr. Seguni Z.S.K, Mikocheni Agricultural Research Institute, Dar es Salaam, Tanzania.

3.2.4 Statistical analysis

Data for field surveys are presented according to plant species, family, location, infestation levels, severity of attack, number of emerged parasitoids, percentage parasitism and number of predators. Infestation by *R. iceryoides* was expressed as the total number of mealybugs of all developmental stages per number of plant part sampled for each locality. Parasitism was expressed as percentage of the number of emerged parasitoid species to the total number of hosts in the samples for each locality. The data on mealybug infestation and parasitism rates were compared across plant parts by subjecting the data to *t* test or one-way ANOVA using the generalized linear model (Proc GLM) after log (x + 1) and angular transformation, respectively to normalize variance before statistical analysis. Means were separated by Tukey honestly significant difference (HSD) test ($P = 0.05$). The overall effect of ant presence was calculated from the regression between ant species on mealybug colony size and number of mummified mealybugs. All computations were performed using SAS 9.1 software (SAS Institute, 2010).

Table 3. 1: Sampling sites for *Rastrococcus iceryoides* and associated natural enemies with geo-referenced positions and altitude

Country/locality	Longitude	Latitude	Eleveation (m a. s. l)
Kenya			
Galana	03° 11' 89" S	040° 06' 86" E	8
Mombasa	04° 03' 61" S	039° 40' 21" E	12
Loka-Chumani	03° 28' 84" S	039° 53' 77" E	14
Lamu	02° 16' 07" S	040° 54' 01" E	18
Mtangani	03° 11' 77" S	040° 05' 25" E	34
Malindi	03° 10' 74" S	040° 07' 23" E	40
Matuga	04° 11' 02" S	039° 33' 38" E	109
Kinango	04° 07' 05" S	039° 25' 27" E	121
Kilifi	03° 42' 01" S	039° 49' 44" E	136
Shimba Hills	04° 15' 24" S	039° 27' 19" E	363
Maungu	03° 33' 45" S	038° 44' 91" E	523
Voi	03° 27' 04" S	038° 22' 02" E	591
Ikanga	03° 22' 61" S	038° 34' 02" E	591
Mwatate	03° 30' 08" S	038° 22' 43" E	843
Kigala	03° 22' 18" S	038° 28' 54" E	854
Ndome	03° 17' 65" S	038° 28' 59" E	866
Kamleza	03° 27' 02" S	037° 41' 65" E	887
Taveta	03° 23' 52" S	037° 40' 61" E	901
Madabogo	03° 27' 12" S	038° 27' 11" E	943
Dembwa	03° 27' 05" S	038° 22' 03" E	1049
Wundanyi	03° 23' 61" S	038° 22' 08" E	1323
Kungu	03° 25' 01" S	038° 21' 09" E	1480
Tanzania			
Bagamoyo	06˚ 36' 23" S	039˚ 05' 13"E	26
Tanga	04˚ 58' 91" S	039˚ 05' 24" E	47
Kibaha	06˚ 43' 84" S	038˚ 46' 07" E	79
Mkuranga	07˚ 04' 05" S	039˚ 15' 63" E	93
Kinondoni	06˚ 45' 80" S	039˚ 06' 25" E	162
Vomero	06˚ 14' 71" S	037˚ 33' 25" E	364
Turiani	06˚ 16' 29" S	037˚ 32' 68" E	366
Mikese	06˚ 45' 04" S	037˚ 52' 46" E	423
Kilosa	06˚ 41' 44" S	037˚ 07' 47" E	441
Ilonga	06˚ 46' 35" S	037˚ 02' 46" E	489
Kyela	09˚ 28' 10" S	033˚ 53' 16" E	503
Morogoro	06˚ 50' 69" S	037˚ 39' 83" E	522

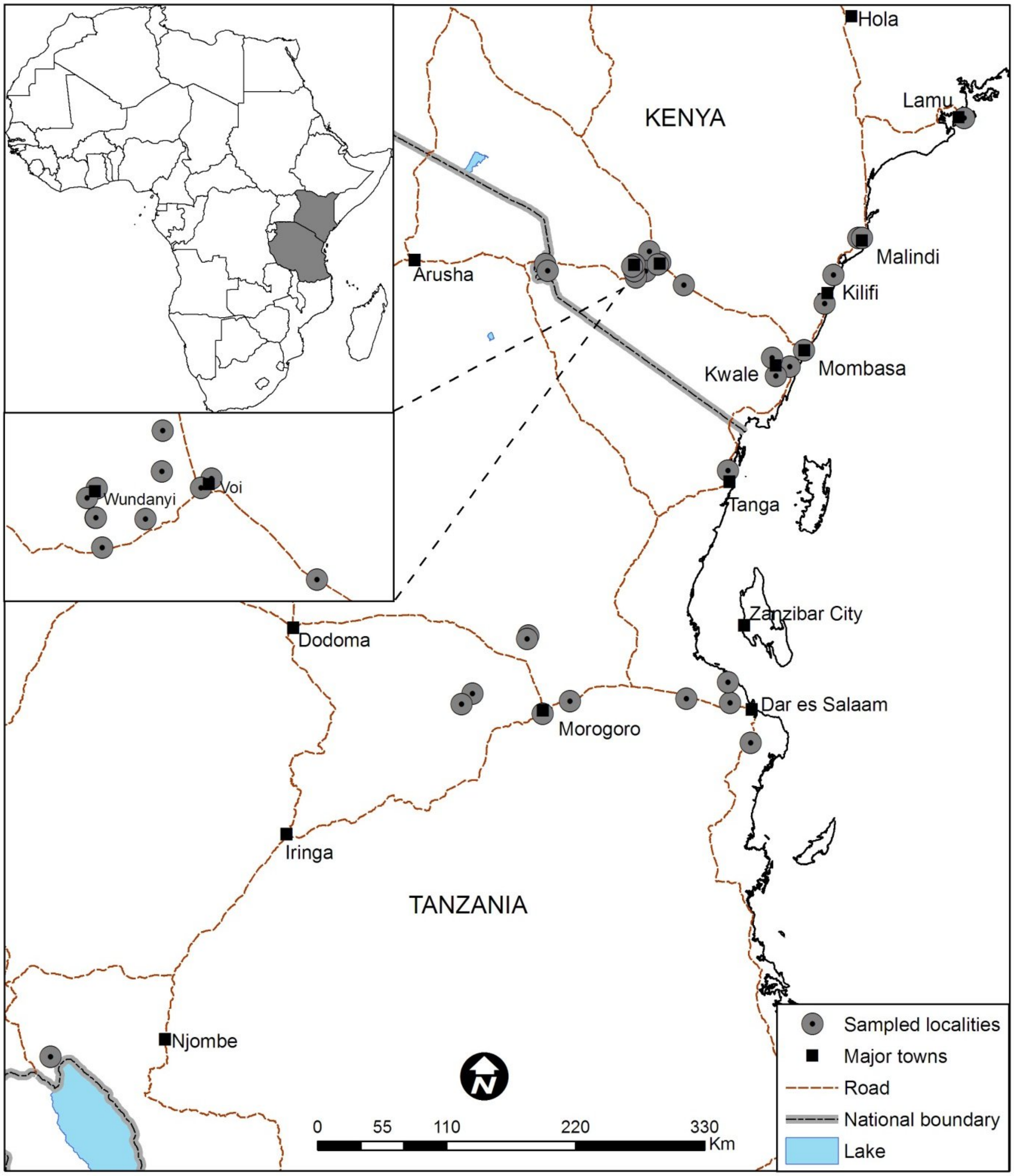

KENYA
TANZANIA
Hola
Lamu
Malindi
Kilifi
Mombasa
Kwale
Arusha
Tanga
Zanzibar City
Dodoma
Dar es Salaam
Morogoro
Iringa
Njombe
Wundanyi
Voi
Sampled localities
Major towns
Road
National boundary
Lake
0
55
110
220
330
Km
N

Figure 3. 1: Map of Kenya and Tanzania showing locations of sites sampled for mealybug.

Table 3. 2 Classification of severity of host plant infestation by *Rastrococcus iceryoides* in the field during the survey

Degree of infestation	Description of severity of infestation
I: Uninfested	0% infestation observed
II: Low	1 – 25% of the host part showed infestation by the mealybug usually on the abaxial surfaces of the foliage
III: Moderate	26–60% of the host part showed mealybug infestation together with sooty mould on both surfaces of foliage or twig
IV: Severe	61–100% of entire foliage, twigs, inflorescences and sometimes fruits, are completely covered by the mealybugs and sooty mould

3.3 Results

3.3.1 Distribution

In the Coast Province of Kenya, out of the 22 localities sampled, *R. iceryoides* was recorded from 12 sites—Mombasa, Malindi, Matuga, Kinango, Kilifi, Voi, Ikanga, Mwatate, Kigala, Ndome, Kamleza and Taveta—but with varying degrees of infestation (Table 3.3). The heaviest infestation on twig of *P. aculeata* was recorded in Kinango (7892 mealybugs/20 twigs). The heaviest infestation on twigs of *M. indica* was recorded in Matuga (3654 mealybugs/20 twigs) followed by Mombasa (971 mealybugs/20 mango twigs) and Malindi (881 mealybugs/20 mango twigs) (Table 3.3).

In Tanzania, *R. iceryoides* was recorded from all localities sampled (Tables 3.1 and Table 3.3). Among all the locations sampled, infestation was heaviest in Morogoro and Kibaha (8325 and 8153 mealybugs/20 mango twigs, respectively) followed by Kinondoni (6868 mealybugs/20 mango twigs) and lowest in Vomero (142 mealybugs/20 twigs) (Table 3.3).

3.3.2 Host-plants

During the survey, *R. iceryoides* was recorded from 29 plant species from 16 families. Twenty-one of these plant species are new records for Africa and the world. Host plants positive for *R. iceryoides* included both cultivated and wild host plants (Table 3.3).

In Kenya, among the plant species sampled, *R. iceryoides* was recorded from only six host plants. These are: *Parkinsonia aculeata* L. [Fabaceae], *M. indica* [Anacardiaceae], *Ficus benghalensis* L. [Moraceae.], *Manilkara zapota* L. [Sapotaceae], *Psidium guajava* L. [Myrtaceae] and *Citrus aurantifolia* Swingle [Rutaceae] (Table 3.3). Among the cultivated host plants, severe infestation was recorded on mango, *M. indica,* in all the localities with mealybugs (nymphs and adults) ranging from 215 to 516 mealybugs/80 leaves and 568 to 3654 mealybugs/20 twigs (Table 3.3). The most important wild host plant was *P. aculeata* with infestation ranging from 11–17 mealybugs/80 leaves and 3467–7892 mealybug/20 twigs (Table 3.3). In the heavily infested plants such as mango and *P. aculeata*, twigs recorded significantly higher mealybugs than the other plant parts: Matuga on *M. indica* (t = -6.94; df = 21; P < 0.0001) and *P. aculeata* (t = -6.96; df = 23; P < 0.0001), Mombasa on *M. indica* (t = -2.85; df =

12; P = 0.0146), Malindi on *M. indica* (t = -5.11; df = 25; $P < 0.0001$), and Kinango on *P. aculeata* (F = 12.25; df = 2,51; $P < 0.0001$) (Table 3.3).

In Tanzania, *R. iceryoides* attack was noted on 27 host plants. Host plants with heavy infestations included *M. indica*, *P. aculeata*, *Osyris lanceolata* Hochst & Steud [Santalaceae], *Caesalpinia sepiaria* Roxb. [Fabaceae], *Artocarpus heteophyllus* Lam., *Cajanus cajan* (L.) Millsp. [Fabaceae], *Annona muricata* L. [Annonaceae] and *Deinbollia borbonica* Scheff. [Anacardiaceae]. Among the cultivated host plants, infestation was severe on mango (211–6054 mealybugs/80 leaves, 142–8325 mealybugs/20 twigs, 2979 mealybugs/5 fruits) and *C. cajan* (87–1452 mealybugs/80 leaves, 457–4672 mealybugs/20 twigs) followed by *P. guajava* (218–435 mealybugs/5 fruits) across localities compared with the other cultivated host plants sampled (Table 3.3). On heavily infested mango (in Morogoro) and pigeon pea (in Kibaha), twigs recorded significantly higher mealybugs than the other plants parts, (t = -2.89; df = 67; P = 0.0051 and t = -4.19; df = 39; P = 0.0002, for mango and pigeon pea, respectively) (Table 3.3).

Other host plants of low to moderate importance in Tanzania include *Artocarpus heterophyllus* Lam. [Moraceae], *Harrisonia abyssinica* Oliv. [Simaroubaceae], *Indigofera spicata* Forsk [Papilionaceae], *Annona squamosa* Linn.[Annonaceae], *Dialium holtzii* Harms [Caesalpiniaceae], *Lecaniodiscus fraxinifolius* Baker [Sapindaceae], *C. aurantifolia*, *C. sinensis* Linn. and *Solanum indicum* Linn. [Solanaceae] with infestation ranging from 34 - 129 mealybugs/80 leaves and 221 - 321 mealybugs/20 twigs, across the various localities sampled.

Rastrococcus iceryoides was also recorded from *Morus alba* Linn. [Moraceae], *Sorindeia madagascariensis* Thou. [Ancardiaceae], *Annona stenophylla* Engl. & Diels. [Annonaceae], *Musca paradisiaca* Linn. [Musaceae], *Annona senegalensis* Pers. [Annonaceae], *Ficus vallis-choudae* Delile [Moraceae], *Dalbergia melanoxylon* Guill & Perr [Papilionaceae], *Flueggea virosa* Voigt [Euphorbiaceae], and *Clerodendrum hohnstonii* Oliv. [Verbenaceae] but infestation on these host plants did not exceed 66 mealybugs/20 twigs.

Other mealybug species were also encountered, although at negligible levels on mango and included: *Icerya seychellarum* (Westwood), *Pseudococcus longispinus* (Targioni-Tozzetti), *Planococcus citri* (Risso), *Ferrisia virgata* (Cockerell)*, Icerya aegyptiaca* (Douglas)*, Phenococcus solenensis* (Tinsley)*, Nipaecoccus nipae* (Maskell) and *Planococcus kenyae* (Le Pelley).

Table 3. 3: Distribution, host plants and infestation of *R. iceryoides* in Kenya and Tanzania

Country/			No. of *R. iceryoides*			Severity of attack			Statistics		
Locality	Plant species	Plant family	Leaves	Twigs	Fruits	S	M	L	*T* or *F*	*df*	*P*
Kenya											
Mombasa	*Mangifera indica* Linn.	Anacardiaceae	422	971	-		+		-2.85	12	0.0146
	***Ficus benghalensis* Linn.	Moraceae	190	358				+	-1.56	14	0.1423
Malindi	*Manilkara zapota* Linn.	Sapotaceae	7	69	-			+	-2.97	8	0.0178
	Mangifera indica Linn.	Anacardiaceae	374	881	-		+		-5.11	25	<0.0001
Matuga	*Mangifera indica* Linn.	Anacardiaceae	516	3654	-	+			-6.94	21	<0.0001
	Citrus aurantifolia Swingle	Rutaceae	3	27	-			+	-2.70	4	0.0539
	Psidium guajava Linn.	Myrtaceae	66	271				+	-0.03	17	0.9729
	Parkinsonia aculeata Linn.	Fabaceae	17	3467	-	+			-6.96	23	<0.0001
Kinango	*Parkinsonia aculeata* Linn.	Fabaceae	11	7892	42	+			12.25	2,51	<0.0001
Kilifi	*Mangifera indica* Linn.	Anacardiaceae	215	568	-		+		-4.25	14	0.0008
Voi	*Mangifera indica* Linn.	Anacardiaceae	161	723	-		+		-3.01	29	0.0021
Ikanga	*Mangifera indica* Linn.	Anacardiaceae	9	23	-			+	-2.11	17	0.0441
Mwatate	*Mangifera indica* Linn.	Anacardiaceae	34	101	-			+	-0.21	21	0.6043
Kigala	*Parkinsonia aculeata* Linn.	Fabaceae	13	3101	-		+		-7.03	32	<0.0001
Ndome	*Mangifera indica* Linn.	Anacardiaceae	26	115	-			+	-2.76	11	0.0201
Kamleza	*Mangifera indica* Linn.	Anacardiaceae	17	72	-			+	-2.18	10	0.0167
Taveta	*Mangifera indica* Linn.	Anacardiaceae	43	215	-			+	-2.44	14	0.0232
Tanzania											
Bagamoyo	*Mangifera indica* Linn.	Anacardiaceae	455	674	-		+		-0.51	17	0.6169
Tanga	*Mangifera indica* Linn.	Anacardiaceae	3603	5154	-	+			-3.55	39	0.0010
	Cajanus cajan Linn.	Fabaceae	98	1578	-		+		-3.86	19	0.0011

Plants parts samples based on 80 leaves, 20 twigs of 10 cm length and 5 fruits; ** = New record for *R. iceryoides* in Africa; - = plants were either not infested and omitted from analysis or not available during sampling; [a]Severity of attack: S = Severe; M = Moderate; L = Low; + = degree of attack.

Table 3.3 continues. Distribution, host plants and infestation of *R. iceryoides* in Kenya and Tanzania

Country/			No. of *R. iceryoides*			Severity of attack			Statistics		
Locality	Plant species	Plant family	Leaves	Twigs	Fruits	S	M	L	*T* or *F*	*df*	*P*
Tanzania											
Tanga	*Psidium guajava* Linn.	Myrtaceae	54	213	218			+	1.51	2,13	0.2567
	Citrus aurantifolia Swingle	Rutaceae	8	38	5			+	16.43	2,7	0.0023
Kibaha	** *Sorindeia madagascariensis* Thouars	Anacardiaceae	4	39	-			+	-5.56	5	0.0026
	** *Annona stenophylla* Engl. & Diels.	Annonaceae	15	66	-			+	-0.99	6	0.3589
	** *Phyllanthus engleri* Pax	Euphorbiaceae	112	837	-		+		-3.15	18	0.0055
	***Artocarpus heterophyllus* Lam.	Moraceae	77	321	-			+	-1.90	20	0.0721
	** *Annona squamosa* Linn.	Annonaceae	13	278	-			+	-3.97	13	0.0016
	Psidium guajava Linn.	Myrtaceae	6	123	435		+		3.33	2,18	0.0587
	Musca paradisiaca Linn.	Muscaceceae	8	0	-			+	1.86	2	0.2036
	** *Annona senegalensis* Pers.	Annonaceae	2	11	-			+	-0.76	2	0.5264
	** *Ficus vallis-choudae* Delile	Moraceae	0	25	-			+	-1.79	3	0.1713
	** *Dialium holtzii* Harms	Caesalpiniaceae	127	566	-		+		-1.51	11	0.1604
	Cajanus cajan (L) Millsp.	Fabaceae	388	3359	-	+			-4.19	39	0.0002
	***Annona muricata* Linn.	Annonaceae	234	1334	-		+		-2.94	9	0.0165
	***Dalbergia melanoxylon* Guill & Perr	Papilionaceae	0	66	-			+	-1.75	3	0.1778
	** *Flueggea virosa* Voigt	Euphorbiaceae	0	23	-			+	-2.49	4	0.0675
	** *Clerodendrum johnstonii* Oliv.	Verbenaceae	1	4	-			+	-0.50	2	0.6667
	** *Lecaniodiscus fraxinifolius* Baker	Sapindaceae	44	231	-			+	-1.60	10	0.1403
	Mangifera indica Linn.	Anacardiaceae	6054	8153	-	+			-2.25	68	0.0277
	** *Solanum indicum* Linn.	Solanaceae	63	314	-			+	-0.86	9	0.4124
	** *Deinbollia borbonica* Scheff.	Sapindaceae	215	2253	-			+	-2.73	36	0.0099
Mkuranga	*Mangifera indica* Linn.	Anacardiaceae	1223	3417	-	+			-2.39	32	0.0231

Plants parts samples based on 80 leaves, 20 twigs of 10 cm length and 5 fruits; ** = New record for *R. iceryoides* in Africa; - = plants were either not infested and omitted from analysis or not available during sampling; [a]Severity of attack: S = Severe; M = Moderate; L = Low; + = degree of attack.

Table 3.3 continues. Distribution, host plants and infestation of *R. iceryoides* in Kenya and Tanzania

Country/ Locality	Plant species	Plant family	No. of *R. iceryoides*			Severity of attack			Statistics		
			Leaves	Twigs	Fruits	S	M	L	*T* or *F*	*df*	*P*
Tanzania											
Kinondoni	*Mangifera indica* Linn.	Anacardiaceae	3865	6868	2979	+			4.70	2,73	0.0120
	Citrus aurantifolia Swingle	Rutaceae	34	122	-			+	-0.53	7	0.6150
	Citrus sinensis Linn.	Rutaceae	118	313	-			+	-1.81	12	0.0952
	***Artocarpus heterophyllus* Lam.	Moraceae	129	326	-			+	-2.23	20	0.0372
	***Morus alba* Linn.	Moraceae	1	5	-			+	-1.0	2	0.4226
	Parkinsonia aculeata Linn.	Fabaceae	24	5567	-	+			-4.82	47	<0.0001
	** *Osyris lanceolata* Hochst. & Steud.	Santalaceae	2	2356	-	+			-3.25	13	0.0063
	** *Harrisonia abyssinica* Oliv.	Simaroubaceae	57	358	-			+	-4.70	7	0.0022
	***Indigofera spicata* Forsk	Papilionaceae	34	221	-			+	-1.57	9	0.1518
	** *Caesalpinia sepiaria* Roxb.	Fabaceae	266	3116	-	+			-3.97	35	0.0003
Vomero	*Mangifera indica* Linn.	Anacardiaceae	335	142	-			+	1.14	20	0.2695
Turiani	*Mangifera indica* Linn.	Anacardiaceae	211	967	-		+		-3.56	26	0.0015
	***Annona muricata* Linn	Annonaceae	5	49	-			+	-3.47	5	0.0179
	Citrus aurantifolia Swingle	Rutaceae	3	21	-			+	-2.47	3	0.0903
Mikese	*Mangifera indica* Linn.	Anacardiaceae	814	3578	-	+			-4.88	41	<0.0001
Kilosa	*Mangifera indica* Linn.	Anacardiaceae	87	237	-			+	0.64	15	0.5326
	Psidium guajava Linn.	Myrtaceae	9	40	-			+	-1.44	6	0.2002
Ilonga	*Mangifera indica* Linn.	Anacardiaceae	62	421	-			+	-4.92	19	<0.0001
Kyela	*Mangifera indica* Linn.	Anacardiaceae	263	1700	-		+		-5.96	23	<0.0001
	Cajanus cajan Linn.	Fabaceae	87	457	-			+	-2.70	14	0.0171
Morogoro	*Mangifera indica* Linn.	Anacardiaceae	2563	8325	-	+	+		-2.89	67	0.0051
	Cajanus cajan (L) Millsp.	Fabaceae	1452	4672	-	+			-2.0	35	0.0530
	Citrus aurantifolia Swingle	Rutaceae	2	28	-			+	-2.67	5	0.0446

Plants parts samples based on 80 leaves, 20 twigs of 10 cm length and 5 fruits; ** = New record for *R. iceryoides* in Africa; - = plants were either not infested and omitted from analysis or not available during sampling; ^{a}Severity of attack: S = Severe; M = Moderate; L = Low; + = degree of attack.

3.3.3 Damage symptoms

Increased severity of attack on the abaxial and adaxial surfaces of the leaf led to distorted, stunted, withering and yellow leaves, which gradually dried up with ultimate premature shedding occurring (Figure 3.2a and Figure 3.2b). During the flowering period, affected panicles were observed to practically dry-up eventually causing the flowers to drop off prematurely as a result of the severe tip die-back effects (Figure 3.2c). On the other hand, immature fruits (less than a month old) were observed to shrivel and dry-up ultimately falling off in due course (Figure 3.2d). High incidence of reduced fruit-settings was commonly observed in heavily infested orchards with shedding of young fruits as a result of early ripening due to increased pressure exerted by the sucking pest on the fruit peduncle (Figure 3.2e and Figure 3.2f). During population outbreaks, high populations of *R. iceryoides* were observed to spread to mature fruit bunches (Figure 3.2g). Intense feeding by the mealybug on fruits resulted in rind pitting and scarring. In cases where the young branches supporting the leaves were heavily infested leaf drop occurred along with twig dieback. The incidence of heavily infested plant's parts drying up was also observed on other host plants, *C. sepiaria*, *O. lanceolata*, *I. spicata*, *P. aculeata,* and *C. cajan*. Symptoms of slow growth, lack of vigour and subsequent plant death under moisture-stress conditions was also observed in the field especially on newly planted mango seedlings in the orchards.

Copious amounts of sugary honeydew were also produced by *R. iceryoides*, which caused blackened-malformed and discolored fruits with severe cracks on the skin upon exposure to intense sunlight (Figure 3.2i and Figure 3.2h). In severe cases, it rendered the leaves completely black (Figure 3.2j), forcing most of the leaves to turn yellow and finally drying up.

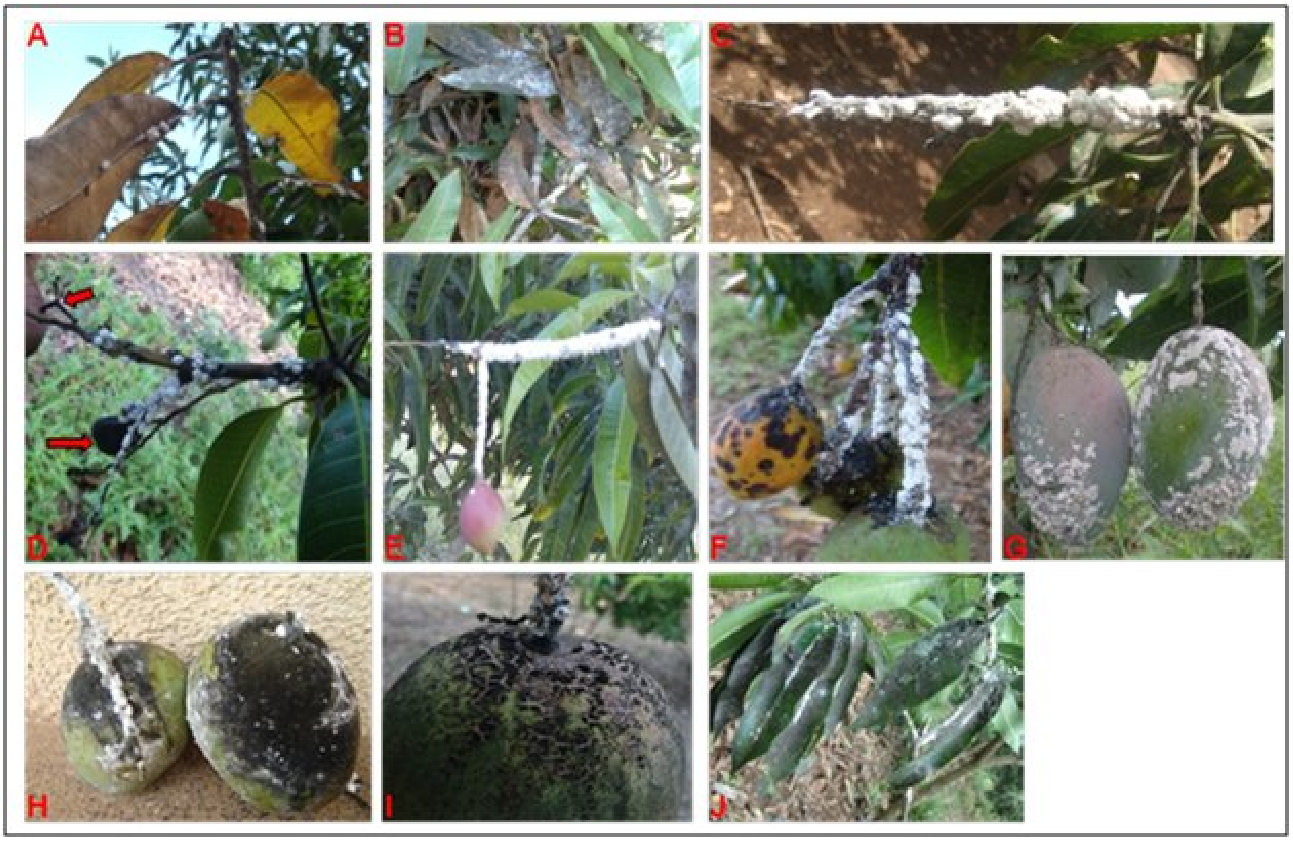
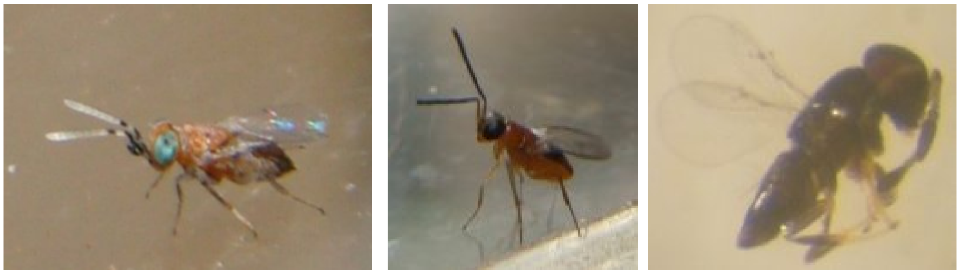


Figure 3. 2: Damage symptoms of the mango mealybug *R. iceryoides*, on the leaves (A, B & J), Inflorescence (C), immature fruits (D-The arrows indicate shriveled or drop-off immature dried fruits) and mature fruits (E, F, G, H & I).

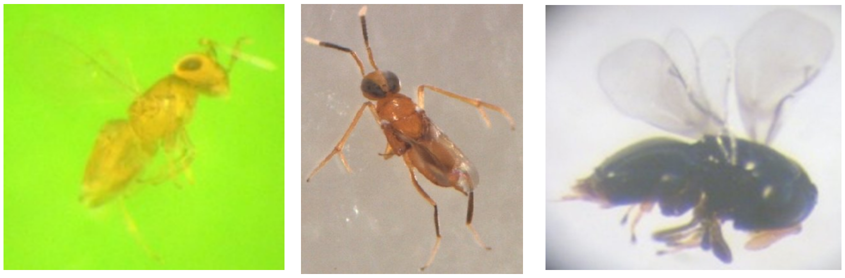
3.3.4 Parasitoid species associated with *R. iceryoides* on different host plants in Kenya and Tanzania

In Kenya, out of 20,021 *R. iceryoides* collected from the six host plant species, 4228 mealybugs were parasitized and yielded a parasitism rate of 21%. Among the mummified mealybugs collected in the field, 76% yielded adult parasitoids. The parasitoid community was composed of three parasitoid species: *Anagyrus pseudococci* Girault (Hymenoptera: Encyrtidae), *Leptomastrix dactylopii* Howard (Hymenoptera: Encyrtidae) and *Leptomastidea tecta* Prinsloo (Hymenoptera: Encyrtidae) with *A. pseudococci* accounting for 99% of the overall percentage parasitism on *R. iceryoides* on the different host plant species sampled. The level of parasitism varied across host plants as well as also host plant parts (Table 3.4). For example, in Matuga, parasitism rate on mango was at 5% on leaves and 20% on twigs with an overall rate of 17%. While at Kinango, parasitism rate on *P. aculeata* was 73% on leaves and 20% on twigs with an overall rate of 20% (Table 3.4).

In Tanzania, a total of 109,824 *R. iceryoides* were collected from 27 host plant species out of which 8529 were parasitized giving a percentage parasitism of 8%. Among the mummified mealybugs, 70% yielded adult parasitoids. Out of these emerged parasitoids, 80% were from *M. indica*. The parasitoid community was composed of five species, *Anagyrus aegyptiacus* Moursi, *Leptomastrix dactylopii* Howard, *Agarwalencyrtus citri* Agarwal, *Aenasius longiscapus* Compere and *A. pseudococci* Girault. The latter accounted for 95% of the overall percentage parasitism of *R. iceryoides* on all the host plant species sampled. The percentage parasitism of the different parasitoid species also varied considerably among the different host plant species and host plant parts (Table 3.4). For example, in Kilosa highest percent parasitism by *A. pseudococci* on *M. indica* was 3 and 27%, followed by Kibaha at 11 and 18% on leaves and twigs, respectively. Overall parasitism rate was 21% in Kilosa and 15% in Kibaha (Table 3.4).



Anagyrus pseudococci *Leptomastix dactylopii* *Agarwalencyrtus citri*

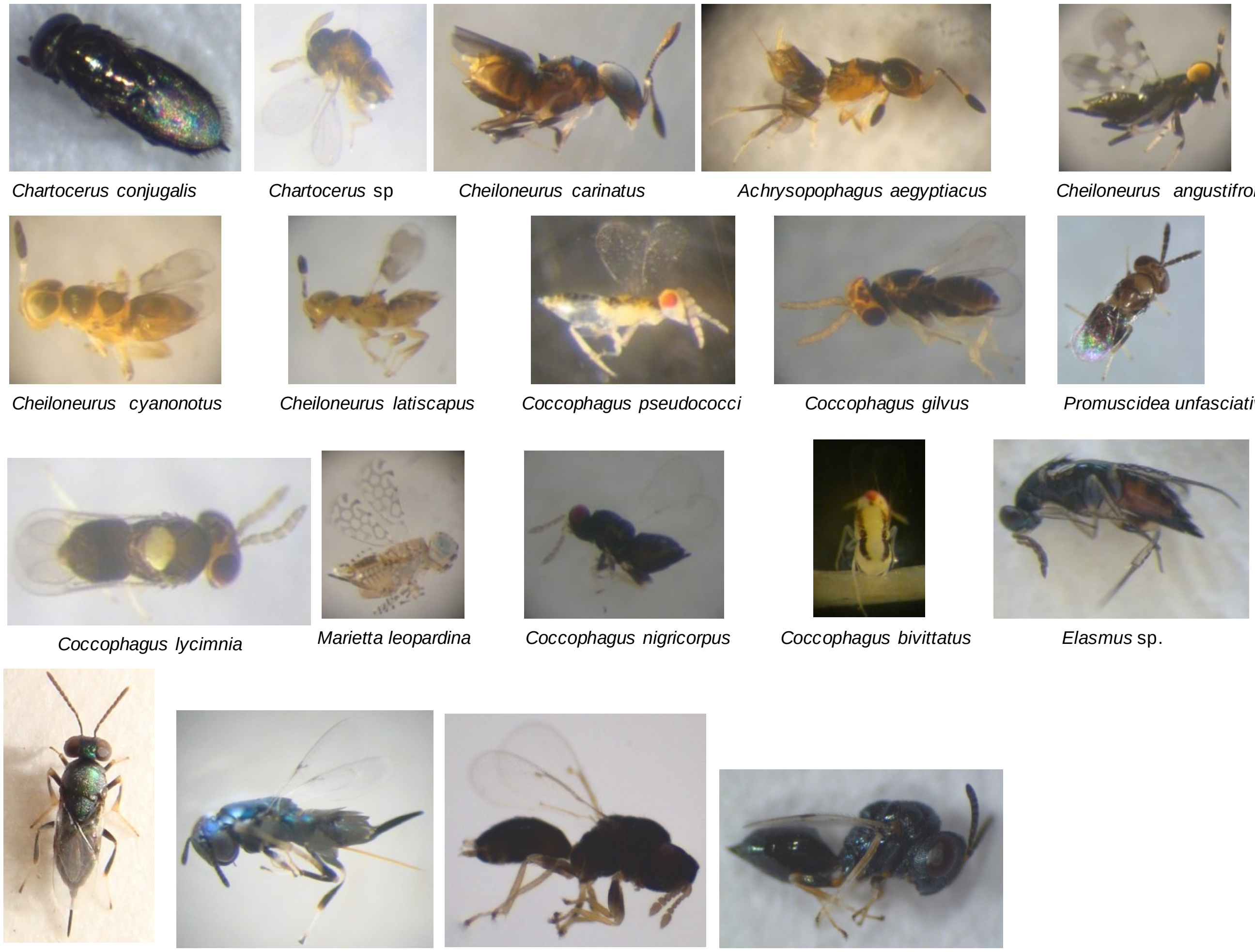


Anagyrus aegyptiacus *Leptomastidea tecta* *Aenasius longiscapus*

Figure 3. 3: Catalogue of indigenous primary parasitoids recovered from *R. iceryoides* in Kenya and Tanzania.

Ninteen species of hyperparasitoids were recorded in Kenya (1 species) and Tanzania (18 species). These included, 5 Encyrtidae (*Achrysopophagus aegyptiacus* Mercet, *Cheiloneurus carinatus*, sp.nov, *Cheiloneurus angustifrons* sp.nov, *Cheiloneurus cyanonotus* Waterston and *Cheiloneurus latiscapus* Girault); 7 Aphelinidae (*Promuscidea unfasciativentris* Girault, *Coccophagus gilvus* Hayat, *Coccophagus pseudococci* Compere, *Coccophagus bivittatus* Compere, *Marietta leopardina* Motschulsky, *Coccophagus lycimnia* (Walker) and *Coccophagus nigricorpus* Shafee); 2 Signiphoridae (*Chartocerus conjugalis* Mercet and *Chartocerus* sp); 1 Elasmidae (*Elasmus* sp.); 3 Pteromalidae (*Pachyneuron* sp. and 2 unidentified species) and 1 Eulophidae (*Tetrastichus flaviclavus* La Salle & Polaszek).

The number of hyperparasitoids found during the survey accounted for 7.57% (n = 487/6432) of the total parasitoid populations collected throughout the survey. Hyperparasitism was sporadic with the dominant species being *C. conjugalis* and *C. cyanonotus*. Among these hyperparasitoids, *Cheiloneurus cyanonotus* Waterston was the only polyphagous species observed to attack parasitized *R. iceryoides* and the pupae of the coccinelid, *Chilocorus nigrita* (Fabricus) with a total of 12 adults parasitoids recovered from 1 pupa of the coccinelid.



Chartocerus conjugalis — *Chartocerus* sp — *Cheiloneurus carinatus* — *Achrysopophagus aegyptiacus* — *Cheiloneurus angustifrons*

Cheiloneurus cyanonotus — *Cheiloneurus latiscapus* — *Coccophagus pseudococci* — *Coccophagus gilvus* — *Promuscidea unfasciativentris*

Coccophagus lycimnia — *Marietta leopardina* — *Coccophagus nigricorpus* — *Coccophagus bivittatus* — *Elasmus* sp.

Gen. et sp. indet. — Gen. et sp. indet. — *Tetrastichus flaviclavus* — *Pachyneuron* sp.

Figure 3. 4: Catalogue of indigenous hyperparasitoids recovered from *R. iceryoides* in Kenya and Tanzania.

Several parasitoid species were also recovered from other mealybug species found co-existing with *R. iceryoides* on mango. The most important primary parasitoid recovered from *Icerya seychellarum* (Westwood) was a parasitic Diptera, *Cryptochetum iceryae* (Williston) (Diptera: Cryptochetidae) and a hyperparasitoid, *Pachyneuron* sp. (Hymenoptera: Pteromalidae) (Figure 3.5). The primary parasitoid of *Ferrisia virgata* (Cockerel) was *Aenasius advena* Compere (Hymenoptera: Encyrtidae) and from *Nipaecoccus nipae* (Maskell) was *Euryishomyia washingtoni* Girault.

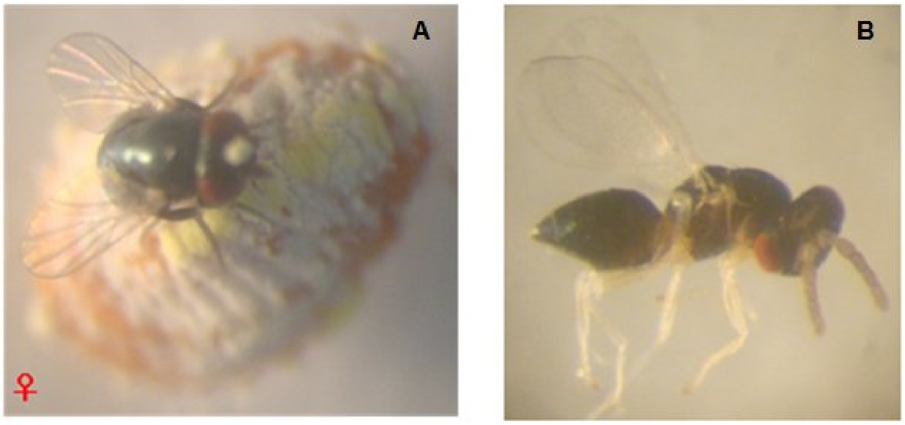


Figure 3. 5: (A) Parasitic Diptera, *Cryptochetum iceryae* (Williston) parasitizing *I. seychellarum*; (B) Hyperparasitoid, *Pachyneuron* sp. recovered from parasitized *I. seychellarum*.

Table 3. 4 Parasitoid complex associated with *R. iceryoides* on different host plants in Kenya and Tanzania

Country/ Locality	Parasitoid species	Plant species	Percentage parasitism			Overall % parasitism
			Leaves	Twigs	Fruits	
Kenya						
Mombassa	*Anagyrus pseudococci* Girault	*Mangifera indica* Linn.	12.32 (422)	8.75 (971)	-	9.83 (1393)
	Anagyrus pseudococci Girault	*Ficus benghalensis* Linn.	4.21 (190)	4.75 (358)	-	4.56 (548)
Matuga	*Anagyrus pseudococci* Girault	*Mangifera indica* Linn.	5.43 (516)	19.65 (3654)	-	17.89 (4170)
	Anagyrus pseudococci Girault	*Psidium guajava* Linn.	3.03 (66)	6.64 (271)	-	5.93 (337)
	Anagyrus pseudococci Girault	*Parkinsonia aculeata* Linn.	17.65 (17)	14.05 (3467)	-	14.06 (3484)
Kilifi	*Anagyrus pseudococci* Girault	*Mangifera indica* Linn.	3.72 (215)	12.68 (568)	-	10.22 (783)
Malindi	*Anagyrus pseudococci* Girault	*Mangifera indica* Linn.	8.29 (374)	10.78 (881)	-	10.04 (1255)
Kinango	*Anagyrus pseudococci* Girault	*Parkinsonia aculeata* Linn.	72.73 (11)	20.12 (7892)	-	20.19 (7903)
	Leptomastrix dactylopii Howard	*Parkinsonia aculeata* Linn.	18.18 (11)	0.09 (7892)	-	0.10 (7903)
	Leptomastidea tecta Prinsloo	*Parkinsonia aculeata* Linn.	9.09 (11)	0.13 (7892)	-	0.14 (7903)
Tanzania						
Kinondoni	*Anagyrus pseudococci* Girault	*Mangifera indica* Linn.	8.38 (3865)	3.13 (6868)	5.1 (2979)	5.04 (13712)
	Anagyrus aegyptiacus Moursi	*Mangifera indica* Linn.	0.21 (3865)	0.31 (6868)	0.10 (2979)	0.15 (13712)
	Leptomastrix dactylopii Howard	*Mangifera indica* Linn.	0.05 (3865)	0.19 (6868)	-	0.14 (10733)
	Agarwalencyrtus citri Agarwal	*Mangifera indica* Linn.	0.08 (3865)	-	-	0.08 (3865)
	Anagyrus pseudococci Girault	*Artocarpus heterophyllus* Lam.	5.43 (129)	2.76 (326)	-	3.52 (455)
	Anagyrus pseudococci Girault	*Parkinsonia aculeata* Linn.	20.83 (24)	5.64 (5567)	-	5.71 (5591)
	Anagyrus pseudococci Girault	***Indigofera spicata* Forsk	5.88 (34)	2.71 (221)	-	3.14 (255)
	Anagyrus pseudococci Girault	*Caesalpinia sepiaria* Roxb.	4.14 (266)	1.64 (3116)	-	1.83 (3382)
Mkuranga	*Anagyrus pseudococci* Girault	*Mangifera indica* Linn.	10.96 (1223)	9.45 (3417)	-	9.85 (4640)
Kibaha	*Anagyrus pseudococci* Girault	** *Phyllanthus engleri* Pax.	7.14 (112)	8.84 (837)	-	8.64 (949)
	Anagyrus pseudococci Girault	*Artocarpus heterophyllus* Lam.	2.60 (77)	4.98 (321)	-	4.52 (398)
	Anagyrus pseudococci Girault	** *Annona squamosa* Linn.	7.69 (13)	12.95 (278)	-	12.71 (291)
	Anagyrus pseudococci Girault	*Psidium guajava* Linn.	0	3.25 (123)	2.53 (435)	2.69 (558)
	Anagyrus pseudococci Girault	** *Dialium holtzii* Harms	5.51 (127)	3.36 (566)	-	3.75 (693)
	Anagyrus pseudococci Girault	*Cajanus cajan* (L) Millsp.	8.51 (388)	2.44 (3359)	-	3.07 (3747)
	Anagyrus pseudococci Girault	** *Lecaniodiscus fraxinifolius* Baker	4.55 (44)	2.60 (231)	-	2.91 (275)
	Anagyrus pseudococci Girault	*Mangifera indica* Linn.	11.22 (6054)	18.43 (8153)	-	15.36 (14207)

Table 3.4 Continues. Parasitoid complex associated with *R. iceryoides* on different host plants in Kenya and Tanzania

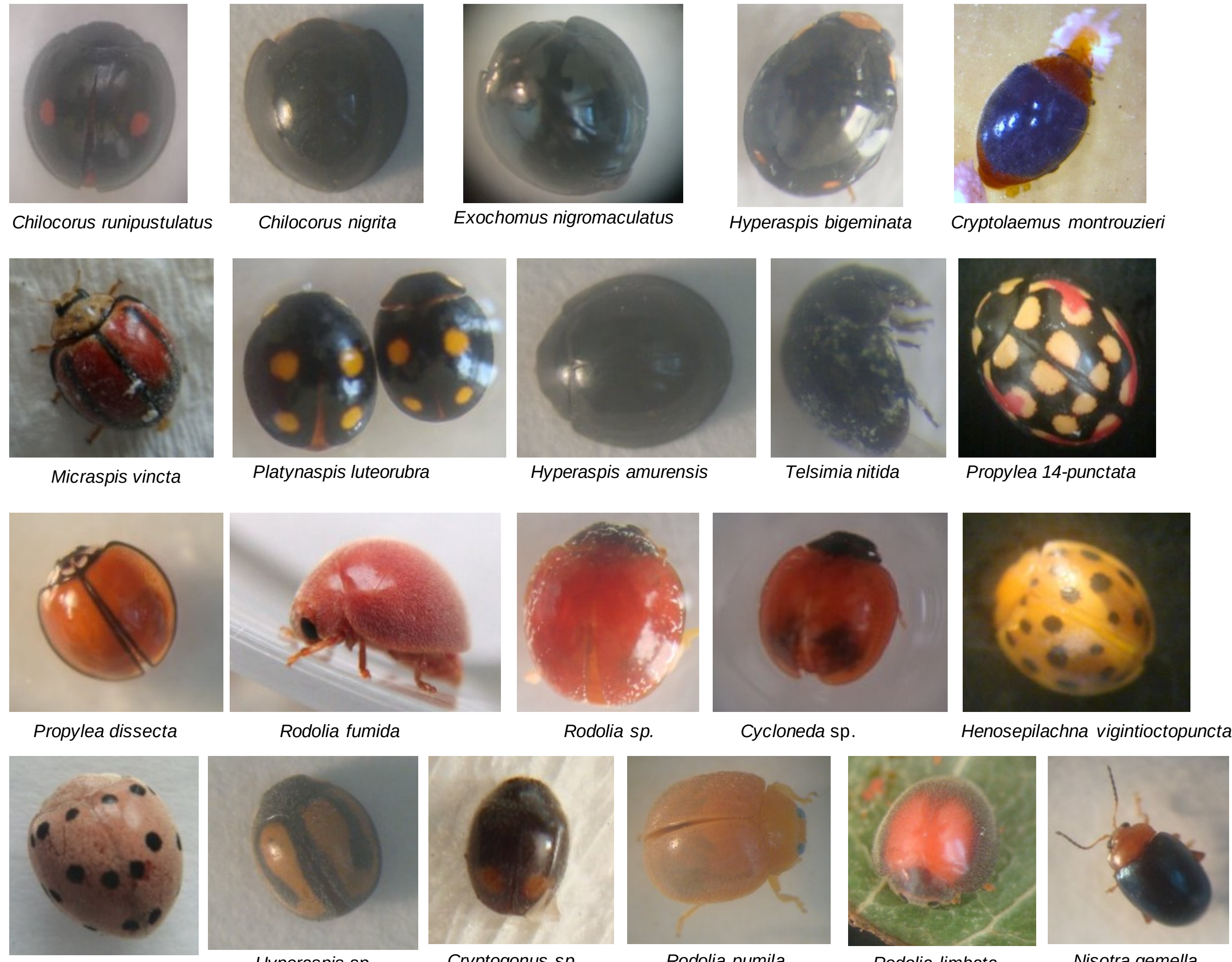
Country/ Locality	Parasitoid species	Plant species	Percentage parasitism			Overall % parasitism
			Leaves	Twigs	Fruits	
Tanzania						
Kibaha	*Anagyrus aegyptiacus* Moursi	*Mangifera indica* Linn.	0.38 (6054)	0.07 (8153)	-	0.20 (14207)
	Leptomastrix dactylopii Howard	*Mangifera indica* Linn.	0.18 (6054)	0.27 (8153)	-	0.23 (14207)
	Agarwalencyrtus citri Agarwal	*Mangifera indica* Linn.	0.03 (6054)	0.06 (8153)	-	0.05 (14207)
	Aenasius longiscapus Compere	*Mangifera indica* Linn.	0.03 (6054)	0.18 (8153)	-	0.12 (14207)
	Anagyrus pseudococci Girault	** *Solanum indicum* Linn.	3.17 (63)	6.69 (314)	-	6.10 (377)
	Anagyrus pseudococci Girault	** *Deinbollia borbonica* scheft	17.21 (215)	13.14 (2253)	-	13.49 (2468)
Bagamoyo	*Anagyrus pseudococci* Girault	*Mangifera indica* Linn.	9.67 (455)	16.62 (674)	-	13.82 (1129)
Morogoro	*Anagyrus pseudococci* Girault	*Mangifera indica* Linn.	2.61 (2563)	5.48 (8325)	-	4.80 (10888)
	Anagyrus pseudococci Girault	*Cajanus cajan* (L) Millsp.	3.86 (1452)	2.10 (4672)	-	2.51 (6124)
Mikese	*Anagyrus pseudococci* Girault	*Mangifera indica* Linn.	9.58 (814)	5.93 (3578)	-	6.60 (4392)
Turiani	*Anagyrus pseudococci* Girault	*Mangifera indica* Linn.	2.37 (211)	5.48 (967)	-	4.92 (1178)
Vomero	*Anagyrus pseudococci* Girault	*Mangifera indica* Linn.	6.57 (335)	9.15 (142)	-	7.34 (477)
Kilosa	*Anagyrus pseudococci* Girault	*Mangifera indica* Linn.	3.45 (87)	27.17 (237)	-	20.68 (324)
Ilonga	*Anagyrus pseudococci* Girault	*Mangifera indica* Linn.	8.06 (62)	9.98 (421)	-	9.73 (483)
Tanga	*Anagyrus pseudococci* Girault	*Mangifera indica* Linn.	5.91 (3603)	19.94 (311)	-	7.03 (3914)
	Anagyrus pseudococci Girault	*Cajanus cajan* (L) Millsp.	3.06 (98)	8.43 (1578)	-	8.11 (1676)
Kyela	*Anagyrus pseudococci* Girault	*Mangifera indica* Linn.	2.66 (263)	4.65 (1700)	-	4.38 (1963)
	Anagyrus pseudococci Girault	*Cajanus cajan* Linn.	8.05 (87)	7.00 (457)	-	7.17 (544)

** = indicate host plants native to Africa; - = indicates infested plant portions that were not available at the time of sampling. Numbers in parentheses represent the actual number of mealybug collected per plant portion during the survey.

3.3.5 Predator species associated with *R. iceryoides* on different host plants in Kenya and Tanzania

During the survey in Kenya and Tanzania, a total of 38 species of predators belonging to 14 families, Coccinellidae, Lycaenidae, Noctuidae, Hemerobiidae, Chrysopidae, Drosophilidae, Chamaemyiidae, Cecidomyiidae, Miturgidae, Salticidae, Sparassidae, Thomisidae, Oxyopidae and Nephilidae (Table 3.5) were found preying on *R. iceryoides* on different host plant. Figure 3.6, illutrates the different predatory beetles recorded during the survey in Kenya and Tanzania. Among the twenty species of predatory beetles, only 4 species were found in Kenya. *Chilocorus nigrita* Fabricus was the most abundant predatory beetle recorded in both countries, followed by *Chilocorus renipustulatus* Scriba, which was restricted to Tanzania. However, *Cacoxenus perspicax* Knab (Diptera: Drosophilidae) (Figure 3.7) was the most widespread and abundant predator species accounting for 78.8 and 89.3% of total predator collections in Kenya and Tanzania, respectively.

The predatory lepidoterans found preying on *R. iceryoides* during the survey were from two families: Lycaenidae and Noctuidae. *Spalgis lemolea* Druce (apefly) (Figure 3.8) was the the only species in the family Lycaenidae. Generally, *S. lemolea* activity was rarely noticed in the field probably because of its ability to camouflage with the mealybug colonies. Among the family Noctuidae, *Pyroderces badia* Hodges and *Thalpochares* sp. were recorded, with their larvae voraciously preying on the eggs (Figure 3.9) and on all the different stages of *R. iceryoides*, respectively. The mealybug-destroying moth, *Thalpochares* sp. builds itself a house, with fine silky webs interwoven with remains of the eaten-out mealybugs. With this protection against its enemies, it is able to walk over the trees and thus devours large number of mealybug populations daily (Figure 3.10).



Chilocorus runipustulatus *Chilocorus nigrita* *Exochomus nigromaculatus* *Hyperaspis bigeminata* *Cryptolaemus montrouzieri*

Micraspis vincta *Platynaspis luteorubra* *Hyperaspis amurensis* *Telsimia nitida* *Propylea 14-punctata*

Propylea dissecta *Rodolia fumida* *Rodolia sp.* *Cycloneda* sp. *Henosepilachna vigintioctopunctata*

Henosepilachna argus *Hyperaspis* sp. *Cryptogonus sp.* *Rodolia pumila* *Rodolia limbata* *Nisotra gemella*

Figure 3. 6: Catalogue of indigenous predatory beetles of *R. iceryoides* in Kenya and Tanzania.

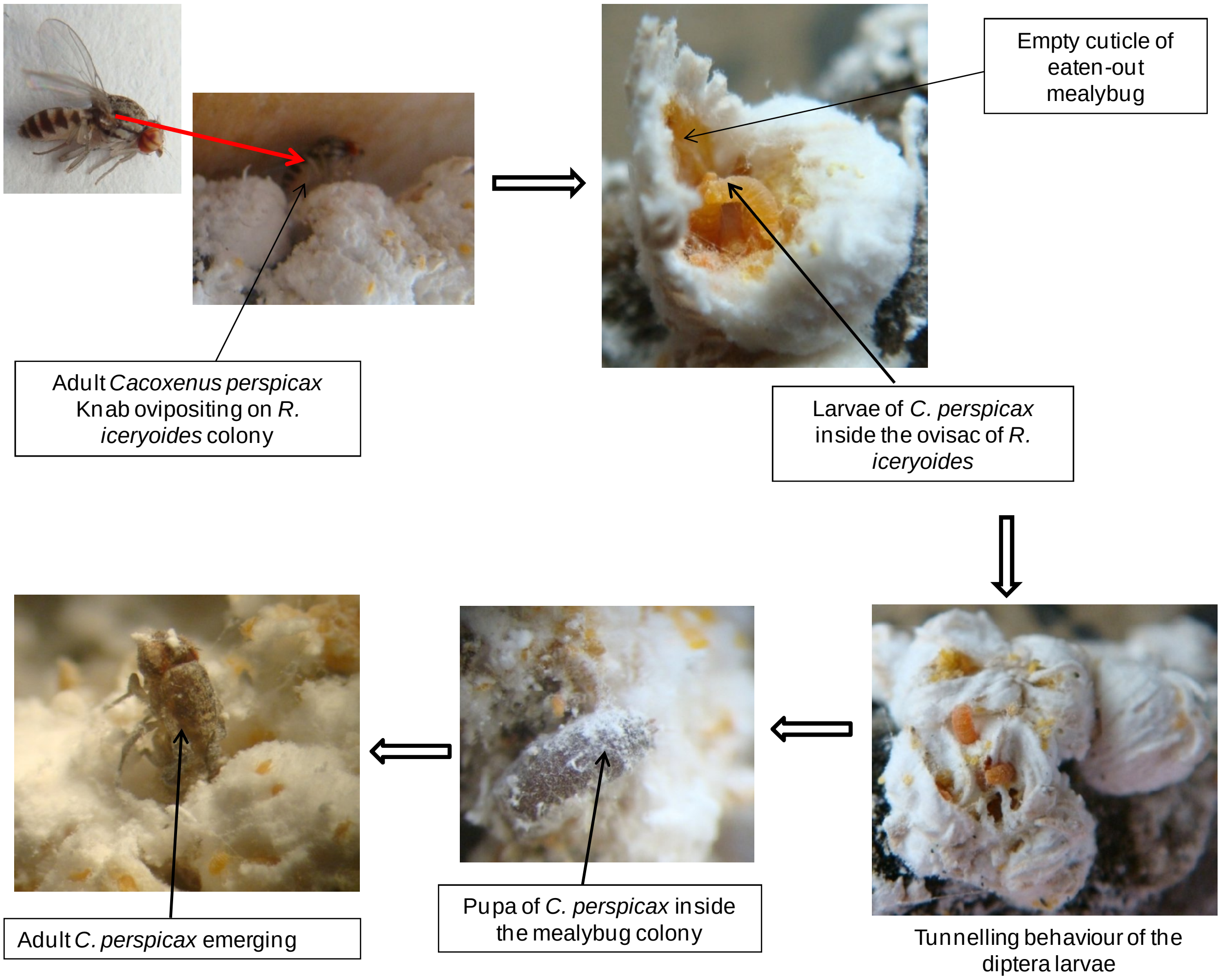
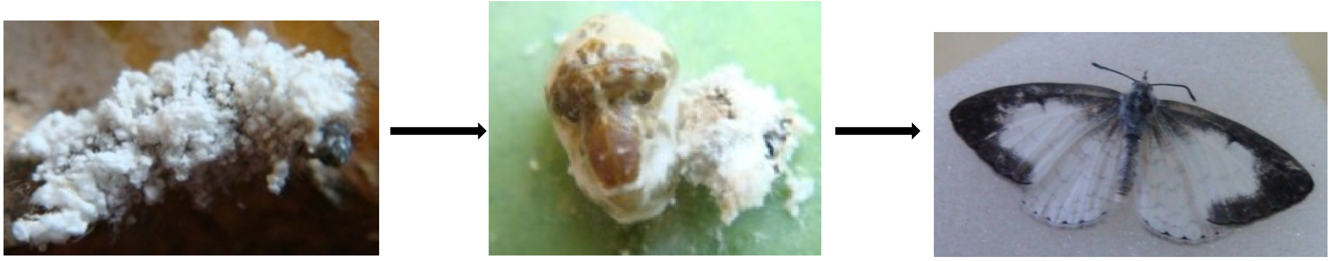

Empty cuticle of eaten-out mealybug
Adult *Cacoxenus perspicax* Knab ovipositing on *R. iceryoides* colony
Larvae of *C. perspicax* inside the ovisac of *R. iceryoides*
Adult *C. perspicax* emerging
Pupa of *C. perspicax* inside the mealybug colony
Tunnelling behaviour of the diptera larvae

Figure 3. 7: Predatory Diptera, *Cacoxenus perspicax* Knab (Diptera: Drosophilidae) of *R. iceryoides*.

Fourteen species of spiders were collected during the study, with *Cheiracanthium inclusum* Hentz*, Orthrus* sp., *Thiodina* sp., *Peucetia viridians* Hentz, *Nephila clavipes* Lat. clavis and *Phidippus audax* Hentz being the most frequently encountered species. The two-clawed hunting spiders, *C. inclusum* (Family Miturgidae) exhibited a remarkable behavioural pattern in their association with *R. iceryoides*. They were found to construct tubular silken retreat along the midrib of mango leaves that were heavily colonized by *R. iceryoides*, as this allowed them to prey on *R. iceryoides* without having to expend energy (Figure 3.11).

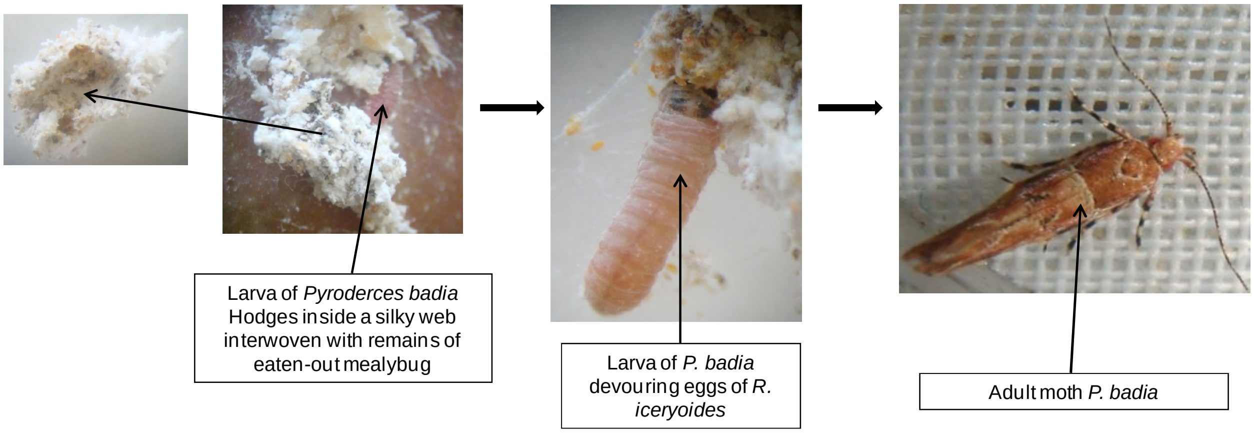


Larva of *Spalgis lemolea* Druce

"Monkey-face" pupa form of *Spalgis lemolea*

Adult *S. lemolea*

Figure 3. 8: Larval, pupa and adult form of the predatory moth *Spalgis lemolea* Druce

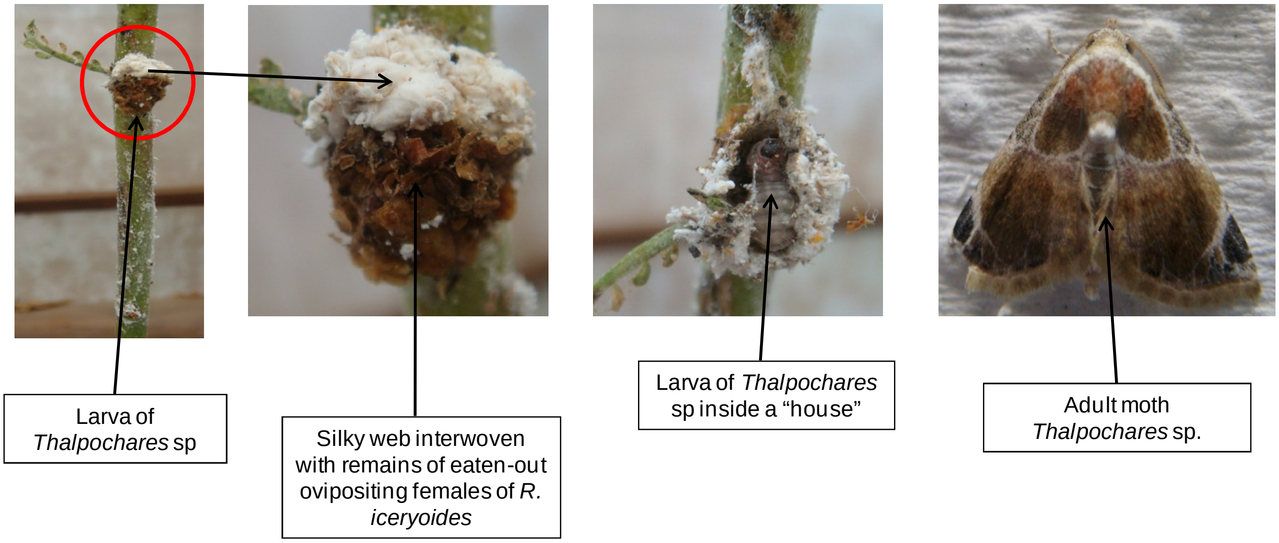


Larva of *Pyroderces badia* Hodges inside a silky web interwoven with remains of eaten-out mealybug

Larva of *P. badia* devouring eggs of *R. iceryoides*

Adult moth *P. badia*

Figure 3. 9: Larva of *Pyroderces badia* Hodges feeding voraciously on eggs of *R. iceryoides*.



Larva of *Thalpochares* sp

Silky web interwoven with remains of eaten-out ovipositing females of *R. iceryoides*

Larva of *Thalpochares* sp inside a “house”

Adult moth *Thalpochares* sp.

Figure 3. 10: Larva of *Thalpochares* sp. after devouring ovipositing females of *R. iceryoides* on a *P. aculeata* plant.

Species of Neuroptera (Table 3.5) were also recorded with the most important familes being Chrysopidae and Hemerobiidae. In this study, three Neuroptera species (*Mallada baronissa* (Navás), *Chrysop*a (*Suarius*) *jeanneli* (Navás) (Chrysopidae) and *Hemerobius* sp. (Hemerobiidae)) were recorded, with the most common being *Hemerobius* sp. Larvae of this species were observed in colonies of *R. iceryoides*, feeding on different nymphal stages as well as on adult mealybug females (Figure 3.12).

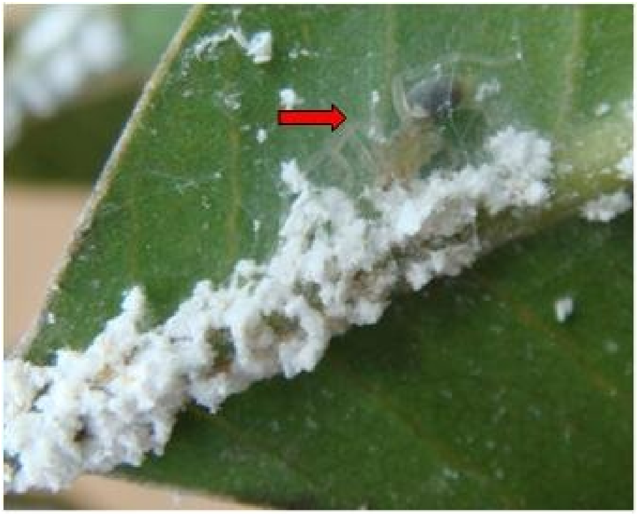


Figure 3. 11: Two-clawed hunting spider, *Cheiracanthium inclusum* Hentz preying on *R. iceryoides* colony on the abaxial surface of the leaf.

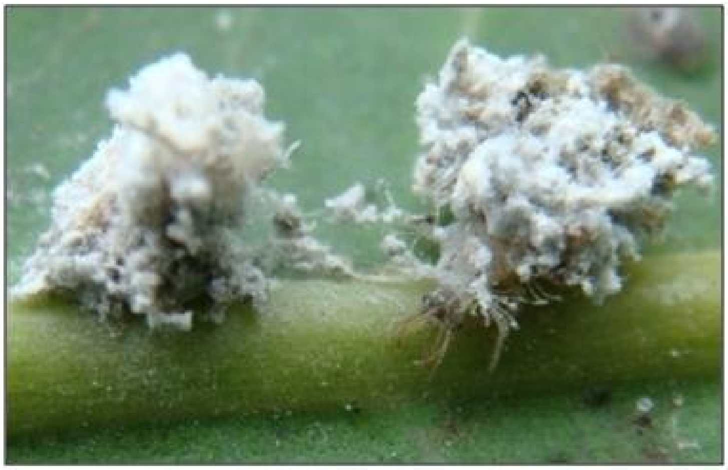


Figure 3. 12: *Hemerobius* sp. larva after devouring an adult oviposition female of *R. iceryoides*.

Table 3. 5: Predators associated with *R. iceryoides* on various host plants in Kenya and Tanzania

Country	Plant species	Family	Predator species	Abundance
Kenya	*Mangifera indica L.*	Chrysopidae	*Mallada baronissa* Navás	3
	Mangifera indica L.	Chrysopidae	*Chrysopa* (*Suarius*) *jeanneli* Navás	8
	Parkinsonia aculeata	Coccinellidae	*Exochomus nigromaculatus* Goeze	35
	Parkinsonia aculeata	Coccinellidae	*Cryptolaemus montrouzieri* Mulsant	7
	Parkinsonia aculeata	Coccinellidae	*Chilocorus nigrita* Fabricius	84
	Ficus benghalensis	Coccinellidae	*Chilocorus nigrita* Fabricius	6
	Parkinsonia aculeata	Coccinellidae	*Propylea dissecta* Mulsant	2
	Mangifera indica L.	Drosophilidae	*Cacoxenus perspicax* Knab	126
	Parkinsonia aculeata	Drosophilidae	*Cacoxenus perspicax* Knab	231
	Mangifera indica L.	Chamaemyiidae	*Leucopis* (Leucopella) *africana* Malloch	11
	Parkinsonia aculeata	Chamaemyiidae	*Leucopis* (Leucopella) *africana* Malloch	27
	***Annona muricata*	Chamaemyiidae	*Leucopis* (Leucopella) *africana* Malloch	5
	***Annona muricata*	Lycaenidae	*Spalgis lemolea* Druce	13
	Parkinsonia aculeata	Salticidae	*Phidippus audax* Hentz	7
	Parkinsonia aculeata	Miturgidae	*Cheiracanthium inclusum* Hentz	4
Tanzania	*Mangifera indica* L.	Coccinellidae	*Chilocorus nigrita* Fabricius	334
	Parkinsonia aculeata	Coccinellidae	*Chilocorus nigrita* Fabricius	63
	Citrus aurantifolia	Coccinellidae	*Chilocorus nigrita* Fabricius	102
	***Annona muricata*	Coccinellidae	*Chilocorus nigrita* Fabricius	15
	Mangifera indica L.	Coccinellidae	*Chilocorus renipustulatus* Scriba	214
	Citrus aurantifolia	Coccinellidae	*Chilocorus runipustulatus* Scriba	56
	Mangifera indica L.	Coccinellidae	*Hyperaspis bigeminata* Randall	178
	Mangifera indica L.	Coccinellidae	*Hyperaspis amurensis* Weise	41
	Mangifera indica L.	Coccinellidae	*Telsimia nitida* Chapin	5
	Mangifera indica L.	Coccinellidae	*Cryptolaemus montrouzieri* Mulsant	65

** = indicate host plants native to Africa

Table 3.5 continues: Predators associated with *R. iceryoides* on various host plants in Kenya and Tanzania

Country	Plant species	Family	Predator species	Abundance
Tanzania	*Mangifera indica* L.	Coccinellidae	*Propylea 14-punctata* Linnaeus	3
	Mangifera indica L.	Coccinellidae	*Micraspis vincta* Gorham	14
	Mangifera indica L.	Coccinellidae	*Henosepilachna vigintioctopunctata* Fabricius	2
	Mangifera indica L.	Chrysomelidae	*Nisotra gemella* Erichson	11
	Mangifera indica L.	Coccinellidae	*Rodolia fumida* Mulsant	23
	Mangifera indica L.	Coccinellidae	*Cryptogonus* sp.	7
	Mangifera indica L.	Coccinellidae	*Henosepilachna argus* Geoffroy,	1
	Mangifera indica L.	Coccinellidae	*Hyperaspis* sp.	1
	Morus alba	Coccinellidae	*Rodolia pumila* Weise	22
	Morus alba	Coccinellidae	*Rodolia limbata* Motschulsky	13
	Mangifera indica L.	Coccinellidae	*Rodolia* sp	6
	Mangifera indica L.	Coccinellidae	*Rodolia pumila* Weise	8
	Morus alba	Coccinellidae	*Cycloneda* sp.	19
	Mangifera indica L.	Coccinellidae	*Platynaspis luteorubra* Goeze	4
	Mangifera indica L.	Lycaenidae	*Spalgis lemolea* Druce	38
	Mangifera indica L.	Noctuidae	*Pyroderces badia* Hodges	11
	Mangifera indica L.	Noctuidae	*Thalpochares* sp.	189
	Parkinsonia aculeata	Noctuidae	*Thalpochares* sp.	46
	Mangifera indica L.	Hemerobiidae	*Hemerobius* sp.	3
	Mangifera indica L.	Drosophilidae	*Cacoxenus perspicax* Knab	1267
	Mangifera indica L.	Chamaemyiidae	*Leucopis* (*Leucopella*) *africana* Malloch	145
	Cajanus cajan L.	Chamaemyiidae	*Leucopis* (*Leucopella*) *africana* Malloch	32
	** *Dialium holtzii*	Chamaemyiidae	*Leucopis* (*Leucopella*) *africana* Malloch	55
	Mangifera indica L.	Chamaemyiidae	*Leucopis* (*Leucopella*) *ardis* Gaimari & Raspi	41
	Cajanus cajan L.	Chamaemyiidae	*Leucopis* (*Leucopella*) *ardis* Gaimari & Raspi	72
	Mangifera indica L.	Cecidomyiidae	*Coccodiplosis* sp.	66
	Morus alba	Cecidomyiidae	*Diadiplosis* sp.	13

** = indicate host plants native to Africa

Table 3.5 continues: Predators associated with *R. iceryoides* on various host plants in Kenya and Tanzania

Country	Plant species	Family	Predator species	Abundance
Tanzania	*Morus alba*	Miturgidae	*Cheiracanthium inclusum* Hentz	47
	Mangifera indica L.	Salticidae	*Orthrus* sp.	15
	Mangifera indica L.	Salticidae	*Opisthoncus* sp.	3
	Mangifera indica L.	Salticidae	*Thiodina* sp.	8
	Mangifera indica L.	Salticidae	*Salticus* sp.	5
	Mangifera indica L.	Salticidae	*Lyssomanes* sp.	3
	Mangifera indica L.	Salticidae	*Phidippus audax* Hentz	23
	Mangifera indica L.	Sparassidae	*Micrommata rosea* Clerck	4
	Mangifera indica L.	Thomisidae	*Thomisus spectabilis* Dolesch	2
	Mangifera indica L.	Oxyopidae	*Peucetia viridians* Hentz	7
	Mangifera indica L.	Oxyopidae	*Oxyopes* sp.	2
	Mangifera indica L.	Nephilidae	*Nephila clavipes* Lat. clavis	6

** = indicate host plants native to Africa

3.3.6 Ant species associated with *R. iceryoides*

Eleven different ant species were found to be closely associated with *R. iceryoides*. These included *Anoplolepis custodiens* (Smith), *Camponotus flavomarginatus* Mayr, *Crematogaster tricolor* st. rufimembrum Santschi, *Linepithema humile* Mayr, *Oecophylla longinoda* Latreille, *Pheidole megacephala* Fabricius, *Atopomyrmex mocquerysi* Bolton, *Lepisiota depressa* (Santschi), *Polyrhachis schistacea* (Gerstäcker), *Iridomyrmex purpureus* (F. Smith) and *Camponotus pennsylvanicus* De Geer. These ants were actively found milking honeydew from the mealybugs (Figure 3.13). Populations of *O. longinoda* and *P. megacephala* had a very strong positive association with *R. iceryoides* as they protected the mealybugs from adverse weather conditions by building tents using plant leaves and organic debris (soil and plant debris) around them**,** respectively. However, *P. megacephala* was frequently observed contructing semi-soil tent buildings around *R. iceryoides* to prevent them from going distances while they frequently visit them from time to time to collect honeydew (Figure 3.14). *Oecophylla longinoda* and *P. megacephala* were also observed transporting *R. iceryoides* from plant to plant or within plant parts (Figure 3.15). On the other hand, *P. megacephala* was also found to transport *R. iceryoides* down to the roots of the plant *O. lanceolata*.

Pheidole megacephala was observed pulling out predatory larvae of *C. perspicax* from the ovisac of gravid females of *R. iceryoides* (Figure 3.16). *Oecophylla longinoda* foragers were also observed to capture and immobilize adult coccinelids (Figure 3.16). *Pheidole megacephala* was the only ant species observed to occasionally prey on *R. iceryoides* and the main predator of *O. longinoda* under field condition. In the absence of ant-attending *R. iceryoides* in the field large numbers of immature life stages were found trapped in excess amount of honeydew produce by the mealybug (Figure 3.17).

The relationship between mealybug colony size and populations of *P. megacephala* and *O. longinoda* is shown in Figure 3. 18. There was a significant negative correlation between percentage parasitism and populations of *P. megacephala* and *O. longinoda* (Figure 3. 18).

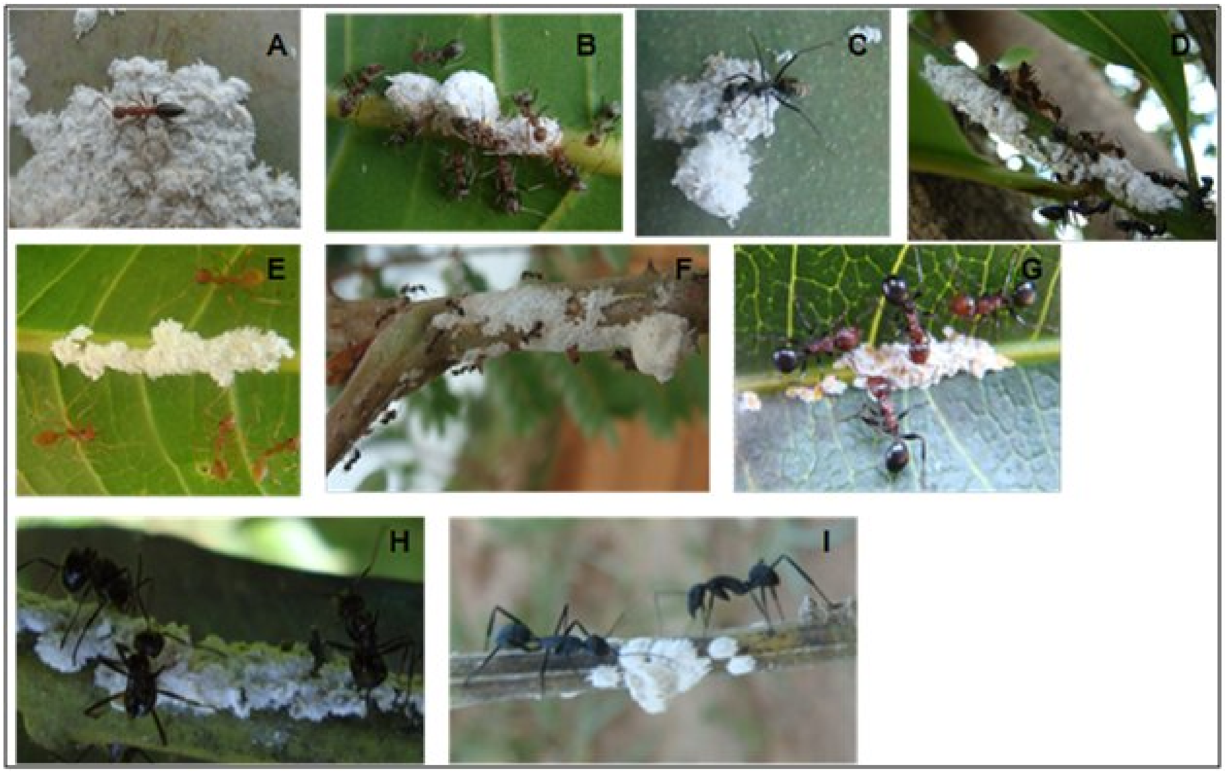


Figure 3. 13: Ant species tending *R. iceryoides* for honey dew on different host plants, (A) *I. purpureus*; (B) *A. custodiens*; (C) *C. flavomarginatus*; (D) *L. humile*; (E) *O. longinoda*; (F) *P. megacephala*; (G) *A. mocquerysi*; (H) *L. depressa* and (I) *C. pennsylvanicus*.

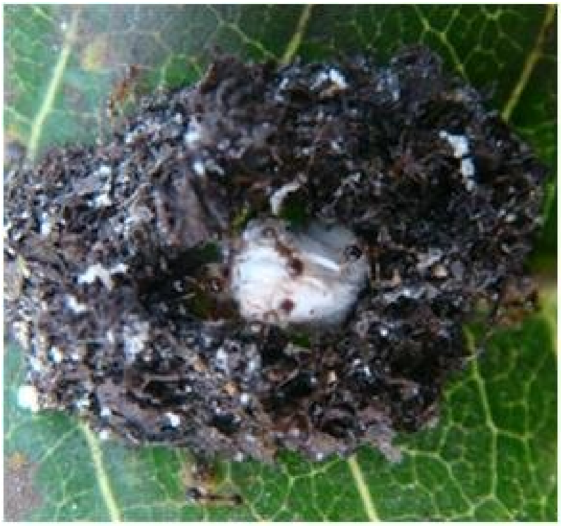


Figure 3. 14: Adult *R. iceryoides* enclosed in an earth-constructed nest of *P. megacephala* to serve as a regularly source for honeydew.

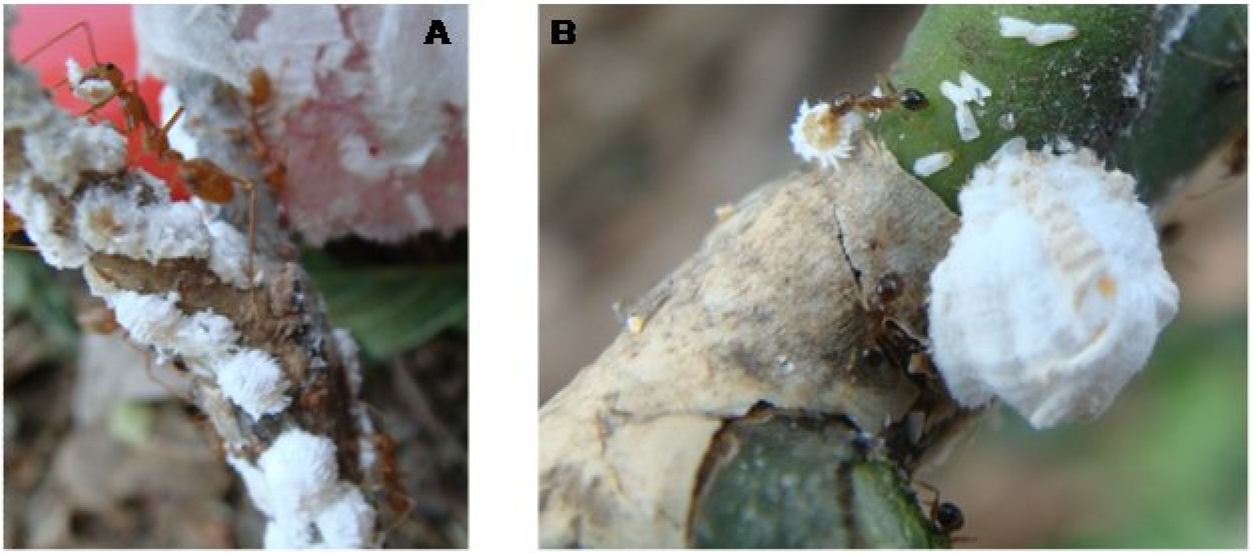


Figure 3. 15: The red weaver ant, *O. longinoda* (A) and *P. megacephala* (B) transporting *R. iceryoides* within the same host plant.

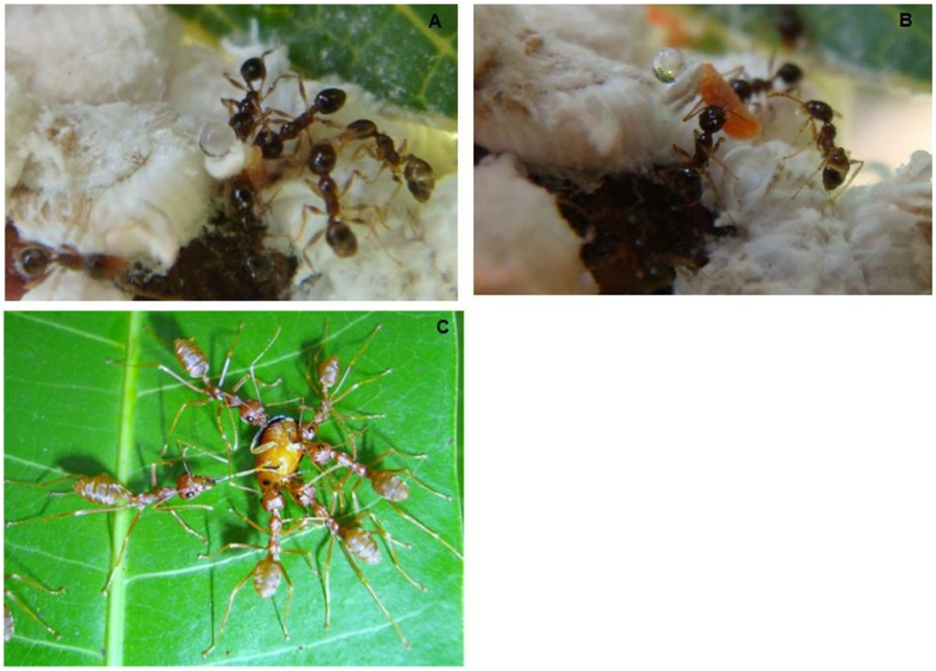


Figure 3. 16: (A): *Pheidole megacephala* foraging for larvae of *C. perspicax* within the ovisac of female *R. iceryoides*; (B): transporting them away as complementary food source and (C) Captive adult coccinelid and attacking *O. longinoda* foragers at the beginning of the immobilization phase of predatory attack.

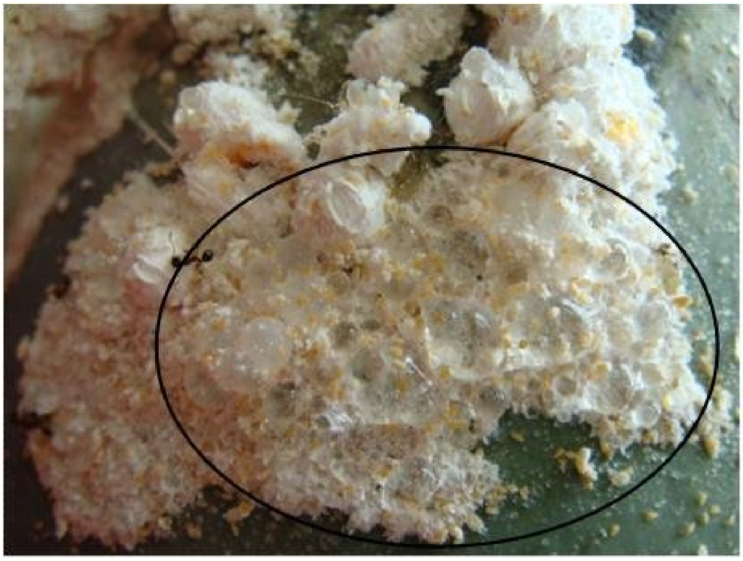


Figure 3. 17: Immature stages of *R. iceryoides* trapped in excess amount of honeydew.

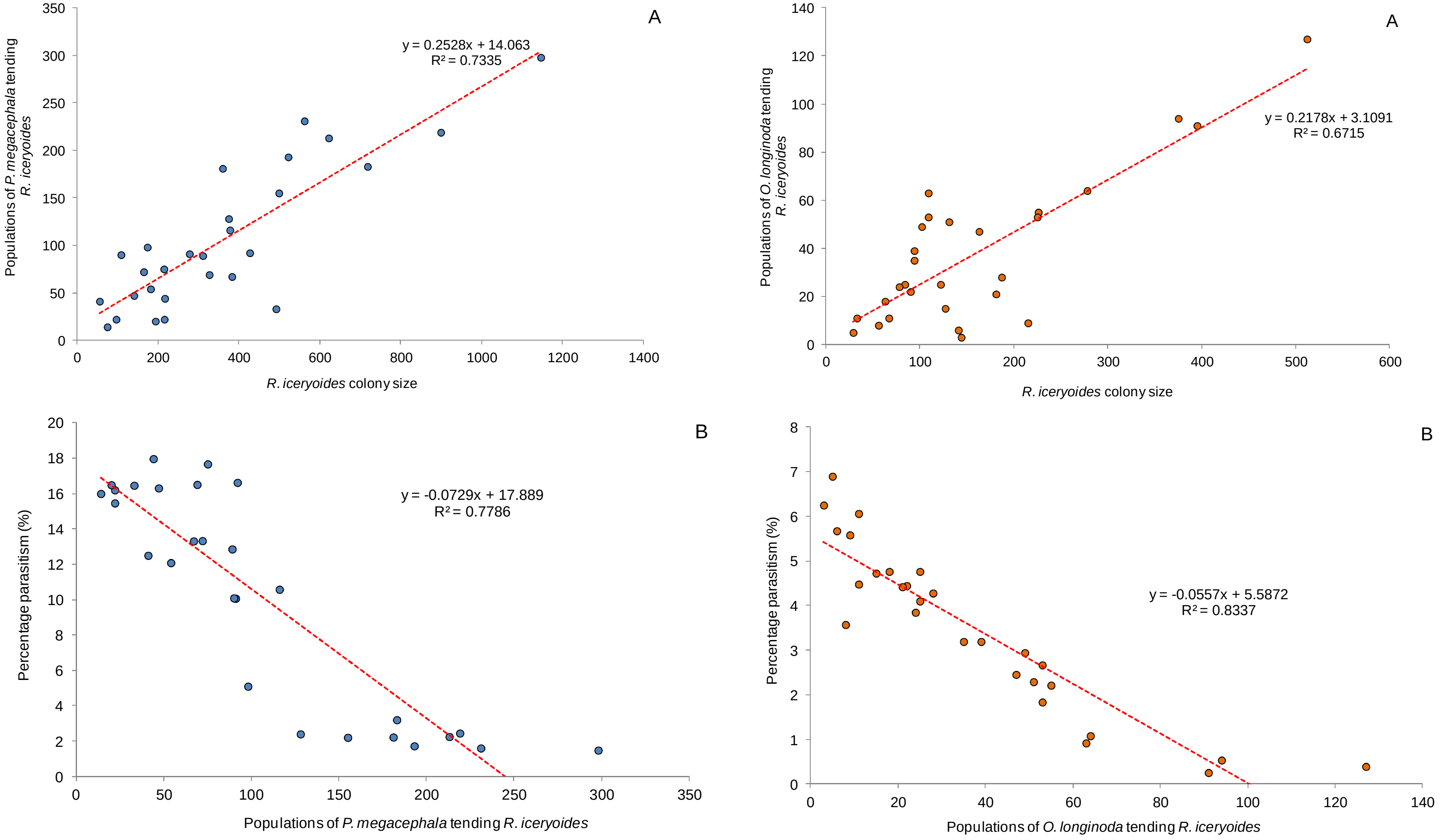


Figure 3. 18: Linear regressions of mealybug colony size (A) and mummified *R. iceryoides* (B), on *P. megacephala* and *O. longinoda* populations in the field.

3.4 Discussion

3.4.1 Distribution

These results showed that *R. iceryoides* is widely distributed across the coastal belt of Kenya and Tanzania. In Kenya, mango infestation extended up to 145 km inland while in Tanzania the pest was found as far as 851 km southwest of the coastal region. In Kenya, heavy infestation was confirmed in Matuga and Kinango both on mango and *P. aculeata*. The high level of *R. iceryoides* infestations in Matuga is particularly disturbing because the locality represents one of the key mango production areas in the country (Griesbach, 2003). Multiple patches of moderate infestation on mango in Mombasa, Kilifi, and Malindi were also observed in Kenya. It is uncertain whether the infestation in these locales is contiguous with that of Matuga or whether they represent discrete populations with limited gene pool but overall, the spread warrants careful attention. In Tanzania, heavy infestations were recorded in Morogoro, Kinondoni, Tanga, Kibaha and Mkuranga on mango (*M. indica*) and three alternative wild host plants, *P. aculeata, O. lanceolata* and *C. sepiaria*. The high level of attack on mango in Kinondoni and Mkuranga demands urgent management attention given the ongoing expansion of the horticulture industry and particularly mango in the region (Nyambo and Verschoor, 2005; Madulu and Chalamila, 2007). These results also provide some evidence of the altitudinal limits of distribution of *R. iceryoides* in both countries. The pest was recorded from as low as 26 meters above sea level (m a.s.l) in Bagamoyo, Tanzania to as high as 901 m a. s. l in Taveta, Kenya.

Although the distribution of insect pests is affected by different abiotic and biotic factors (temperature, humidity, host plants and presence of competitors), despite the wide availability of preferred host plants (*M. indica* and *P. aceleata*) in Madabogo, Dembwa, Wundanyi and Kungu (located at 943 to 1480 m a.s.l), *R. iceryoides* was absent at these sampling sites suggesting that the pest may not occur above these altitudes. The data suggest that *R. iceryoides* may be pre-adapted to surviving in low and mid altitudes similar to its native range of India (Rawat and Jakhmola, 1970; Williams, 1989; Narasimham and Chacko, 1991; Narasimham and Chacko, 1988; Tanga, unpublished data). Although the precise date of introduction of *R. iceryoides* to both Kenya and Tanzania is unknown (Williams, 1989), it is highly probable that current widespread distribution and spread of the mango mealybug populations is assisted by fruit and plant material transported across the region in commercial and private vehicles as is the case with the introduction of *R. invadens* into West and Central Africa (Agounké et al. 1988).

3.4.2 Host plants

Rastrococcus iceryoides was recorded from 29 plants species including cultivated and wild host plants from 16 families, 21 of which are new records for Kenya and Tanzania. The major plant families infested based on level and severity of attack includes Anacardiaceae, Fabaceae, Sapindaceae and Santalaceae. Plants from the family Annonaceae, Euphorbiaceae and Caesalpiniaceae were moderately infested while attack on the Moraceae, Solanaceae, Myrtaceae, Rutaceae, Muscaeceae, Papilionaceae, Simaroubaceae, Verbenaceae, and Sapotaceae was generally low. In its first description, CABI (1995) listed six host plants of *R. iceryoides* in Tanzania namely mango (*M. indica*), cacao (*Theobroma cacao* Linn.), *Albizia lebbeck* Linn. (Indian siris), cotton (*Gossypium* spp.) and rain-tree (*Samanea saman* (Jacq.) Merr.). The additional host plant records from this survey clearly suggests that *R. iceryoides* is an emerging polyphagous invasive mealybug pest in Tanzania and Kenya. Several *Rastrococcus* species have been reported from the different host plant families listed in this study. For example, following the invasion of *R. invadens* in West Africa, Agounké et al. (1988) recorded 45 plant species from 22 families as host of the insect in Togo and Benin. In Nigeria, Ivbijaro et al. (1992) reported *R. invadens* from over 20 species of host plants in 12 different plant families. Host status is a dynamic phenomenon and this list is by no means exhaustive and given that the genus *Rastrococcus* to which *R. iceryoides* belongs attack several host plant species (Williams, 1989; Williams, 2004; Ben-Dov, 1994), it is envisaged that this list is likely to increase.

Mangifera indica recorded the heaviest attack by *R. iceryoides* from among the host plants sampled within the family Anacardiaceae. In *R. invadens*, out of the 45 plant species recorded by Agounké et al. (1988), the author also found that attack on mango was usually high in addition to citrus, banana, breadfruit and guava. Based on the severity of attack, Ivbijaro et al. (1992) also reported that mango breadfruit, guava, sweet orange, lime and grapefruit was the most preferred host plants of *R. invadens* in Nigeria.

Heavy infestation of *R. iceryoides* was recorded among all the plant species sampled from the family Fabaceae. This included *P. aculeata, C. cajan* and *C. sepiaria* in order of severity of attack. In Asia, *P. aculeata* and *C. cajan* are known to be heavily infested by *R. iceryoides* (Ben-Dov, 1994) and these findings concur with previous observations. The heavy infestation of *P. aculaeta* is perhaps surprising given that the plant is not native to Asia, rather an invasive tree indigenous to tropical America (Cochard and Jackes, 2005). Nevertheless, plants that are generally water stressed easily favour high populations of mealybug (Calatayud et al., 2002; Shrewsbury et al., 2004; Lunderstadt, 1998; Gutierrez et al., 1993) and *P. aculeata* is known to thrive in drought prone environment with limited amount of water (Floridata, 2001). Fully grown *P. aculeata* can flower throughout the year (WNS, 2011) and can harbor several successive generations of the pest that will ultimately move to mango, pigeon pea and other cultivated host plants when conditions become favourable. In Kenya and Tanzania, *P. aculeata* also thrives as an ornamental tree, mostly utilized as shade trees around the homesteads and sometimes in close proximity to mango orchards. Management methods targeting *R. iceryoides* must also take into cognizance the presence of *P. aculeata* and possible infestation by *R. iceryoides*.

The cat's claw, *C. sepiaria* is recorded here for the first time as a preferred host harboring large populations of *R. iceryoides*. The observed high levels of infestation on *C. sepiaria* although remarkable is perhaps not surprising given that the plant species is native to tropical Southern Asia. It is an Indo-Malayan species, indigenous to India (the putative home aboriginal of *R. iceryoides*) and Burma, Sri Lanka, eastern China and South-east Asia down to the Malay Peninsular (Brandis, 1907). The observed high levels of infestation on the Fabaceae can also be generally attributed to nitrogen accumulation in the plant family (Harris, 1982). For example, Hogendrop et al. (2006) and, Rae and Jones (1992) reported that the life history parameters of the citrus mealybug, *Planococcus citri* Risso and pink sugar-cane mealybug, *Saccharicoccus sacchari* (Cockerell) were affected by increase level of plant nitrogen content. Hogendrop et al. (2006) demonstrated that higher nitrogen concentrations, in the form of supplemental fertilizers led to an increased in the performance of citrus mealybugs as defined by increased egg loads, larger mature females, and shorter developmental times.

Among the Sapindaceae, *D. borbonica* was heavily infested during the survey and can be considered as important reservoir host plant for *R. iceryoides*. High infestation levels were especially recorded in Kibaha, Tanzania (2253 mealybug/10 cm twig). *Deinbollia borbonica* is a perennial tree that occurs throughout the year and found to be a crucial off-season host plant for *R. iceryoides* particularly when mango, the primary cultivated host plants was off-season. Several plant species from the Sapindaceae family (e.g., *Nephelium lappaceum* Linnaeus, *Harpullia* sp., *Guioa pleuropteris* Blume, *Heterodendrum* sp., and *Nephelium lappaceum* Linnaeus) have also been found to be heavily infested by different *Rastrococcus* species including *R. jabadiu* Williams, *R. neoguineensis* Williams & Watson, *R. spinosus* Robinson, *R. stolatus* Froggatt and *R. tropicasiaticus* Williams, respectively (Williams, 1989; Ben-Dov, 1994; Williams, 2004).

Osyris lanceolata from the family Santalaceae was observed to be heavily attacked by *R. iceryoides*. From literature, there are no records of mealybug attack from this plant species and this report is perhaps the first record of *R. iceryoides* infestation from this plant family. On young plants, in addition to the leaves and twigs, heavy infestation was observed on the stem at 10 cm above the ground level. In Kenya, a root decoction of *O. lanceolata* is used to treat diarrhea while in Tanzania, a decoction of the bark and heartwood is used to treat sexually transmitted diseases and anaemia (Orwa et al., 2009).

In the Annonaceae, *R. iceryoides* was found to attack *A. stenophylla*, *A. senegalensis*, *A. muricata* and *A.squamosa*. Ben-Dov (1994) reported *A. squamosa* as a major host plant of *R. iceryoides* in India but the occurrence of the mealybug on *A. stenophylla*, *A. senegalensis*, *A. muricata* is a new record for the insect. Studies elsewhere have shown that other species of *Rastrococcus* such as *R. invadens*, *R. spinosus* are pestiferous on this family (Ben-Dov, 1994; Boussienguent and Mouloungou, 1993; Williams, 2004). Plant species belonging to the family Annonaceae (and especially *A. muricata*) are economically important export horticultural crops in Kenya and Tanzania. In fact numerous Annonaceous acetogenins from these plants have been reported to possess insecticidal, pesticidal, antimalarial, cell growth inhibitory, antiparasitic, antimicrobial and cytotoxic activities (Fujimoto et al., 1998; Colman-Saizarbitoria et al., 1995; Oberlies et al., 1997; Chih et al., 2001). Recently, these compounds have attracted increased attention as potential antineoplastic agents due to their ability to kill tumour cells (Fang et al., 1993). During the survey, infestations on *A. muricata* and *A. squamosa* by *R. iceryoides* on the stem and leaves was associated with noticeable deformation and distortion of the terminal growth, twisting and curling of leaves, leaf wrinkling and puckering and premature fruit drop. The damage on these important plant species therefore requires careful attention.

Phyllanthus engleri and *F. virosa* from the family Euphorbiaceae were observed to be moderately infested by *R. iceryoides*. This plant species is very common and scattered throughout the Tanzania mainland, Mozambique, Zambia and Zimbabwe (Christopher et al., 2002). There are no records of mealybug attack from these plant species in literature and this is perhaps the first record of *R. iceryoides* attack on this family in Africa. Among the two plant species, *P. engleri* was more infested compared to *F. virosa,* but infestation levels were generally low. In Tanzania, *P. engleri* is an important medicinal plant; the leaves and fruits are chewed together for treating cough and stomach-ache while the roots are boiled and the concoction is drank to treat bilharzias, sexually transmitted diseases (STDs), menstrual problems and abdominal and chest pain (Christopher et al., 2002).

Two crops in the family Myrtaceae and Rutaceae that had low to moderate infestation records namely *Citrus* spp. and *C. aurantifolia*; and *P. guajava*, respectively warrant discussion. The family Myrtaceae is known to host a variety of mealybug species worldwide including several species of *Rastrococcus* (Williams, 2004; Ben-Dov, 1994) but *P. guajava* was the only plant species sampled in our study. Moderate infestation of *R. iceryoides* was recorded on this plant in Kenya and Tanzania. In West and Central Africa, *P. guajava* has also been reported as a major host plant of *R. invadens* (Ivbijaro et al., 1992). In the Rutaceae, *R. iceryoides* was only recorded from *C. aurantifolia* in Kenya while in Tanzania; the insect was recorded from *Citrus sinensis* and *C. aurantifolia.* Although infestation was generally low in this study, reports from other studies indicate that several citrus species have been recorded as major host plants of mealybugs from the genus *Rastrococcus*. For example, *R. invadens* is reported to be a major host of *Citrus paradisi* Macfad, *C. maxima* Merr., *C. limon* (L.) Burm. f., *C. reticulata* Blanco, *C. grandis* Osbeck (Williams, 1989; Ben-Dov, 1994; Boussienguent and Mouloungou, 1993), in addition to *C. sinensis* and *C. aurantifolia*, (Ivbijaro et al., 1992).

3.4.3 Parasitoids

Several parasitoid species have been reared from *R. iceryoides* (Tandon and Lal, 1978; Narasimham and Chako, 1988). In this study a total of six indigenous parasitoid species were recovered from *R. iceryoides* in Kenya and Tanzania with *A. pseudococci* clearly the most dominant and widespread in both countries. Despite its widespread distribution across the different localities sampled, percentage parasitism did not exceed 20%. Tandon and Lal (1978) listed *R. iceryoides* as host mealybug of *A. pseudococci*, however, Noyes and Hayat (1994) noted that this was a misidentification. The current study however confirms that *R. iceryoides* is an important host insect of *A. pseudococci* and should be considered a suitable candidate for biological control of the insect pest. Globally, *A. pseudococci* have been reported from twelve countries (Noyes and Hayat, 1994) excluding the countries of this survey, which implies that the results presented herein add Kenya and Tanzania to the list of countries where the parasitoid exists. In Texas, Europe and Pakistan, *A. pseudococci* has been credited with successful biological control of *Planococcus citri* on citrus and grapes (Tingle and Copland, 1989; Noyes and Hayat, 1994). Among all the host plant species sampled, the highest percent parasitism by *A. psuedococci* on *R. iceryoides* was from mealybugs infesting mango and *P. aculeata*. This study provides information that predicts the distribution of parasitism across host plants, which is crucial for rational conservation and augmentation of the parasitoid. Therefore, management of this parasitoid, through either augmentation and or conservation may be able to concentrate parasitism where and when it will exert the most control. In the case of *R. iceryoides*, one such target location would be *P. aculeata* (since it is used as ornamental shade plants by growers) in the vicinity of mango orchards. Augmentative releases and or conservation of *A. pseudococci* directed at *R. iceryoides* before their spread into mango crop should be both an effective and timely strategy for suppressing the population of the mealybug.

Parasitism by the other parasitoid species encountered during the survey did not exceed 1%. The reason for the general low level of parasitism by the parasitoid species is not well understood. Many factors including host and parasitoid suitability, age, sex, climatic conditions and host plants influence parasitism success. Indeed, all these factors have been found to be crucial for successful parasitism by most encyrtid parasitoids on mealybugs (Blumberg, 1997; Islam and Copland, 1997; Sagarra and Vincent, 1999; Daane et al., 2004a; Daane et al., 2004b; Karamaouna and Copland, 2000, Cross and Moore, 1992; McDougall and Mills, 1997; Persad and Khan, 2007). Although the need to conserve all the natural enemies reared from *R. iceryoides* will be critical for the overall management of the insect, the lack of efficient coevolved natural enemies capable of suppressing *R. iceryoides* populations to levels below economically damaging levels calls for exploration for natural enemies in the putative aboriginal home of Southern Asia and their introduction into Africa for classical biological control of the pest. Such an approach should be considered as high priority in seeking a long term solution to the management of *R. iceryoides* in Africa.

Ninteen hyperparasitoid species attacked *R. iceryoides* parasitized by the primary parasitoids with *C. conjugalis* and *C. cyanonotus* developing high populations. Field survey of *R. invadens* in West Africa also revealed several hyperparasitoids attacking mealybugs parasitized by *G. tebygi* with four species developing high populations (Boavida and Neuenschwander, 1995). In West Africa, Moore and Cross (1992) identified *Chartocerus hyalipennis* as the major secondary parasitoids associated with *Anagyrus mangicola* Noyes and *G. tebygi*. In a similar study on the hyperparasitism of both *G. tebygi* and *Epidinocarsis lopezi* (DeSantis) in Togo, *C. hyalipennis* rather than *M. leopardina* contributed mainly to hyperparasitism of the two parasitoids (Agricola and Fisher, 1991). *Cheiloneurus* species, *Marietta leopardina* and *Pachyneyron* species are believed to be hyperparasites through *Anagyrus* spp. and *L. dactylopii* (Whitehead, 1957) while the *Tetrastichus* sp has been reported as hyperparasites of *R. invadens* through *G. tebyi* in Africa (Ukwela, 2009). Most of the hyperparasitoid species from the family Aphilinidae recorded in our study has been reported as hyperparasites of *R. iceryoides*, among other species of mealybugs in India (Hayat, 1998). Low parasitism of *R. iceryoides* by the primary parasitoids can be attributed in part to the presence of hyperparasitoids. Similar findings of low parasitism of *R. invadens* by *G. tebygi* due to activities of hyperparasitoids under laboratory and field conditions have been reported (Agricola and Fischer, 1991; Moore and Cross, 1992). Secondary parasitism (hyperparasitism) is a common phenomenon in insect host-parasitoid systems and a high percentage of secondary parasitism of Pseudococcidae is not unusual in natural and agricultural habitats with economically important crops like mango and citrus orchards (Ukwela, 2009). Secondary parasitoids are generally assumed to have major implications for the biological control of pest insects because of their negative effects on the population dynamics of the beneficial primary parasitoids (Lucky et al., 1981; May and Hassell, 1981; Hassell and Waage, 1984; Hassel, 1978; Greathead, 1986), although few studies have demonstrated this conclusively (Sullivan, 1987). The knowledge of the level of hyperparasitism of the primary parasitoids by the hyperparasitoids can be useful in planning further biological control activities on *R. iceryoides*.

3.4.4 Predators

Among the predators recovered from *R. iceryoides* colonies the predaceous drosophilid *C. perspicax* was the most abundant species. *Cacoxenus perspicax* has also been reported to be associated with only high pink hibiscus mealybug, *Maconellicoccus hirsutus* (Green) (Hemiptera: Pseudococcidae) densities in Australia (Goolsby et al., 2002), which is in accordance with our observations. However, the host range of *C. perspicax* and its impact on *M. hirsutus* is not known. An extensive search of the literature failed to reveal any published work why these flies are strongly attracted to high density mealybug colonies except that by Nicholas and Inkerman (1989). Nicholas and Inkerman (1989) explains that mealybug exudates are highly acidic (pH 3) and their continuous production allows ethanol production by yeast cells, which in turn promotes the rapid growth of acetic acid bacteria. The coproduction of ketogluconic acids and γ-pyrones with associated lowering of the pH also increases the selection against most other microorganisms, including the mealybug parasite *Aspergillus parasiticus*. In contrast to suppressing mold attack, the acetic acid bacteria and yeast cells stimulate the predation of mealybug by larvae of *C. perspicax* (Inkerman et al., 1986). If a parallel can be drawn with the fruit fly larvae that feed on necrotic prickly-pear (*Opuntia* spp.) tissues (Baker et al., 1982), then acetic acid bacteria alone could be sufficient for the complete development of the flies (Vacek, 1982). Baker et al., (1982) further reported that yeast species can sustain flies, and yeast produce volatile compounds that are particularly important attractant to fly. This report by Baker et al. (1982) confirms the possible reason why these flies were mostly found in heavily infested orchard with significant impact on the populations of *R. iceryoides*.

The larvae of *C. perspicax* were particularly active voracious predators of eggs within the ovisac to free-living adults in undisrupted colonies. Within the ovipositing female mealybug ovisac, 5 to 8 larvae were recovered. However, significant behavioural similarities were observed between *Cacoxenus* sp., *Leucopis (Leucopella) africana* Malloch and *Leucopis (Leucopella) ardis* Gaimari & Raspi. The 1st and 2nd instar larvae were observed to have low dispersal capacity and both stages tend to stay within *R. iceryoides* colony, while the 3rd instar larvae were more mobile tunnelling through the ovisac and exposing the *R. iceryoides* eggs to adverse weather conditions. As a result, the real mortality rates inflicted by colony disruptive behaviour by these predatory larvae were probably higher than their simple consumption rate. At present, difficulty in rearing these predators is the major obstacle in their study to ascertain their principal role in biological control.

Coccinellidae (Coleoptera) was the major group with the highest number of species but showed a highly generalistic feeding behaviour. This could probably be the reason why coccinelids are rarely as successful in the biological control of mealybug as hymenopterous parasitoids (Moore, 1988). Among the predatory beetles, the only species that showed some level of host specificity was *Hyperaspis bigeminata* Randall, whose larvae and adults chewed holes through the felt-like test of the ovisac and feeding exclusively on the eggs within the ovisac of gravid female *R. iceryoides*. Apart from *Chilocorus nigrita* and *Cryptolaemus montrouzieri,* the other 16 species are new records for the East Africa fauna preying on *R. iceryoides*. The presence of the *Rodalia* sp. was probably due to infestation by *I. seychellarum*, since they have been widely used in the control of *I. seychellarum* in other parts of the world (Butcher, 1983; Caltagirone and Doutt, 1989; Waterhouse, 1993).

3.4.5 Ants association with *R. iceryoides*

Eleven species of ants were found to be closely associated with *R. iceryoides* in the field. Several authors have already pointed out the negative impact of ants, notably, *L. humile*, *Crematogaster* spp. and *Anoplolepis* spp. on mealybug parasitoids (Horton, 1918; Kriegler and Whitehead, 1962; Smit and Bishop, 1934; Steyn, 1954; Samways et al., 1982). For example, Joubert (1943) noted that the parasite *Coccophagus gurneyi* Compere was severely hindered by *L. humile* in controlling *P. maritimus* (Ehrhorn) and Compere (1940) with the incidence of *Saisetia oleae* Olivier in the Cape between 1936 and 1937 greatly increasing due to the presence of *L. humile*. This implies that ants might have interfered with the parasitoid activities either by direct attack (including consumption of adults, larvae or eggs) or incidental disturbance, as such causes them to lay fewer eggs than would probably happen in the absence of ants (Martinez-Ferrer et al., 2003; Barlett, 1961). Samways et al. (1982) found that *A. custodiens*, while tending soft brown scale on citrus trees caused incidental increases in the population of red scale

Aonidiella aurantii (Maskell). This observation is in accordance with our findings, as mealybug populations tended by *P. megacephala* and *O. longinoda* was found to increase with increased ant density. Percentage parasitism on the other hand was found to reduce significantly with increase in *P. megacephala* and *O. longinoda* density. However, there is unequivocal evidence that ants can protect scale insects from natural enemies, especially parasitic wasps (Bartlett, 1961; Buckley and Gullan, 1991; Bach, 1991) and predatory beetles (Das, 1959; Bartlett, 1961; Burns, 1973; Bradley, 1973; Hanks and Sadof, 1990; Bach, 1991).

The different ant species recorded during the study were observed tending the mealybug for honey. Several other studies confirm that honeydew produced by many mealybugs, provides ants of numerous species with a stable source of energy (Way and Khoo, 1992; Nixon, 1951; Way, 1963; Buckley, 1987a; Buckley, 1987b). Most associations are facultative for both partners but some associations are apparently obligate (Tho, 1978; Ward, 1991) and many ants that tend mealybugs to obtain honeydew have also been reported to prey on them, either regularly or only under particular circumstances (Shanahan and Compton, 2000; DeBach et al., 1951; Folkina, 1978). However, ants whether regarded as pest species or not, frequently affect plant health and reproductive output indirectly via the phytophagous insect that they tend and defend. The mealybugs remove plant sap, which led to damaged plant tissues or injection of toxins (Nixon, 1951; Steyn, 1954; Briese, 1982), and generally contaminate fruit and foliage with honeydew that becomes blackened with sooty moulds which may impair photosynthesis and sometimes lead to leaf abscission.

In this study, it was also found that the different ant species removed honeydew, which improved the sanitation of the mealybug aggregations by reducing physical fouling caused by both the honeydew and the sooty moulds that grew on them. In colonies of mealybug that were not attended by ants, younger nymphal stages (particularly, crawlers) become engulfed in their own honeydew and die in large numbers. Several authors have confirmed these findings and demonstrated that the removal of honeydew prevent contamination, which is especially detrimental to first-instar nymphs (Cudjoe et al., 1993; Daane et al., 2006b; Daane et al., 2007; Gullan and Kosztarab, 1997; Moreno et al., 1987; Flander, 1951; Way, 1954b; Bess, 1958; Das, 1959). However, it is not clear whether death of the mealybugs resulted from asphyxiation or from some effect of the fungal growth which usually follows honeydew contamination.

During this survey *O. longinoda* and *P. megacephala* were observe to transport *R. iceryoides* to new feeding sites on the same plants or to uninfested plants, thus greatly facilitating the spread of *R. iceryoides* populations. Records of scale insect transport by *O. smaragdina* and *O. longinoda* has been reported by Das (1959) and Way (1954b). When mealybug populations were low, *P. megacephala* and *O. longinoda* built protective structures over *R. iceryoides*, which they were attending possibly to limit predatory and parasitic attacks. Smit and Bishop (1934) argued that the shelters were of primary benefit for the ants although they also conferred limited benefit to the mango mealybug by reducing exposure to natural enemies. This could be true because on many occasions during this study, parasitized mealybugs were collected from them and even predatory beetle larvae fed on adult female ovisacs underneath these shelters, particularly within fruit bunches. Other authors have reported that these shelters are of benefit to the scale insects by providing protection from bad weather (Briese, 1982; Way, 1954b), excluding predators and parasitoids (Wheeler, 1910; Strickland, 1950; Way, 1954b; Clarke et al., 1989; Nixon, 1951; Das, 1959; Way, 1963; Sugonyayev, 1995) and reducing the incidence of disease.

CHAPTER FOUR

Effect of host plant on life history and population growth parameters of *Rastrococcus iceryoides* Green (Hemiptera: Pseudococcidae)

ABSTRACT

The effect of four cultivated host plants namely *Mangifera indica* L., *Cajanus cajan* (L.) Millspaugh, *Coffee arabica* L. and *Cucurbita moschata* Duchesne, and two important ornamental plants namely *Parkinsonia aculeata* L. and *Ficus benjamina* Roxb., on the biology of the mango mealybug *Rastrococcus iceryoides* Green (Hemiptera: Pseudococcidae) was studied in a screen house at 22.3 ± 5.07˚C, 40 - 80% relative humidity and 12L:12D photoperiod. The development, survivorship, longevity, reproduction and life table parameters of *R. iceryoides* differed significantly among the host plants. The shortest developmental period (from egg to adult) was recorded on *M. indica* (23.5 ± 0.34 days for females and 25.3 ± 0.19 days for males), whereas the longest was recorded on *F. benjamina* (33.0 ± 0.62 days for females and 37.3 ± 0.65 days for males). The highest egg to adult female *R. iceryoides* survivorship was recorded on *C. moschata* (79.6 ± 1.4%) and the lowest was on *C. arabica* (30.9 ± 1.1%). The highest fecundity was observed on *C. moschata*. The sex ratio was female-biased on *C. moschata*, *M. indica*, *C. cajan* and *P. aculeata*, but there was a slightly higher number of males on *F. benjamina* and *C. arabica*. The highest adult longevity of females was 67.35 ± 0.678 days on *C. moschata*, whereas that on *P. aculeata*, *C. cajan* and *F. benjamina* did not differ significantly. Body size of *R. iceryoides* was influenced by the different host plant types. Maximum values of the intrinsic rate of natural increase (r_m), finite rate of increase (λ) and the shortest mean generation time (GT) and doubling time (T_d) were recorded on *M. indica*. The highest net reproductive rate (R_o) was recorded on *C. moschata* and the lowest on *C. arabica*. The results strongly suggest that *M. indica*, *C. moschata*, *P. aculeata* and *C. cajan* were the most preferred host plants. Results of this study indicated that host plant can largely influence the population dynamics of *R. iceryoides*, and our findings are useful in understanding the roles of host plants in integrated management of *R. iceryoides*, including exploitation of these host plants in push-pull control.

***Key-words*:** *Rastrococcus iceryoides*; Host plants; Development; Survivorship; Reproduction

4.1 Introduction

Rastrococcus iceryoides Green is an invasive pest of mango in East Africa (mainly Tanzania and coastal Kenya) and northern Malawi (Williams, 1989; Luhanga and Gwinner, 1993; CABI, 2000). In Southern Asia, the putative aboriginal home of *R. iceryoides*, the pest is believed to be highly polyphagous and has been reported from over 65 host plants from 35 families (Williams, 1989; Ben-Dov, 1994). In Kenya and Tanzania, recent observation showed that the insect attack 29 host plants from 16 families with *Mangifera indica* as the most preferred cultivated plant and *Parkinsonia aculeata* as the preferred non-cultivated host plant (Tanga, unpublished data). As with other mealybug species, *R. iceryoides* sucks sap from leaves, young shoots, inflorescences and fruits. Mealybug activity also results in shedding of mango fruit-lets. They also excrete sugary honeydew on which sooty mould develops thus reducing fruit marketability. As a result of sooty mould, export opportunities are often impaired due to quarantine regulations (CPC, 2002). Sooty mould that fouls the leaves reduces photosynthetic efficiency and can cause leaf drop. In village homesteads, heavy infestations usually render the trees unsuitable for shade. In Kenya, Tanzania and Malawi, damage can range from 30% to complete crop failure in unmanaged orchards (CABI, 2000; C.M. Tanga, unpublished data). In Tanzania, the pest has become the major target for majority of insecticidal sprays on mango (in addition to pruning and burning of infested plant parts) (Willink and Moore, 1988; C.M. Tanga, unpublished data). In addition to health concerns attributed to chemical pesticides, resource-limited farmers cannot afford to use them. Chemical pesticide also does not provide adequate control owing to the waxy coating of mealybugs. Some growers have resorted to cutting down mango trees as a result of heavy *R. iceryoides* infestation while others have abandoned mango cultivation altogether. It has been speculated that the intensity of damage by *R. iceryoides* may have been due to the expansion of mango production and the introduction of hybrid cultivars, which are highly susceptible to attack by the pest (Boussienguet and Mouloungou, 1993).

Different host plant species have also been shown to affect the life history parameters of different mealybug species. For example, the mortality of the citrus mealybug *Planococcus citri* (Risso) was reported to be higher on green than red or yellow variegated *Coleus blumei* Bellevue (Bentham) plants, and development was faster and fecundity higher on red variegated plants (Yang and Sadof, 1995). The developmental time of *Planococcus kraunhiae* (Kuwana) was shorter when reared on germinated *Vicia faba* L. seeds than on leaves of a *Citrus* sp. L. and on *Cucurbita maxima* Duchesne, and it survived better when reared on germinated *V. faba* seeds than on citrus leaves (Narai and Murai, 2002). The pink hibiscus mealybug, *Maconellicoccus hirsutus* (Green), was able to develop equally well on *Cucurbita pepo* L. as on *C. maxima* (Serrano and Lapointe, 2002).

The current *R. iceryoides* host plant data from Africa (Tanga, unpublished data) are based on field observation of damage. There are no comparative demographic data and biological performance of the mealybug on the different host plants to determine the true value of the plant species as host of *R. iceryoides*. Host plants that slow or accelerate the development of the insect are likely to be considerable relevance to the development of management methods. Studies on the biology and life table parameters of *R. iceryoides* on different host plants should provide information in understanding the population dynamics of the insect.

The main objective of this study was to investigate the development and reproduction of *R. iceryoides* on four cultivated host plants namely mango (*Mangifera indica* L., Anacardiaceae), Pigeon pea (*Cajanus cajan* (L.) Millspaugh, Fabaceae), Arabica coffee (*Coffea arabica* L., Rubiaceae) and crookneck squash (*Cucurbita moschata* Duchesne, Cucurbitaceae), and two important ornamental plants namely Jerusalem thorn (*Parkinsonia aculeata* L., Fabaceae) and Weeping fig (*Ficus benjamina* Roxb., Moraceae). Another objective was to develop life table structure for the insect and estimate parameters for population increase on the different host plants to guide pest management decisions. The host plants selected represents some of the most economically important plants both in terms of horticulture, beverage and ornamental or shade plants in Kenya and Tanzania.

4.2 Materials and Methods

4.2.1 Host plant

Six host plants were used in this study, namely mango, pigeon pea, arabica coffee, butternut squash, Jerusalem thorn and weeping fig. Twelve-month old mango and coffee seedlings were obtained from the commercial nursery of Kenya Agriculture Research Institute's (KARI) and Coffee Research Foundation (CRF) in Ruiru, Kenya, respectively. The Weeping fig and Jerusalem thorn of same age were obtained from Tropical Nursery, Nairobi, Kenya and

Malindi, Kenya respectively. The production polythene bags of each seedlings were removed in the screen house and the seedlings were subsequently transplanted into white plastic containers (35 cm height x 29 cm top diameter x 20 cm bottom diameter or 19 cm height x 21 cm top diameter x 12.8 cm bottom diameter) in a soil mix containing sieved forest soil and sand (1:1 by volume). The pigeon pea plants were propagated from seeds (va. ICEAP00040) (Høgh-Jensen, 2007) obtained from KARI Seed Unit (KSU), Nairobi, Kenya. The butternut squashes (fruit) were purchased from a local grocery store in Kasarani, Nairobi, Kenya. Seedlings of all the plant species were placed on benches in a screen house (2 m height by 2.9 m diameter) at the International Centre of Insect Physiology and Ecology (*icipe*), Nairobi, Kenya. Plants were fertilized with farmyard manure, a common agronomic practice by the growers and watered on alternate days.

4.2.2 Insect culture

Colony of *R. iceryoides* was initiated from a cohort of 300 adult mealybugs collected from heavily infested mango orchard in Mombasa, Coastal Province, Kenya in February 2008. Insect were transported to the laboratory at *icipe*, Nairobi, Kenya. The insect were reared on mature butternut squashes (purchased from a local grocery store) in the laboratory maintained at room temperature of 25-26^{0}C, photoperiod of 12 h L: 12 h D, and 40-70% relative humidity (RH). The colony was maintained on an open table surface (76 cm wide x 245 cm length) in the laboratory for over 20 generations before the start of the experiment. The sides of the table were blocked with plywood (10.5 cm height x 245 cm length) to prevent the crawling insects from falling off the table. The colonies were maintained by exposure of uninfested butternuts to adult females with fully developed ovisacs. Eggs hatched within 6-8 days and newly emerged nymphs are allowed to colonize the uninfested butternuts. This procedure is repeated on a weekly basis. After every 6 months, fresh wild *R. iceryoides* from mango are injected into the established colonies to ensure broader genetic diversity.

4.2.3 Maintenance of *R. iceryoides* on the study plant materials

For the bioassay, insect were reared on the different host plants for at least 3 generations in the screen house to allow them adapt to the new host and to remove maternal effects (Lacey,

1998) before commencement of the experiments. Approximately 40 adult female mealybugs (18 d old) with well-formed ovisacs were obtained from the stock colony to infest each of the different host plants under investigations. The ovisacs were carefully teased open with blunt probes under a stereomicroscope and the number of eggs present in each ovisac counted. The eggs were then refolded into the fine cottony ovisac before inoculation. After the first generation on these host plants, subsequent uninfested plants were similarly infested with ovisacs from their respective cultures. In the screen house, the plants were maintained in large cages (30 cm length x 30 cm width x 60 cm height) consisting of a glass top and screened mesh (30 cm length x 30 cm width x 60 cm height) on the sides. Experimental conditions in the screen house were: 22.3 ± 5.07 ˚C, 40 - 80% RH and 12L: 12D photoperiod.

4.2.3.1 Assessment of *R. iceryoides* development, survivorship and sex ratio on the different host plants

Thirty eggs (collected within 12 h) were obtained from a single female ovisac arising from the different host plant species and transferred to the seedlings of the various host plants using a fine-tipped paintbrush (American Painter 4000, Loew-Cornell, Englewood Cliffs, NJ). Mango, arabica coffee, Jerusalem thorn and weeping fig seedlings were 12 months old at commencement of the experiments while pigeon pea were at 3 months. In the case of butternut squash, the insects were maintained on fruits similar to the rearing conditions and used as check. After inoculation, each host plant seedling and fruit were housed individually in wooden cages (30 cm length x 30 cm width x 60 cm height).

Host plants was checked twice daily for egg hatch and exuviae to identify emergence of nymphal instars. The sex of each individual mealybug was determined during the latter part of the second instar when the males finally shed-off the white mealy-covering on their body and change their colour from orange to pale yellow with light ashy deposit on their body. Development of the males at this point continued with their body completely devoid of lateral processes and the duration of development of each sex could be recorded separately. The following data were collected for each host plant: (1) developmental duration for each stage, (2) the number of emerging adult males and females (3) offspring sex ratio ([♀ / (♀ + ♂)]), and (4) percentage survival of each of the immature stages. Five replicates were maintained for each host plant species.

4.2.3.2 Morphometric analysis

Fifty randomly selected nymphal instars of each developmental stage from the plant species under investigation were treated and slide-mounted using the methodology of Watson and Kubiriba (2005). The body length was measured along each insect's dorsal midline from the vertex of the head to the tip of the abdomen. The width was measured across the middle surface of the insect. All measurements were carried out using a Leica EZ4D stereomicroscope with an integral digital camera [Leica Microsystems (Switzerland) Limited].

4.2.3.3 Reproduction and Longevity

Forty randomly selected newly emerged virgin adult females (24 h old) derived from nymphs reared on each host plant species were evaluated to determine the effect of host plant on *R. iceryoides* reproduction and longevity. Within each host plant treatment, half of the mealybugs (i.e. 20 females) were held alone to assess asexual reproduction and the other half (i.e. 20 females) were used to assess sexual reproduction. Each female used for sexual reproduction was transferred individually to plastic Petri dishes (5 cm in diameter and 1 cm height) with a wet cotton ball at the side together with three newly emerged males (24 h old) from the same plant species and allowed to mate for 24 h. After mating, females were transferred to their respective host plants and observe daily until they died. The total number of eggs produced by each female was recorded daily. The eggs were kept separately in transparent polyvinyl chloride (PVC) cylinder, (4 cm in diameter x 10 cm height x 0.21 mm thick) lined with pieces of moistened black filter paper (3.5 by 1.5 cm) to prevent desiccation of the eggs and egg hatch was determined every 12 h for a period of 7 days. Emerging nymphs from each daily cohort of eggs were removed using a Carmel hair brush (#000) with the help of a magnifier hand lens (size: 100 mm in diameter). Females for asexual reproduction were also observed daily until they died.

The following data were collected for each host plant (1) pre-oviposition, oviposition, and post-oviposition periods (2) adult longevity and (3) daily egg production. Standard life table parameters including age-specific fertility (m_x; mean number of female progeny per female per day) and female survivorship (l_x; the fraction of females surviving to age x) were calculated from daily records of mortality and fecundity of cohorts on each host plant. For each of the two reproductive stages (sexual and asexual), each female was considered a replicate.

4.2.4 Statistical analysis

Statistical analysis was performed using a general linear model (PROC GLM) of SAS for all experiments to compare the data from all host plant treatments. Data for the developmental times, pre-oviposition, oviposition, post-oviposition periods, adult female longevity, egg production and size of *R. iceryoides* were subjected to a one-way analysis of variance (ANOVA). The sex ratios and survival rates of *R. iceryoides* were arcsine transformed to normalize data (Sokal and Rohlf, 1981) before analysis of variance. The means were separated by Student-Newman-Keuls (SNK) procedure at a significant threshold of 0.05 (PROC GLM; SAS 9.1 Institute, 2010). Percentage survival estimates were based on mealybug found alive during each counting period, divided by preceding counts for each host plant species. Fertility life table for each host plant species was constructed following the method described by Carey (1993). Demographic parameters (intrinsic rate of increase (r_m), net reproductive rate (R_o), mean generation time (GT), doubling time (T_d) and finite rate of increase (λ)) were estimated using the Jackknife program (Maia et al., 2000). Differences between life table parameters across the different host plant species based on Jackknife estimates of variance for each parameter value (Meyer et al., 1986) were separated using SNK.

4.3 Results

4.3.1 Development, survivorship and sex ratio

There were significant differences in the developmental times of *R. iceryoides* reared on six host plant species (Table 4.1). Egg development took 7.8 to 8.7 d across the different host plants and was shortest on *M. indica* (7.8 d). The development of the first instar nymphs ranged from 4.8 d (*C. moschata*) to 9.6 d (on *F. benjamina*). Male second instar development was shortest on *M. indica*, *C. moschata*, and *P. aculeata* (4.8-5.0 d) and longest on *F. benjamina*

(11.4 d). Similarly, female second instar had the shortest development on *P. aculeata* (4.8 d) and longest on *F. benjamina* (8.1 d). The development by third male instar ranged from 3.4 d on *C. moschata* to 9.5 d on *C. arabica* while female development was shorter on *C. cajan* and also longer on *C. arabica*. *Mangifera indica* was the most conducive for development of fourth instar males (4.7 d) and males took 8.7 d to complete development on *F. benjamina*. Egg to adult development was shortest on *M. indica* (25.3 and 23.5 d) for males and females, respectively. *Rastrococcus iceryoides* recorded the longest development on *F. benjamina*. Second-instar, pre-pupa and pupa males did not produce cocoon.

Egg survival was highest on *M. indica*, *C. moschata* and *F. benjamina* (85-90%) compared with the other host plants while first instar survived more on *C. moschata*, *P. aculeata* and *C. cajan* (80-84%) (Table 4. 2). Survival of second male instar was highest on *C. cajan* (90%) and females survived more on *C. moschata*, *P. aculeata* and *C. cajan* (86%, 86%, 87%, respectively). Third instar males survived more on *C. moschata*, *P. aculeata* and *C. cajan* (91%, 90%, and 91%, respectively) while third female and fourth instar male survival was highest on *C. moschata* (90%). Overall, egg-adult female survival was significantly higher on *C. moschata*, *P. aculeata* and *C. cajan* (80%, 74%, 78%, respectively) compared with the other plants while egg-adult males survival was highest on *C. moschata* (88%) and *C. cajan* (88%) than on the other plant species.

Table 4. 1: Mean number of days (± SEM) for each developmental stage of *R. iceryoides* reared on six host species

			Second		Third		Fourth	Egg-adult	
Plant species	Egg	First	Male	Female	Male	Female	Male	Male	Female
C. moschata	8.4 ± 0.20ab	6.1 ± 0.21c	4.8 ± 0.18c	5.7 ± 0.16c	3.4 ± 0.11d	5.8 ± 0.19b	5.8 ± 0.18d	26.2 ± 0.17deA	25.9 ± 0.36cB
M. indica	7.8 ± 0.19b	5.5 ± 0.15c	4.9 ± 0.13c	5.1 ± 0.17cd	4.2 ± 0.14c	5.1 ± 0.16cd	4.7 ± 0.23e	25.3 ± 0.19eA	23.5 ± 0.34dB
P. aculeata	8.4 ± 0.15ab	5.8 ± 0.16c	5.0 ± 0.15c	4.8 ± 0.18d	4.6 ± 0.18c	5.5 ± 0.14bc	6.4 ± 0.11c	28.4 ± 0.43cA	24.4 ± 0.21dB
C. cajan	8.4 ± 0.18ab	5.8 ± 0.19c	4.9 ± 0.12c	5.4 ± 0.23cd	4.6 ± 0.15c	4.8 ± 0.16d	5.6 ± 0.22d	27.4 ± 0.28cdA	24.4 ± 0.43dB
F. benjamina	8.7 ± 0.22a	9.6 ± 0.27a	11.4 ± 0.37a	8.1 ± 0.32a	8.5 ± 0.28b	6.7 ± 0.28a	8.7 ± 0.29a	37.3 ± 0.65aA	33.0 ± 0.62aB
C. arabica	8.3 ± 0.21ab	6.7 ± 0.22b	9.6 ± 0.26b	6.6 ± 0.17b	9.5 ± 0.42a	6.7 ± 0.15a	7.4 ± 0.26b	34.4 ± 0.58bA	28.3 ± 0.37bB
F	46.38	145.66	96.16	63.81	113.23	38.63	84.47	230.22	77.58
df	5, 894	5, 894	5, 294	5, 294	5, 294	5, 294	5, 294	5, 294	5, 294
P	0.0431	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001

Means within a column followed by the same lower case letters are not significantly different at α = 0.05 (Student-Newman-Keuls)

Meas within a row followed by the same upper case letters are not significantly different at α = 0.05 (Student-Newman-Keuls) for cumulative males and females.

Table 4. 2: Mean (± SEM) percentage of survival (%) for each life-history stage of *R. iceryoides* reared on six different host plant species

			Second		Third		Fourth	Egg to adult	
Host plant	Egg	First	Male	Female	Male	Female	Male	Female	Male
C. moschata	89.7 ± 1.4a	84.4 ± 2.3a	80.1 ± 1.8b	86.2 ± 1.0a	91.2 ± 0.6a	90.1 ± 1.0a	94.0 ± 1.1a	79.6 ± 1.4a	87.9 ± 0.6a
M. indica	88.4 ± 1.4a	66.1 ± 3.0b	74.0 ± 2.3b	70.3 ± 1.7b	83.6 ± 2.2b	80.5 ± 3.2b	87.3 ± 1.8b	64.4 ± 1.6b	79.9 ± 1.0c
P. aculeata	78.4 ± 1.0b	80.1 ± 0.5a	83.6 ± 2.3ab	85.9 ± 0.9a	90.3 ± 1.0a	88.1 ± 0.8ab	88.8 ± 0.7ab	74.9 ± 2.2a	84.2 ± 0.7b
C. cajan	83.7 ± 1.5ab	84.3 ± 1.4a	90.1 ± 1.9a	86.6 ± 1.1a	91.4 ± 1.4a	89.0 ± 0.8ab	90.0 ± 0.7ab	78.2 ± 2.4a	87.8 ± 0.9a
F. benjamina	84.8 ± 1.7a	37.3 ± 3.0d	76.0 ± 3.3b	37.4 ± 3.3d	71.1 ± 2.1c	32.9 ± 5.1d	73.2 ± 1.9c	32.2 ± 1.9c	68.5 ± 0.8e
C. arabica	83.6 ± 2.6ab	48.2 ± 1.8c	73.7 ± 3.0b	51.2 ± 2.7c	88.4 ± 0.6a	54.6 ± 3.3c	89.3 ± 0.7ab	30.9 ± 1.1c	76.6 ± 0.6d
F	6.07	75.51	6.60	112.06	26.00	69.50	20.95	121.21	94.18
df	5, 24	5, 24	5, 24	5, 24	5, 24	5, 24	5, 24	5, 24	5, 24
P	0.0002	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001

Means within a column followed by the same lower case letters are not significantly different at α = 0.05 (Student-Newman-Keuls)

4.3.2. Longevity and reproduction

The mean pre-oviposition, oviposition and post-oviposition periods of *R. iceryoides* were significantly affected by host plant species (Table 3). The duration of pre-oviposition period was highest on *F. benjamina* (29.40 d) while the shortest duration was encountered on *M. indica* (20.35 d). The longest oviposition period of *R. iceryoides* was recorded on *C. moschata* (36.75 d) compared to the other host plant species (Table 4.3) and the mealybugs ceased to oviposit on *C. arabica* after 15 d. Post-oviposition period of *R. iceryoides* was longest on *C. arabica* (15.70 d) and shortest on *P. aculeata* (7.15 d) (Table 4.3).

The females reared on *C. moschata* laid the highest number of eggs (811.3) than did females reared on *M. indica* (716.8), *P. aculeata* (655.4), *C. cajan* (618.6), *F. benjamina* (364.4) and *C. arabica* (267.9). There was no significant difference in the number of egg laid by *R. iceryoides* when reared on *P. aculeata* and *C. cajan*. Daily egg production was highest on *M. indica* (46.6 eggs) although this did not differ significantly from egg production on *P. aculeata*, *C. cajan*, and *C. moschata* (37.7-39.3 eggs/female/day) (Table 4.3). Unmated adult female mealybugs did not lay eggs on any of the six host plant species tested.

Longevity of mated female mealybugs ranged from 56.0 days on *P. aculeata* to 67.35 d on *C. moschata*. No difference in mated adult females' longevity was observed when *R. iceryoides* was reared on *P. aculeata* (59.30 d), *C. cajan* (57.75 d) and *F. benjamina* (58.85 d). Longevity of unmated adult females ranged from 70.80 d on *C. arabica* to 90.50 d on *C. moschata*. There was no significant difference in unmated adult female longevity on *M. indica* (87.3 d) and *C. moschata* (90.5 d), *P. aculeata* (84.6 d) and *M. indica*, as well as on *C. cajan* (81.0 d) and *F. benjamina* (78.6 d) (Table 4.3). Longevity of unmated female mealybugs were significantly higher when compared with mated females reared on the same host plant, for all host plants tested (Table 4.3).

The offspring sex ratio [♀ / (♀ + ♂)] of *R. iceryoides* reared on *M. indica*, *P. aculeata*, *C. cajan* and *C. moschata* were female biased and ranged from 56-64% (Table 4.3). On *F. benjamina* and *C. arabica*, offspring sex ratio ranged from 44-49% (Table 4.3).

Table 4. 3: Mean (± SEM) sex ratio, duration (days ± SEM) of pre-oviposition, oviposition and post-oviposition periods, longevity and reproduction rate of *R. iceryoides* reared on six host plant species

Plant species	Pre-oviposition period	Oviposition period	Post-oviposition period	Reproductive rate		Longevity		Sex ratio (Females)
				Fecundity (eggs/female life)	Oviposition rate (eggs/female/day)	Mated female	Unmated female	
C. moschata	24.2 ± 0.4c	36.8 ± 0.4a	6.4 ± 0.3d	811.3 ± 7.3a	37.7 ± 3.5ab	67.4 ± 0.7aA	90.5 ± 0.8aB	63.6 ± 1.3a
M. indica	20.4 ± 0.4e	32.5 ± 0.3b	8.6 ± 0.3c	716.8 ± 12.7b	46.6 ± 4.3a	61.4 ± 0.5bA	87.3 ± 1.0abB	58.3 ± 1.2b
P. aculeata	21.7 ± 0.3d	30.5 ± 0.5c	7.2 ± 0.3d	655.3 ± 20.8c	39.3 ± 3.3ab	59.3 ± 0.7cA	84.6 ± 1.6bB	57.1 ± 1.7b
C. cajan	22.1 ± 0.3d	27.7 ± 0.2d	8.1 ± 0.2c	618.6 ± 17.3c	37.7 ± 3.5ab	57.8 ± 0.5cA	81.0 ± 1.4cB	56.2 ± 1.4b
F. benjamina	29.4 ± 0.3a	16.8 ± 0.3e	12.7 ± 0.3b	364.4 ± 15.2d	28.0 ± 3.6b	58.9 ± 0.5cA	78.6 ± 1.2cB	48.5 ± 0.6c
C. arabica	25.4 ± 0.3b	15.0 ± 0.4f	15.7 ± 0.3a	267.9 ± 15.5e	25.4 ± 3.0b	56.0 ± 0.5dA	70.8 ± 0.7dB	43.6 ± 0.5d
F	92.91	597.16	147.27	184.32	3.94	48.24	36.36	25.12
df	5, 114	5, 114	5, 114	5, 114	5, 114	5, 114	5, 114	5, 24
P	< 0.0001	< 0.0001	< 0.0001	< 0.0001	0.0016	< 0.0001	< 0.0001	< 0.0001

Means within the same column followed by the same lower letters and within the same row followed by the same upper case letters do not differ significantly by Student-Newman-Keuls test (α = 0.05).

4.3.3 Morphometric analysis

The body size of the different developmental instars of *R. iceryoides* was significantly influenced by host plant species (Table 4.4). Adult female *R. iceryoides* reared on C. *moschata* (3.930 mm) were significantly larger in body length than females reared on the other host plants. Adult females with the smallest body length were recorded when *R. iceryoides* was reared on *C. arabica* (2.243 mm). Adult female body width did not differ significantly among *R. iceryoides* reared on *M. indica*, *P. aculeata*, *C. cajan* and *C. moschata* (2.634-2.677 mm) (Table 4.4).

4.3.4 Age-specific fecundity and age-specific survivorship

The curves of age-specific fecundity (m_x) peaked soon after the onset of reproduction and varied considerably among the different host plant species (Figure 4.1). The age-specific fecundity for *R. iceryoides* reared on *M. indica* peaked on day 24, *P. aculeata* on day 25, *F. benjamina* on day 33 and *C. arabica* on day 29. Age-specific fecundity observed for *R. iceryoides* reared on *C. moschata* and *C. cajan* were remarkably different, each having two peaks (Figure 4.1). Major peaks for *R. iceryoides* reared on *C. moschata* were on day 30 and 33 while on *C. cajan*, a major peak was recorded on day 27 and a minor peak on day 31. The age-specific survivorship (l_x) curves decreased gradually and asymptotically as *R. iceryoides* aged (Figure 4.1). On *M. indica,* 50% of mortality occurred on day 43, and the entire mealybug cohort died on day 62. On *C. moschata*, 50% of mortality occurred on day 48, and all mealybugs died on day 68.

4.3.5 Population growth statistics

The net reproductive rate (R_o) on *C. moschata* was 1.6, 1.4, 1.6, 6.5 and 10.5 times higher than on *M. indica*, *P. aculeata*, *C. cajan, F. benjamina* and *C. arabica*, respectively. The intrinsic rate of increase (r_m), population doubling time (T_d), net reproduction rate, generation time and infinite rate of increase (λ) were also significantly influenced by host plant species tested (Table 4.5). The intrinsic rate of increase was higher on *M. indica* (0.178) and the population was expected to double in 3.9 days. The lowest r_m was recorded on *C. arabica* (0.102) with a doubling time of 6.8 days. *Mangifera indica* recorded the lowest generation time of 31 days and the highest duration occurred on *F. benjamina*. The infinite rate of increase was 1.11 on *F. benjamina* and *C. arabica* and 1.20 on *M. indica* and *C. cajan* (Table 4.5).

Table 4. 4: Morphometric measurements of body size of each developmental stage of female *R. iceryoides* reared on six host plants species

Host plants	Egg		First		Second		Third		Adult	
	Length (mm)	width (mm)	Length (mm)	Width (mm)	Length (mm)	Width (mm)	Length (mm)	Width (mm)	Length (mm)	Width (mm)
C. moschata	0.27 ± 0.004a	0.17 ± 0.002a	0.64 ± 0.002a	0.28 ± 0.003a	1.81 ± 0.004b	1.31 ± 0.005a	2.89 ± 0.11a	1.57 ± 0.020b	3.93 ± 0.011a	2.68 ± 0.037a
M. indica	0.24 ± 0.004bc	0.17 ± 0.005a	0.55 ± 0.003b	0.28 ± 0.003a	2.26 ± 0.003a	0.89 ± 0.008c	2.84 ± 0.004a	1.63 ± 0.005a	3.87 ± 0.021b	2.64 ± 0.004a
P. aculeata	0.23 ± 0.006bc	0.17 ± 0.004a	0.51 ± 0.003d	0.26 ± 0.002c	1.77 ± 0.004c	0.97 ± 0.020b	2.61 ± 0.00 9b	1.27 ± 0.004c	3.74 ± 0.009c	2.63 ± 0.009a
C. cajan	0.24 ± 0.006b	0.17 ± 0.004a	0.53 ± 0.004c	0.26 ± 0.002c	1.79 ± 0.013b	0.98 ± 0.016b	2.65 ± 0.007b	1.28 ± 0.005c	3.76 ± 0.006c	2.65 ± 0.009a
F. benjamina	0.22 ± 0.004c	0.16 ± 0.004a	0.53 ± 0.002c	0.27 ± 0.003b	1.74 ± 0.003d	0.87 ± 0.003c	2.02 ± 0.010d	1.22 ± 0.006d	3.41 ± 0.006d	2.52 ± 0.009b
C. Arabica	0.21 ± 0.005d	0.14 ± 0.005b	0.42 ± 0.002e	0.26 ± 0.002c	1.44 ± 0.003e	0.81 ± 0.004d	2.14 ± 0.051c	1.08 ± 0.027e	2.24 ± 0.007e	1.80 ± 0.039c
F	22.25	12.3	375.14	14.79	570.3	235.8	269.72	225.28	683.45	218.37
df	5, 294	5, 294	5, 294	5, 294	5, 294	5, 294	5, 294	5, 294	5, 294	5, 294
P	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001

Means within the same column followed by the same lower case letters are not significantly different at α = 0.05 (Student-Newman-Keuls)

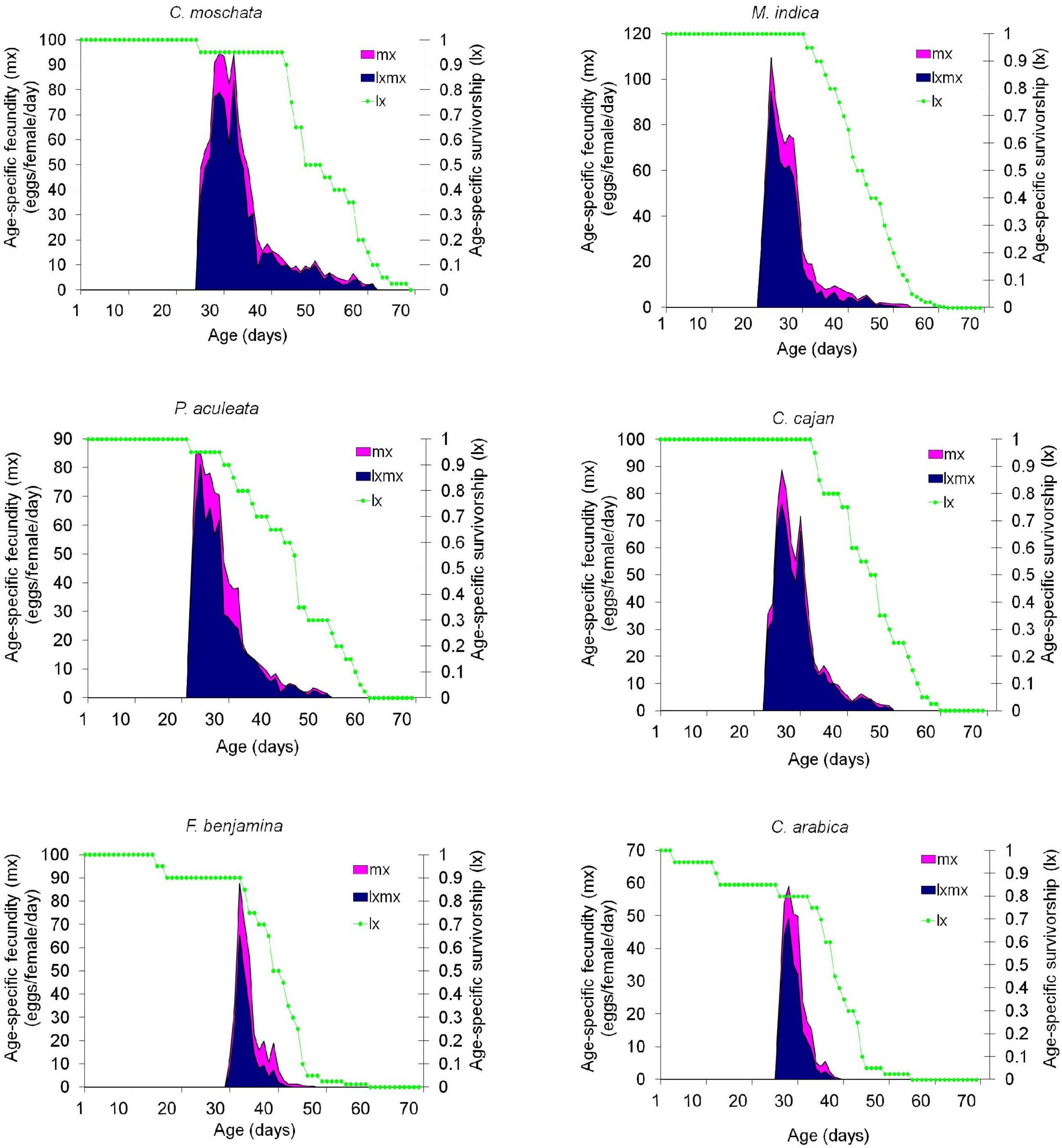


Figure 4. 1: Age-specific fecundity (m_x), age-stage specific maternity (l_xm_x), and age-specific survivorship (l_x) of *R. iceryoides* reared on six host plant species.

Table 4. 5: Effects of various host plant species on life table parameters of *R. iceryoides*

Host plants	R_o	r_m	T_d	GT	λ
M. indica	240.95 ± 4.541c	0.178 ± 0.003a	3.90 ± 0.063d	30.812 ± 0.501e	1.20 ± 0.004a
P. aculeata	274.33 ± 3.611b	0.172 ± 0.001b	4.03 ± 0.010c	32.64 ± 0.056c	1.19 ± 0.001b
C. cajan	240.53 ± 3.329c	0.175 ± 0.001a	3.96 ± 0.013d	31.33 ± 0.081d	1.20 ± 0.001a
C. moschata	378.95 ± 11.850a	0.169 ± 0.002b	4.10 ± 0.051c	35.13 ± 0.603b	1.18 ± 0.003b
F. benjamina	58.28 ± 1.911d	0.108 ± 0.001c	6.42 ± 0.049b	37.64 ± 0.098a	1.11 ± 0.001c
C. arabica	36.12 ± 1.093e	0.102 ± 0.001d	6.79 ± 0.058a	35.17 ± 0.124b	1.11 ± 0.001c

For each parameter, mean ± SE within column followed by the same letter do not differ significantly according to Student-Newman-Keuls test ($P < 0.05$). r_m = Jackknife estimate of the intrinsic rate of increase (female eggs/female/day), R_o = net reproductive rate (female offspring/female/generation), GT = mean generation time (days), T_d = doubling time (days) and λ = infinite rate of increase for population (female offspring/female/day).

4.4 Discussion

Results of this study showed that the six host plant species tested support the development of *R. iceryoides*. However, the results established that although the six host plants were acceptable hosts with mealybugs developing to adulthood, the host plants differ significantly in their suitability for *R. iceryoides* development and weight of the insect. Previous field studies have suggested that *M. indica*, *P. aculeata* and *C. cajan* were the most heavily infested host plants by *R. iceryoides* (Williams, 1989; Luhanga and Gwinner, 1993; Gado and Neuenschwander, 1993; CABI, 2000; Neuenschwander and Ajuonu, 1993; Tanga, unpublished data). In this study, *M. indica*, *P. aculeata*, *C. cajan* and *C. moschata* were observed to be the optimal host for larval development and survival. van Lenteren and Noldus (1990) stated that shorter pre-reproductive period and increased reproductive capacity of an insect on a host reflect the suitability of the plant. It was observed that on the most suitable host plants (*M. indica*, *P. aculeata*, *C. cajan* and *C. moschata*), *R. iceryoides* grew faster and had higher progeny production, probably due to the high nutritional status of these plant species when compared to the less suitable host plants. These findings also strongly corroborates with the observations by Boavida and Neuenschwander (1995) who also reported shorter pre-reproductive period and higher progeny production for *Rastrococcus invadens* Williams when reared on its most suitable host plant, *M. indica*. Matokot et al. (1992) showed that the development of *R. invadens* Williams (Homoptera: Pseudococcidae) vary considerably when reared on *M. indica*, *Ficus* sp., frangipani and *Citrus* spp. Marohasy (1997) reported no difference in development, survival, and fecundity of cohorts of *Phenacoccus parvus* Morrison, when reared on *Lantana camara* L., *Lycopersicon esculentum* Miller, and *Solanum melongena* L., but *Gossypium hirsutum* L., *Ageratum houstonianum* Miller, and *Clerodendrum cunninghamii* Benth were identified as less suitable host plants. By and large, the results of this investigation provide strong indication that *R. iceryoides* host plant species will have a significant impact on population dynamics thereby affecting the timing and extent of mealybug damage to these hosts. *Rastrococcus iceryoides* development on highly suitable plant such as *M. indica* and *C. cajan* may result in rapid development and greater numbers of mealybugs surviving to adulthood hence more damage on these host plants. The observation has significant implications for management of the pest on the suitable host plants. *Parkinsonia aculeata* is an important ornamental shade plants used by growers in the vicinity of *M. indica* and *C. cajan* crops. An important biological control method using mealybug parasitoids could be conservation and or augmentative releases of appropriate parasitoids on *P. aculeata* for parasitoid population build up and subsequent suppression of *R. iceryoides* population before their spread into the cultivated crop.

Although development, survival and reproduction were poor on *F. benjamina* and *C. arabica*, these host plants supported establishment of *R. iceryoides*. It is probable that some constituent compounds or physiological barriers inherent in this host plant species significantly reduced feeding and consequent reduction in development and reproductive performance of *R. iceryoides*. Despite these observations, *C. arabica* especially warrants careful monitoring given the previous history of invasion and impact of *Planococcus kenyae* Le Pelley on coffee in East Africa and its subsequent classical biological control by *Anagyrus kivuensis* Compere (Greathead, 1971; 2003). Base on these findings, it is likely that more important host plants will be shown to support development of *R. iceryoides* as further studies are carried out.

Rastrococcus iceryoides sex ratio was significantly affected by host plant species that the insect was reared on; which was female biased on *M. indica*, *P. aculeata*, *C. cajan* and *C. moschata*. This suggests that maternally-influenced sex ratio distortion or mortality of either sex during egg and nymphal development are dependent on the host plant species used. Contrary, to our study, developmental studies on *R. invadens* revealed a significantly male-biased sex ratio with male and female ratio ranging from 2.1:1 to 3.3:1 on its most preferred host plant, *M. indica* (Sahoo and Ghosh, 2000). Sex ratio was male biased in the less suitable host plants (*F. benjamina* and *C. arabica*). The findings may be of practical significance to potential economic losses on these plants as a high proportion of males will result in less damage in the present generation and fewer off-springs in subsequent generations (Moore, 2004).

The morphometric studies revealed that the body size of *R. iceryoides* was greatly influenced by the host plant type on which the mealybug was reared. As with the developmental studies, mealybugs reared on *M. indica*, *P. aculeata*, *C. cajan* and *C. moschata* had significantly larger body size than those reared on *F. benjamina* and *C. arabica.* Body size is influenced, among other factors, by different host plant species due to differences in nutritional quality, chemical constituents as well as physical differences in the plant structures that adversely affect development, reproduction, survival, behaviour and distribution of phytophagous insect (Slansky and Rodriguez, 1987b; Bethke et al., 1998). Larger individual mealybugs have the potential to cause more damage individually, as food intake is positively correlated with body weight (C. Tanga, unpublished data). Positive relationships between body size and subsequent fecundity are common in other insects (Haukioja and Neuvonen, 1985; Sopow and Quiring, 1998; Ekesi et al., 2007) and evidence suggests that similar relationships exist in female *R. iceryoides* (C. Tanga, unpublished data). On other mealybug species, it has been reported that mealybugs feeding on host plant species with high nitrogen concentrations have increased egg loads, larger matured females, and shorter developmental time (Klinguaf, 1987; Bethke et al., 1998; Hogendorp et al., 2006). Conversely, it is likely that adult mealybugs emerging from suboptimal host plants tested may have less potential to inflict damage on the plants if their numbers, size and fecundity are lower.

The intrinsic rate of natural increase (r_m) is the most important parameter for describing the growth potential of a population under given climatic and food conditions, because, it reflects the overall effect of development, reproduction and survival (Southwood and Handerson, 2000). The results from this study indicated that *M. indica* and *C. cajan* are the most suitable among the plants tested for *R. iceryoides* (r_m of 0.178 and 0.175, respectively). For *R. invadens*, Boavida and Neuenschwander (1995) reported r_m values of 0.070-0.078 on *M. indica*. In addition to helping predict the population growth potentials, the life table findings from the different host plants in this study have practical implications to more efficient and effective production of the mealybugs for parasitoid mass rearing and releases. Reproductive values (m_x), would be helpful in determining the best host plants for rearing. To judge from the R_o and r_m values, mass rearing would be suitable on the four most optimal host plants.

Insecticides do not generally provide adequate control of mealybugs owing to their waxy coating. Biological control with natural enemies is most recommended option (Neuenschwander, 2003; Moore, 2004). Explorations are already underway in India to introduce efficient natural enemies for the suppression of *R. iceryoides*. A practical implication of the current study is the potential interaction between host plant effect on mealybug development and the level of control by the mealybug parasitoids. For example, the encyrtid parasitoid, *Anagyrus pseudococci* has been observed to allocate fewer eggs to mealybugs developing on suboptimal hosts with the capacity to kill lesser proportion of *R. iceryoides* when development is delayed or where average nymphal population is reduced (Tanga et al., unpublished data).

In summary, the present study have demonstrated that development, survival, morphometric and demographic parameters of *R. iceryoides* are optimal on host plants such as *M. indica*, *P. aculeata*, *C.cajan* and *C. moschata* compared to *F. benjamina* and *C. arabica*. Demographic parameters on the most suitable host plants showed that these host plants will be excellent for mass rearing of *R. iceryoides* parasitoids for field releases. These observations also provide important information for future management of the mealybug. On non-crop host plants such as *P. aculeata*, targeted management methods including parasitoid conservation and augmentation on this host should result in the build-up of the parasitoid populations ahead of the mango fruiting season before heavy infestations on the mango fruits start. Overall, the information provided in this investigation should be essential in understanding the dynamics of the mealybug *R. iceryoides* and form vital part of an integrated management plan that allows for targeted suppression of the mealybug in East Africa.

CHAPTER FIVE

Effect of plant species on life history parameters and bio-control potential of *Anagyrus pseudococci* Girault (Hymenoptera: Encyrtidae), parasitoid of *Rastrococcus iceryoides* (Hemiptera: Pseudococcidae) in Africa

ABSTRACT

Anagyrus pseudococci Girault (Hymenoptera: Encyrtidae) is a solitary, koinobiont endoparasitoid of several mealybug species**.** The effect of five host plants (*Mangifera indica, Cucurbita moschata, Parkinsonia aculeata, Cajanus cajan and Ficus benjamina*) on host acceptability for oviposition (as measured by % parasitized nymphs) and suitability (as measured by day to mummification, percent mummified host, percent parasitoid adult eclosion, sex ratio and pre-maginal developmental time) for immature development of this parasitoid in the invasive mango mealybug *Rastrococcus iceryoides* (Hemiptera: Pseudococcidae) was investigated**.** Effect host plant on fitness trait (parasitoid size, egg load and longevity) and life table parameters was also assessed. Although *A. pseudococci* accepted mealybugs regardless of the host plant, level of acceptability varied significantly, with the highest and lowest percent parasitized nymphs on butternut and weeping fig respectively. Host suitability was also strongly affected by the host plant and largely mirrored host acceptability for all the parameters evaluated. Wasps reared on mealybugs maintained on butternut, were biggest (for both sexes), lived longest and their females were more fecund, while those reared from mealybugs maintained on weeping fig were of inferior quality with regard to all fitness parameter evaluated. In general female had larger body size and lived longer than male reared on meaybug maintained on the same host plant for all host plant tested. The impact of the tested host plant was also evident on all life table parameters of the parasitoid. The parasitoid achieved a greater intrinsic rate of natural increase (r_m), net reproductive rate (R_o), finite rate of increase (λ) in addition to a shorter mean generation time (G) and population doubling time (T_d) on mealy bug maintained on butternut and the reverse was true for those maintained on weeping fig. The findings are discussed in view of improvement laboratory mass rearing, as well as field enhancement of the parasitoid performance.

Key words: Host plants, *Anagyrus pseudococci*, host acceptability, host suitability, life table parameters.

5.1 Introduction

The mango mealybug, *Rastrococcus iceryoides* Green (Hemiptera: Pseudococcidae) is an important pest of mango, (*Mangifera indica* L.). The pest is native to Southern Asia, however, it was, accidentally introduced into Africa (mainly Tanzania, Kenya and northern Malawi) where it rapidly spread and became a serious pest on mango, several ornamentals, forest trees and food crops (Williams, 1989; Luhanga and Gwinner, 1993; C.M. Tanga, unpublished data). *Rastrococcus iceryoides* has also been reported as serious pest to mango and *Albizia lebbek* seedlings in nurseries (Zaman and Maiti, 1994; Pillai et al., 1991). In Tanzania, Kenya, and Malawi, mango losses range from 30% to complete crop failure in unmanaged orchards (CABI, 2000; C.M. Tanga, unpublished data). In the former country (Tanzania) where the pest is widely spread across several agroecological zones, *R. iceryoides* has become a major target for insecticidal sprays on mango in addition to pruning and burning of infested plant parts (Willink and Moore, 1988; C.M. Tanga, unpublished data). Apart from health and environmental hazard caused by to chemical pesticides, they also don't provide adequate control owing to the waxy coating of mealybugs. Due to unaffordability or inaccessibility of these chemical pesticides, some African mango growers, have resorted to cutting down their mango trees as a result of heavy *R. iceryoides* infestation while others have abandoned mango cultivation altogether.

Being alien invasive pest classical biological control is likely to be the best management option for *R. iceryoides* in Africa, considering the fact the pest is of no economic importance in its native home range (Southern Asia), where it is reported to be attacked by several natural enemies (Tandon and Lal, 1978; Narasimham and Chako, 1988; Tandon and Srivastava, 1980) with up to 40% parasitism. However, before embarking on introduction of coevolved natural enemies the composition as well as the efficacy of indigenous natural enemies which may have formed new associations with this pest in Kenya and Tanzania had to be established. This information was generated during a survey conducted in east Africa by Tanga et al (in press). Out of the several natural enemies reported by the authors during the survey, only *A. pseudococci* was found to be the most dominant and widely distributed parasitoid in both countries. However, information regarding the effect of the first trophic level, host plant, on the preference and performance of the third trophic level, *A. pseudococci* on *R. iceryoides* is lacking. The objective of the present study is to evaluate the effect of the of host plant on acceptability for ovipostion and suitability of *R. iceryoides* for the immature development of *A. pseudococci* and also to assess the effect the host plant on fitness trait and life table parameter of this parasitoid.

5.2 Materials and Methods

5.2.1 Host plant

Five host plant species; mango (*Mangifera indica* L.) (Anacardiaceae), Jerusalem thorn (*Parkinsonia aculeata* L.) (Fabaceae), weeping fig (*Ficus benjamina* Roxb.) (Moraceae), pigeon pea (*Cajanus cajan* (L.) Millsp.) (Fabaeae) and butternut (*Cucurbita moschata* Duchesne) (Cucurbitaceae) were used. These plants were selected because of their known association with the invasive pest in the field. All plants used in this experiment were purchased from a commercial nursery in Nairobi and maintained in the screen house (287 cm height x 256 cm length x 252 cm width) at the International Centre of Insect Physiology and Ecology (*icipe*), Nairobi, Kenya, to keep them free from other pests. In the screen house the plants were kept in either plastic containers (35 cm height x 29 cm top diameter x 20 cm bottom diameter or 19 cm height x 21 cm top diameter x 12.8 cm bottom diameter) or large polythene bags (26 cm top opening x 28 cm height). These plants were fertilized with farmyard manure and were watered on alternate days.

5.2.2 Host insect

The colony was initiated from a cohort of 300 adult mealybugs which were collected from mango orchards in coastal Kenya and brought to the laboratory at the International Centre of Insect Physiology and Ecology (*icipe*), Nairobi, Kenya in February 2008. In the laboratory the insect were reared on mature fruit of butternut squash purchased at a local grocery store for about 20 generations before the start of the experiment. For colony maintenance weekly or biweekly infestation of 10-20 butternuts was carried out and after every 6 month fresh wild mealybug isolates from the field were injected in the already established colonies to ensure a broader genetic diversity in the laboratory population.

For the experiments, 30 adult female mealybugs with well-formed ovisacs (similar age) obtained from the stock colony were placed on each of the different host plant species in large cages (30 cm length x 30 cm width x 60 cm height) with fine screen sides and glass tops in the screen house. The cultures on respective host plant species were maintained under screen house conditions at 22.3 ± 1.07 ˚C, 40 - 80% relative humidity (RH), under a photoperiod of 12L: 12D for at least 3 generations to allow them to adapt to their new host, and to minimize their associative learning to their rearing host (butternut squash).

5.2.3 Parasitoid

The parasitoid colony was initiated from a cohort of 93 parasitoids (72 ♀ and 21 ♂), which were collected from the same host plant and location as that of the host and brought to the laboratory at *icipe* where it was reared on third and adult instars of *R. iceryoides* fed on butternut fruits in Perspex cages (30 cm length x 30 cm width x 30 cm height) maintained at ambient condition (26 - 28^{o}C, 45 - 60% RH, and photoperiod of 12L: 12D). An opening (13.5 cm diameter) was made on the front side of the cage to which a sleeve, made of organza material was fixed. The same material was fixed to an opposite opening (20 cm diameter) of the cage to allow for ventilation. A third opening (20 cm diameter) was fixed with a sliding door where newly infested butternuts with *R. iceryoides* were place into the cages regularly to maintain the parasitoid colony. The parasitoids were provided streaks of pure honey as food.

Prior to the experiment the parasitoid were collected from the stock colony in the laboratory and conditioned under screen house conditions using the same procedure described for *R. iceryoides* above. Every two weeks, 20 *A. pseudococci* adult females were released into Persplex clip cages (12 cm length x 12 cm width x 12 cm height). An opening (5.5 cm diameter) was made on the front side of the cage to which a sleeve, made of organza material, was fixed. The same material was fixed to another opening (5.5 cm diameter) on the opposite side of the cage to allow for ventilation. Two small holes (1 cm in diameter) were also made on the top and bottom of the cages to accommodate the stem of the respective plant species. Cotton wool was fixed on these small openings before placing the plant stem to avoid injuring the plant. Each of these host plant species before introducing the female wasp supported 200 - 300 third instar stages (L3) of *R. iceryoides*. Insects were maintained at 22.3 ± 1.07 ˚C, 40 - 80% RH, with 12L: 12D photoperiod under screen house conditions. The parasitoid colonies were reared on the established cultures of *R. iceryoides* from the different host plant species for at least 3 generations before the start of the experiment. At the start of the experiment, 12 days after the release of the parasitoids, mummies were collected from respective host plants and individually placed in gelatin capsules. Mummies were observed twice daily and newly (< 24 h) emerged parasitoids were used for experiments.

5.2.4 Bioassays

5.2.4.1 Effect of host plant on *R. iceryoides* acceptability for oviposition and suitability for immature development of *A. pseudococci*

Naïve, fed, 3day-old wasps (5♀ and 5♂) emerging from *R. iceryoides* reared on each of the host plant were introduced into the test cages containing 100, 3rd instar *R. iceryoides* nymphs maintained on respective host plant. The parasitoids were left to forage and oviposit for 24 hours at ambient conditions described above. Thereafter, the parasitoids were removed and the exposed mealybugs were allowed to continue feeding on the respective host plants for three days. All the surviving *R. iceryoides* were later dissected in phosphate buffer solution under a stereomicroscope and number of nymph containing at least one parasitoid egg was recorded. Also the number superparasitized nymph and encapsulated egg were noted. This experiment was replicated eight times for each plant species. Percent parasitized nymph was computed.

To further test for the effect of host plant on *R. iceryoides* suitability for immature development of *A. pseudococci*, other sets of 100, 3rd instar *R. iceryoides* nymphs were exposed to the parasitoids. Number, and status of the parasitoid used, and the duration and conditions of the exposure were the same as that described above for host acceptability, except in this experiment the hosts were allowed to develop till mummification and parasitoid eclosion. After host mummification, the mummies from each host plant were collected, counted and stored separately in transparent plastic cups (4 cm height, 5.5 cm base diameter and 7.5 cm top diameter) until parasitoid emergence. The number of emerging wasps, and their sex were recorded and their left hind tibia length was measured. Percent mummified nymph was computed based on the initial number of exposed host (100 nymph) while the percent parasitoid emergence was computed based on the number of the mummified nymphs for each host plant. Sex ratio (percent female offspring) was computed as percentage emerging females over the total emerging wasps. The experiment was replicated 8 times for each host plants.

5.2.5 Effect of host plant on some fitness traits of *A. pseudococci*

5.2.5.1 Egg load and female body size

Newly emerged mated *A. pseudococci* females from the different host plants were held separately with access to pure honey and water in Persplex cages (15 cm length x 12 cm width x 12 cm height). Females reared on mealybugs maintained on various host plant were dissected and egg load were recorded at various ages to test for ovigeny. Females were dissected at ages (0, 1, 3, and 9 day-old) and upon death (> 20 day). Female was placed in a drop of phosphate buffer solution on a glass slide. The specimens were then dissected and observed at 35x with a Leica EZ4D stereomicroscope with an integral digital camera [Leica Microsystems (Switzerland) Limited]. Only matured oocytes with well-defined neck connecting the two bulbs were counted. After the number of mature eggs was recorded, the wasps' left hind tibia (LHT) was then mounted temporarily using PBS and measured to the nearest 0.0025 mm under the same magnification.

5.2.5.2 Parasitoid adult longevity of non-ovipositing wasps fed on four different diets

Four groups of twenty parasitoid wasps (10♀ ,10♂) that had emerged from mealybug-infested host plants on the same day (08.00 - 11.00 am), were aspirated and kept in cage (20 cm length x 20 cm width x 20 cm height) for each host plant separately. The cages were kept under the same ambient conditions describe above. The four groups, for each host plant were offered one of the four following diets: (i) distil water only; (ii) pure honey; (iii) 50% honey solution (50%hony: 50 distilled water); and (iv) no provisions (starved). Water only and 50% honey solution were supplied in filter-paper wicks soaked in respective solution inside small vials containing 10 ml of the solution (3.5 cm height x 2 cm top and bottom diameters) with a hole of 1 cm diameter cut on the cap. The water only and 50% honey solution were changed every two days to prevent growth of mould. Pure honey was supplied by streaking it on the underside of the top cages and the parasitoid were monitored daily. Dead wasps were removed and their longevity was recorded. Thereafter, their left hind tibia lengths were measured as described above. Individuals that drowned in excess solution were excluded.

5.2.6 Life table experiment and calculation of demographic growth parameters

One pair of newly emerged fed *A. pseudococci* adults originating from each host plant cultures was introduced into each test Perspex clip cages described above containing twenty nymph of the suitable host stage (third instar) from respective host plant. The parasitoids were left to forage and oviposit for 24 h. The adult female parasitoids were removed and transferred to another infested host plant with 20 new mealybugs each day for another 24 h. This process was repeated for each host plant for the entire life span of the parasitoid female. These observations were replicated using 10 females ($n = 10$ parasitoid) for each host plant. Upon dying, left hind tibia of each female was measured as describe above. For each exposure the mummified mealybugs were carefully removed and transferred into transparent plastic cups (4 cm height, 5.5 cm base diameter and 7.5 cm top diameter) separately for each host plant. The lid of the cup and two side cut windows (4 cm in diameter) were covered with very fine mesh to allow for enough ventilation as well as to prevent escape of parasitoids. The mummies were check twice a day for parasitoids emergence. Parameters recorded during this experiment are: days to mummification, development time, the number of offspring emerging daily, total number of offspring for each parasitoid female during its life time, pre-reproductive, reproductive and post-reproductive periods, and female longevity. Data obtained from this experiment were used to generate demographic growth parameters; the intrinsic rate of natural increase (r_m), net reproductive rates (R_o), mean generation time (G); population doubling time (T_d), finite rate of increase (λ).

5.2.7 Statistical analysis

Percent parasitized nymph, percentage mummified mealybugs, percent emerged adult wasp, sex ratio (percent female offspring) and life time fecundity were analyzed using one way analysis of variance using a general linear model (SAS Institute, 2010). Developmental time, adult wasp size, and longevity of host deprived wasps were analyzed using two way ANOVA, with host plant and sex as factors. Also egg load and longevity of ovipositing females were analyzed using two way ANOVA with host, and female age as factors. Count data were $\log_{10}$ transformed, while the percentages were arcsine transformed before statistical analysis (Sokal and Rohlf, 1981). When ANOVAs were significant, means were separated using the Student-Newman-Keuls (SNK) test. Demographic parameters namely intrinsic rate of increase (r_m), net reproductive rate (R_o), mean generation time (GT), doubling time (T_d) and finite rate of increase (λ) were calculated using the Jackknife procedure described by Hulting et al. (1990).

5.3 Results

5.3.1 Effect of host plant on *R. iceryoides* acceptability for oviposition and suitability of the immature development of *A. pseudococci*

An acceptable host was defined as a host containing at least one parasitoid egg. *Anagyrus pseudococci* females accepted *R. iceryoides* third nymphals regardless of the host plant on which they were cultured (Table 5.1). However, acceptability varied significantly among host plants with the highest percentage parasitized nymphs when the host was cultured on butternut (79.13±1.19%), Jerusalem thorn (74.25±1.52) and mango (73.13±2.48), and lowest on weeping fig (58.75±2.17) (Table 5.1). Superparasitism was recorded on hosts maintained on butternut, Jerusalem thorn and mango with an average percent superparasitism of 2.4±1.02, 1.7±0.77 and 1.1±0.73, respectively. No encapsulation of either egg or larva by *R. iceryoides* was recorded on any of the tested host plants.

Host suitability (as measured by day to mummification, percent mummified host, percent adult parasitoid eclosion, sex ratio and pre-maginal developmental time) was strongly influenced by host plant, and it was largely mirrored host acceptability. Number of days to mummification was significantly shortest when the host was maintained on butternut (9.4 ± 0.38 days), Jerusalem thorn (9.93 ± 0.36 days) and mango (10.47 ± 0.48 days) followed by that on pigeon pea (11.20 ± 0.38 days), and was longest when the host was maintained on weeping fig (12.73 ± 0.34 days) (Table 5.1). While percent parasitoid eclosion and sex ratio were highest when the host was maintained on butternut (83.0 ± 1.72% and 65.79 ± 1.45%, for percent parasitoid eclosion and sex ratio respectively), Jerusalem thorn (74.50 ± 4.47% and 62.25 ± 2.75%, for percent parasitoid eclosion and sex ratio respectively) and mango (68.25 ± 2.17% and 59.64 ± 1.01%, for percent parasitoid eclosion and sex ratio respectively), followed by pigeon pea (67.25 ± 5.04% and 59.53 ± 1.70% for percent parasitoid eclosion and sex ratio respectively) and was lowest when the host was maintained on weeping fig (38.63 ± 2.60% and 57.07 ± 2.33%, for percent parasitoid eclosion and sex ratio respectively). However, sex ratio of the parasitoid offspring was female biased irrespective of the host plant on which the host was maintained (Table 5.1)

Anagyrus pseudococci completed development on *R. iceryoides* maintained on all host plants tested. However, the duration of pre-maginal developmental time varied considerably across host plant (Table 5.2). Comparing the same sex across different host plants, both sexes took significantly shorter time to emerge when the host was maintained on butternut (15.52 ± 0.25 and 15.76 ± 0.14 days, for males and females, respectively). While those maintained on weeping fig required the longest time to complete their development (21.50 ± 0.38 and 23.73 ± 0.17, for males and females, respectively). The overall mean of developmental time of the parasitoid was also significantly shortest when the parasitoid was reared on host maintained on butternut and longest when the parasitoid was maintained on host maintained on weeping fig (Table 5.2). On the same host plant females took significantly longer time to develop than males for all host plan tested except on butternut where female and male developmental time were comparable (Table 5.2). Peaks of males' emergence were one day earlier than those of females when the parasitoid was reared on host maintained on any of the tested host plant except for Jerusalem thorn (Figure 5.1).

5.3.2 Effects of host plant on parasitoid fitness parameters

5.3.2.1 Parasitoid adult size

Body size, as measured by left hind tibia length, was strongly influenced the host plant of the on which the mealybug was maintained for both parasitoid sexes (Table 5.1). Comparing the same sex across different host plants, both sexes attained significantly the largest body size when the host was maintained on butternut (0.415 ± 0.006 and 0.452 ± 0.002 mm, for males and females, respectively) and Jerusalem thorn (0.385 ± 0.004 and 0.450 ± 0.003 mm, for males and females, respectively). While those emerged from hosts maintained on weeping fig were significantly the smallest compared to all other hosts (0.304 ± 0.003 and 0.400 ± 0.002 mm, for males and females, respectively (Table 5.1). On the other hand, males reared on hosts maintained on pigeon pea and mango were comparable with regard to their body size, also female reared on host maintained on Jerusalem thorn, pigeon pea were not significantly different. Female wasps emerged from on *R. iceryoides* were significantly larger than males when reared on the same host plant, for all host plant tested (Table 5.1).

Table 5. 1: Effect of five host plants on biological parameters of *A. pseudococci* produced from 3rd instar *R. iceryoides*. Values represented as Mean ± SE

Host plant	Parasitized nymph (%)	Time to mummification (days)	Host mummified (%)	Adult eclosion (%)	Sex ratio (%)	Tibia length [mm]		Statistics (♀ and ♂)		
						Female	Male	*F*	*df*	*P*
M. indica	73.13 ± 2.48ab	10.47 ± 0.48bc	81.5 ± 3.02ab	68.25 ± 2.17ab	59.64 ± 1.01ab	0.443 ± 0.002bA	0.371 ± 0.002cB	393.74	1, 31	< 0.0001
C. moschata	79.13 ± 1.19a	9.40 ± 0.38c	89.75 ± 1.46a	83.0 ± 1.72a	65.79 ± 1.45a	0.452 ± 0.002aA	0.415 ± 0.006aB	56.0	1, 37	< 0.0001
P. aculeata	74.25 ± 1.52ab	9.93 ± 0.36bc	83.75 ± 3.73ab	74.50 ± 4.47ab	62.25 ± 2.75ab	0.450 ± 0.003abA	0.385 ± 0.004bB	172.94	1, 30	< 0.0001
C. cajan	70.75 ± 1.52b	11.20 ± 0.38b	77.38 ± 3.91b	67.25 ± 5.04b	59.53 ± 1.70b	0.443 ± 0.003bA	0.360 ± 0.003cB	263.46	1, 33	< 0.0001
F. benjamina	58.75 ± 2.17c	12.73 ± 0.34a	48.38 ± 2.67c	38.63 ± 2.60c	57.07 ± 2.33c	0.400 ± 0.002cA	0.304 ± 0.003dB	523.61	1, 33	< 0.0001
F	16.36	11.03	23.06	26.72	2.9	76.47	100.64			
df	4, 35	4, 70	4, 35	4, 35	4, 35	4, 118	4, 46			
P	< 0.0001	< 0.0001	< 0.0001	< 0.0001	0.0359	< 0.0001	< 0.0001			

Means in the same row followed with different letters are significantly different (Student-Newman-Keuls test, $P < 0.05$)

Means followed by the same upper case on the same column are not significantly different

Table 5. 2: Egg-adult development time, longevity, oviposition time and lifetime fecundity (mean ± SE) of female *A. pseudococci* on third instar *R. iceryoides* reared on five different host plant species

Host plant			Mean development time (in days ± s.e.)			Statistics (♀ and ♂)		
	Reproductive period (days)	Lifetime fecundity (progeny #)	♂♀	♂	♀	*F*	*df*	*P*
M. indica	12.1 ± 0.75bc	27.6 ± 1.00bc	19.51 ± 0.12c	18.81 ± 0.19bB	19.83 ± 0.12cA	21.86	1, 49	< 0.0001
C. moschata	10.6 ± 0.64c	34.6 ± 1.10a	15.68 ± 0.13e	15.52 ± 0.25dA	15.76 ± 0.14eA	0.80	1, 61	0.3759
P. aculeata	12.3 ± 0.40bc	30.1 ± 1.66ab	18.47 ± 0.19d	17.22 ± 0.33cB	19.08 ± 0.14dA	37.04	1, 53	< 0.0001
C. cajan	13.9 ± 0.31ab	25.2 ± 1.50bc	20.23 ± 0.17b	19.41 ± 0.17bB	20.68 ± 0.21bA	15.90	1, 46	0.0002
F. benjamina	15.3 ± 0.40a	22.2 ± 3.17c	23.03 ± 0.24a	21.50 ± 0.38aB	23.73 ± 0.17aA	38.59	1, 36	< 0.0001
F	11.79	6.50	253.63	67.77	329.14			
df	4, 45	4, 45	4, 250	4, 79	4, 166			
P	< 0.0001	0.0003	< 0.0001	< 0.0001	< 0.0001			

Means in the same row followed with different letters are significantly different (Student-Newman-Keuls test, $P < 0.05$)

Means followed by the same upper case on the same column are not significantly different

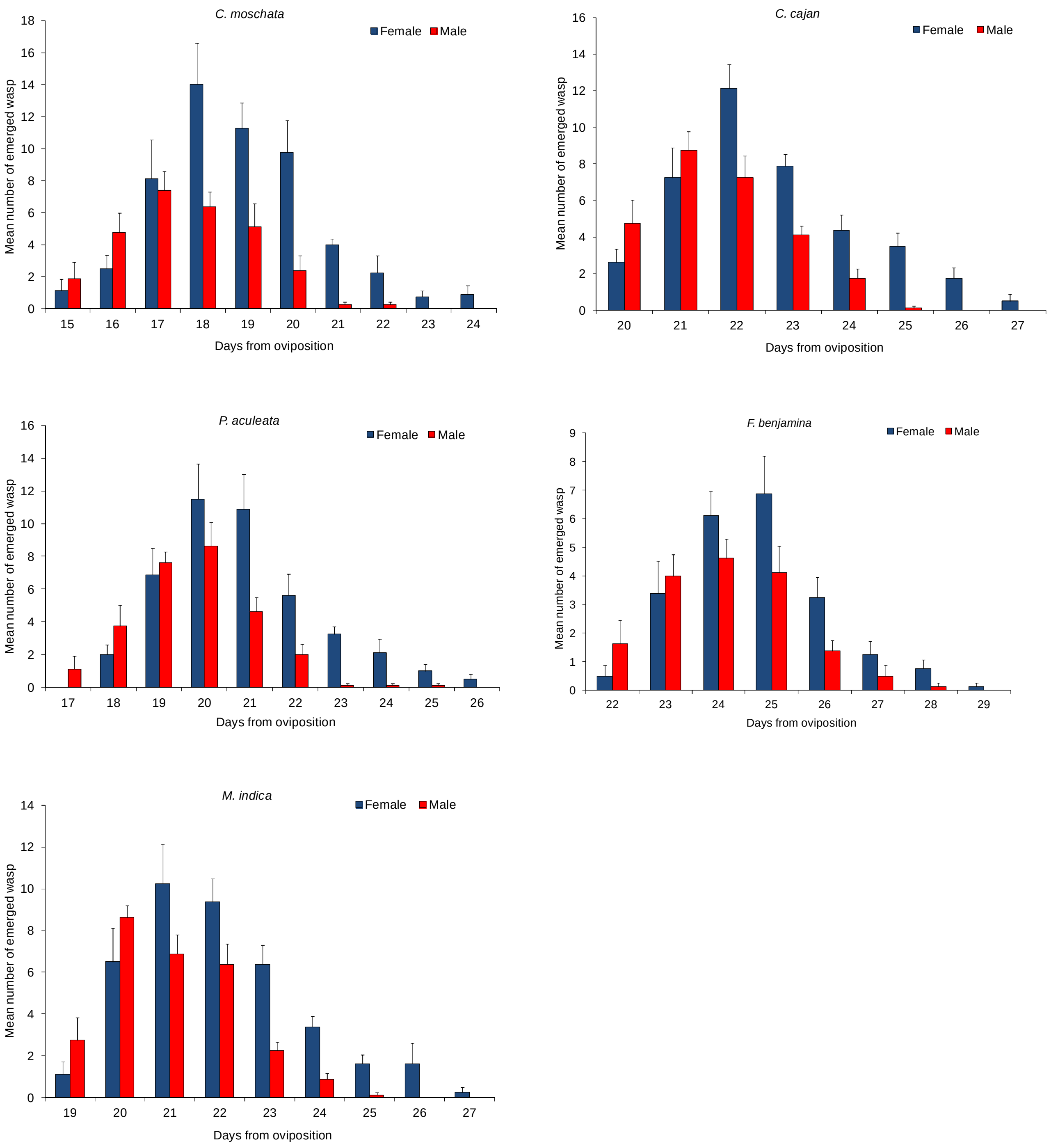


Figure 5. 1: Number of *A. pseudococci* males and females emerging on different days after 24 h exposure period to *R. iceryoides* cultured on five different host plants.

5.3.3 Parasitoid adult longevity

5.3.3.1 Longevity of ovipositing female (host provided), and lifetime fecundity

The survival of female wasps reared on and offered hosts that were maintained on any of the host plant followed a type I survivorship curve (Figure 5.2). However, the overall mean life span of *A. pseudococci* females were significantly longest when the parasitoids were reared on and offered hosts maintained on weeping fig (16.8 ± 0.66 day) and pigeon pea (15.2 ± 0.36 day) and it shortest when the parasitoids were reared and offered hosts on butternut (11.4 ± 0.72 days). However, female parasitoid reared and offered host on mango and Jerusalem thorn had a similar life expectancy.

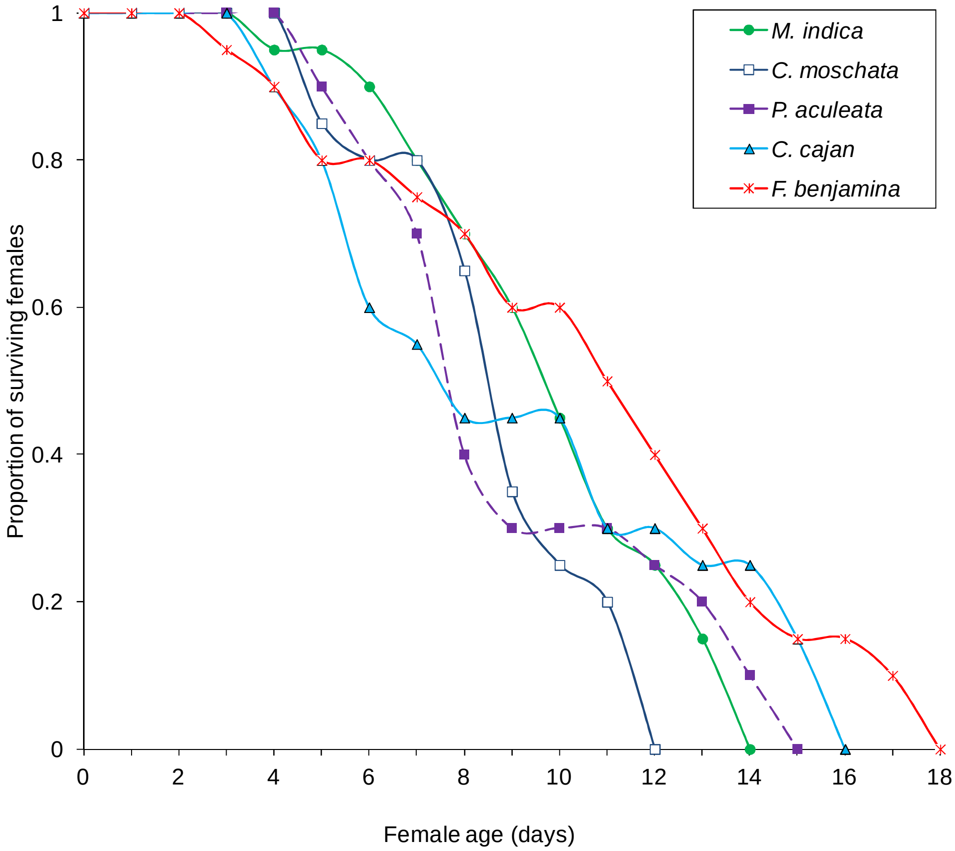


Figure 5. 2: Survival of ovipositing females of *A. pseudococci* reared on different mealybug-infested host plants at 22.3 ± 1.07 ˚C, 40 - 80% RH, with 12L: 12D photoperiod.

5.3.4 Longevity of non-ovipositing female (host deprived) under different feeding regime

Host plant of the rearing hosts, feeding regime as well as their interaction had a strong influence on the life expectancy of the parasitoid wasps (Table 5.3). Comparing the same sex for the same feeding regime across different host plants, both sexes live significantly longest when the hosts were maintained on butternut (47.2 ± 1.7 days) and Jerusalem thorn (41.8 ± 2.0 days), and shortest on weeping fig (32.0 ± 3.0 days) (Table 5.3). While longevity of the males were comparable across all the host plants, except for weeping fig on which it was shortest (17.6 ± 1.3). Comparing males and females reared on the same host plants; females lived significantly longer than males when reared on hosts maintained on butternuts (47.2 ± 1.7 and 31.7 ± 2.5, for female and male, respectively), Jerusalem thorn (41.8 ± 2.0 and 31.1 ± 2.3, for female and male, respectively) and weeping fig (32.0 ± 3.0 and 17.6 ± 1.3, for female and male, respectively). Whereas, longevity were comparable for both sexes, when the wasps were reared on mango and pigeon pea (Table 5.3).

There was a possible influence of body size (using left hind tibia as an indictors) on adult longevity when examined separately for each host plant and feeding treatment combinations (Table 5.4). Significant regressions between left hind tibia length and longevity were detected in several host plant/feeding treatment combinations, especially for water or pure honey nourished females (Table 5.4). Water and 50% honey solution nourished males generally showed insignificant relationships between left tibia length and adult longevity. Hind tibia length accounted for > 60% of the variation in most host plant when adult male and female parasitoids were fed on pure honey except for weeping fig (Table 5.4).

Table 5. 3: Mean (± SEM) of longevity (in days) of female and male *A. pseudococci* adults reared from five different host plants subjected to various feeding treatments

Host plant	Feeding treatment								Statistics					
	Starved		Water		Pure honey		50% honey solution		Female			Male		
	F	M	F	M	F	M	F	M	*F*	*df*	*P*	*F*	*df*	*P*
M. indica	1.8 ± 0.25aB	1.7 ± 0.21aC	4.2 ± 0.33aB	3.2 ± 0.33aC	38.3 ± 2.44bcA	34.4 ± 2.43aA	34.4 ± 2.52aA	22.2 ± 2.29aB	120.32	3, 36	< 0.0001	87.53	3, 36	< 0.0001
C. moschata	2.1 ± 0.21aC	1.4 ± 0.16aC	4.6 ± 0.4aC	2.3 ± 0.21aC	47.2 ± 1.74aA	31.7 ± 2.51aA	30.6 ± 1.74abB	24.1 ± 1.82aB	300.54	3, 36	< 0.0001	97.25	3, 36	< 0.0001
P. aculeata	1.6 ± 0.22aC	1.3 ± 0.15aC	4.3 ± 0.26aC	2.8 ± 0.36aC	41.8 ± 2.03abA	31.1 ± 2.33aA	32.7 ± 2.18aB	22.6 ± 2.46aB	182.36	3, 36	< 0.0001	74.62	3, 36	< 0.0001
C. cajan	1.5 ± 0.17aB	1.2 ± 0.13aC	3.6 ± 0.22aB	2.6 ± 0.27aC	35.6 ± 2.84bcA	30.2 ± 2.64aA	30.1 ± 3.01abA	24.4 ± 2.37aB	72.35	3, 36	< 0.0001	69.66	3, 36	< 0.0001
F. benjamina	1.8 ± 0.2aC	1.4 ± 0.16aB	4.2 ± 0.29aC	2.8 ± 0.33aB	32.2 ± 3.04cA	17.6 ± 1.33bA	23.4 ± 2.19bB	16.7 ± 1.33aA	61.14	3, 36	< 0.0001	82.74	3, 36	< 0.0001
F	0.85	1.25	1.41	1.18	5.60	8.15	3.14	2.19						
df	4, 45	4, 45	4, 45	4, 45	4, 45	4, 45	4, 45	4, 45						
P	0.5007	0.3036	0.2446	0.3338	0.0010	< 0.0001	0.0232	0.0850						

Mean longevity of females or males followed by the same upper case letter are not significantly different among feeding treatments within a specific host plant species.

Mean longevity of females or males followed by the same lower case letter are not significantly different among host plants within a specific feeding treatment (Student-Newman-Keuls test, $P < 0.05$).

Table 5. 4: Regression coefficients (R^2) and *P*-values for the linear regression analyses between wasp size (tibia length) and longevity of female and male *A. pseudococci* adults subjected to various host plant/feeding treatment combinations

Sex	Feeding treatment	Host plant									
		M. indica		*C. moschata*		*P. aculeata*		*C. cajan*		*F. benjamina*	
		R^2	*P*	R^2	*P*	R^2	*P*	R^2	*P*	R^2	*P*
Female	Starved	0.3208	0.0878	0.5106	0.0202*	0.4284	0.0400*	0.7565	0.0011*	0.2328	0.1578
	Water	0.5621	0.0125*	0.8011	0.0005*	0.6099	0.0077*	0.2455	0.1453	0.5667	0.0120*
	Pure honey	0.8065	< 0.0004*	0.7146	0.0021*	0.6378	0.0056*	0.9301	< 0.0001*	0.0021	0.8999
	50% honey solution	0.1056	0.3595	0.9777	< 0.0001*	0.7720	0.0008*	0.0351	0.6045	0.0214	0.6870
Male	Starved	0.4238	0.0415*	0.4833	0.0256*	0.0660	0.4738	0.4252	0.0410*	0.0904	0.3986
	Water	0.0865	0.4094	0.0178	0.7134	0.3746	0.0600	0.0229	0.6763	0.1370	0.2925
	Pure honey	0.9516	< 0.0001*	0.7812	0.0007*	0.8508	0.0001*	0.8012	0.0005*	0.2155	0.1765
	50% honey solution	0.0619	0.4883	0.9098	< 0.0001*	0.0985	0.3771	0.0444	0.5588	0.0238	0.6706

Asterisks show statistically significant relations between wasp size and longevity

5.3.5 Effect of host plant and female age on egg load

Host plant of the rearing host and female's age as well their interaction ($F = 6.86$; $df = 8, 322$; $P < 0.0001$) had a significant effect on parasitoid egg load (Figure 5.3). Females reared on hosts maintained on butternut were significantly more fecund than those reared on mealybugs maintained on any other host plant for all female age groups evaluated (7.08 ± 0.52, 28.63 ± 0.96, 16.79 ± 1.05, and 2.93 ± 0.49, for 1-day old, 3-day old, 9-day old and at death respectively), while those reared on hosts maintained on weeping fig had significantly the lowest egg load for all female age group (3.88 ± 0.4, 16.16 ± 0.92, 11.4 ± 0.94, and 0.21 ± 0.09, for 1-day old, 3-day old, 9-day old and at death respectively) (Figure 5.3). Among the wasps reared on mealybugs maintained on the same host plant, three day-old females had the highest egg load for all rearing host plant (28.63 ± 0.96, 22.84 ± 1.06, 26.04 ± 0.73, 24.36 ± 1.02, and 16.16 ± 0.92 for butternut, mango, Jerusalem thorn, Pigeon pea and weeping fig respectively) (Figure 5.3). When egg load was regressed against female body size, it was found increase linearly with adult female body size for all host plant except on pigeon pea (Figure 5.4).

5.3.6 Demographic growth parameters

Rastrococcus iceryoides host plants had a strong influence on various growth parameters of *A. pseudococci* (Table 5.5). Host plants significantly affected the intrinsic rate of increase (r_m), population doubling time (T_d), and infinite rate of increase (λ). Net reproductive rate (R_o) was 1.7, 1.4, 2.2 and 4.0 times higher for females reared and allowed to oviposit on butternut than those on mango, Jerusalem thorn, pigeon pea and weeping fig plants, respectively. While intrinsic rate of increase (r_m) was 1.3, 1.4, 1.7 and 2.6 times higher for females reared and allowed to oviposit on butternut than those on mango, Jerusalem thorn, pigeon pea and weeping fig plants, respectively. Population doubling time (T_d) on butternut was 61.23% shorter than that on weeping fig, and the mean generation time (GT) was 2 - 9 days less when using butternut compared to the other host plants. For example, the net reproduction (R_o) decreased from 21.753 ± 0.137 female/female/generation on butternut to 5.476 ± 0.066 female/female/generation on weeping fig. While the mean generation time (GT) increased from 20.95 ± 0.331 days on butternut to 29.83 ± 0.279 days on weeping fig. Also the doubling time increased from 4.715 ±

0.106 days on butternut to 12.16 ± 0.162 days on weeping fig. Both the finite rate of increase and the intrinsic rate of increase (r_m) reached their maximum on butternut with 0.147 ± 0.033 female/female/day and 1.158 ± 0.052 female/female/day, respectively.

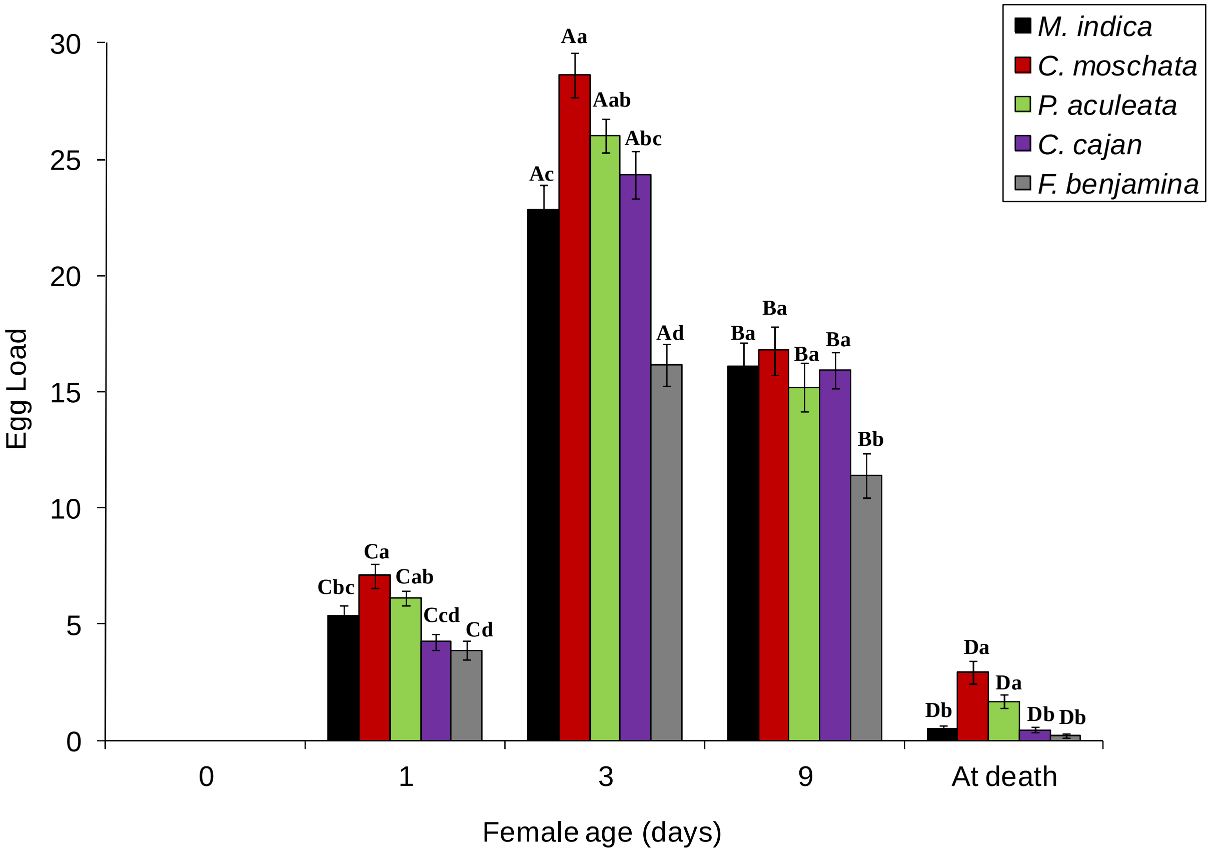


Figure 5. 3: Egg maturation and resorption by *A. pseudococci* females provided honey in the absence of hosts. Bars show mean egg loads ± SEM. Sample sizes for days 0, 1, 3, 9 and at death (> 30 day) are for *C. moschata*: 35, 25, 30, 19 and 30; *M. indica*: 23, 25, 25, 15 and 24; *P. aculeata*: 18, 25, 28, 15 and 28; *C. cajanus*: 21, 25, 25, 15 and 27; *F. benjamina*: 17, 25, 25, 15 and 29.

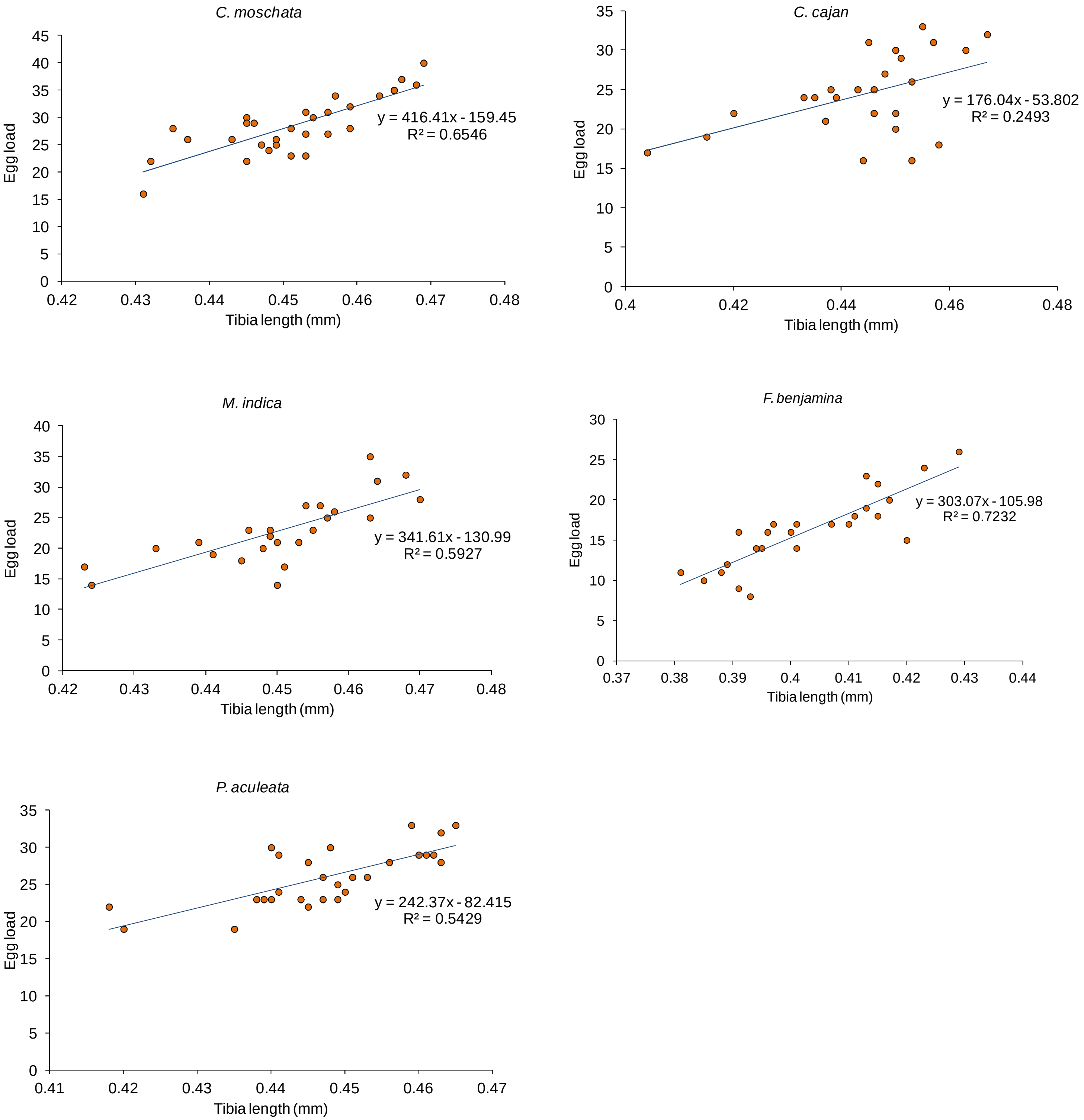


Figure 5. 4: Influence of the size of *A. pseudococci* females (tibia length) reared from *R. iceryoides* maintained on different host plants on their egg loads. GLM based on Poisson distribution for 3 day-old females.

Table 5. 5: Life table parameters of the parasitoid *Anagyrus pseudococci* ovipositing on 3rd instar *R. iceryoides* reared on five host plant species

Host plant	r_m	R_o	GT	T_d	λ
M. indica	0.111 ± 0.016b	13.018 ± 0.181c	23.12 ± 0.206d	6.245± 0.094d	1.117 ± 0.022b
C. moschata	0.147 ± 0.033a	21.753 ± 0.137a	20.95 ± 0.331e	4.715 ± 0.106e	1.158 ± 0.052a
P. aculeata	0.108 ± 0.015c	15.453 ± 0.206b	25.35 ± 0.264c	6.418 ± 0.101c	1.114 ± 0.020b
C. cajan	0.086 ± 0.006d	09.851 ± 0.064d	26.60 ± 0.202b	8.060 ± 0.304b	1.090 ± 0.006c
F. benjamina	0.057 ± 0.011e	5.476 ± 0.066e	29.83 ± 0.279a	12.16 ± 3.762a	1.059 ± 0.011d

Mean ± SE within column followed by the same letter do not differ significantly by Student-Newman-Keuls test ($P < 0.05$). r_m = Jackknife estimate of the intrinsic rate of increase (female eggs/female/day), R_o = net reproductive rate (female offspring/female/generation), GT = mean generation time (days), T_d = doubling time (days) and λ = infinite rate of increase for population (female offspring/female/day).

5.4 Discussion

The effect of host plant on the preference and performance of the parasitoid has been well document for many host/parasitoid systems (Souissi and Le Rü, 1997a, b; Souissi et al., 1998a, b; Souissi, 1999; Prince et al., 1980). Among the widely reported aspect of the host plant-parasitoid interaction is the effect of host plant on various steps of parasitization process (habitat location, host location, host acceptability and host suitability) (Vinson, 1976; Nordlund et al., 1988; Turlings et al., 1991; Vinson and Williams, 1991; Vet and Dicke, 1992, Mohamed et al., 2003; 2006). In this study, although *A. pseudococci* females accepted mealybug that were maintained on all host plants tested, it showed marked preferences for hosts reared on butternut, despite the fact that the parasitoid were reared on their respective test host plant for several generations prior to start of any experiment in order to eliminate or minimize experiences as the result of associative leaning. Similar results of differential host acceptability were reported for the congenic parasitoids *A. kamali* in association with other melaybug species when they were cultured on different plant species reported (Souissi and Le Rü, 1997; Souissi et al., 1998). Also the acceptability (% parasitism) of the encyrtid parasitoid *Apoanagyrus lopezi* De Santis (Hymenoptera: Encyrtidae) for its host [cassava mealybug, *Phenacoccus manihoti* Matile Ferrero (Homoptera: Pseudococcidae)] varied significantly when cultured on different host plant species (Souissi et al., 1998). Differential host acceptability of the same mealy species (*R. iceryoides*) was also reported for other related parasitoid species.

The low acceptance level of hosts reared on weeping fig in our study may have been caused by the architecture of the weeping fig stem which is covered with a thick layer of fibrillary waxes, and possess lots of shortly papillose-hairs that could hamper the foraging ability of the parasitoid, resulting in less host encounters, or by the low quantity and quality of the odor (karimones) emitted from weeping fig as result of low feeding activity of the mealybug on this host plant or by the inferior quality of the mealybug in term of its smaller size as well as its poor nutritional quality, or by combination of the three (plant architecture, low plant odor and inferior host quality). These factors either separately or in combination were reported to have a great influence of host acceptability by several parasitoid species (Hulspas-Jordaan and van Lenteren, 1978).

Chemical and physical characteristics of the leaf surface (Hulspas-Jordaan and van Lenteren, 1978), size and trophic characteristics of the host, abundance and composition of the honeydew (Budenberg, 1990), and host cuticular secretions (Takahashi et al., 1990), all of which are likely to vary considerably with the plant species, variety and physiological state (Takabayashi et al., 1991).

In many parasitoid-host associations, parasitoids chose hosts that are more profitable to their progeny (e.g. Charnov and Skinner, 1985; Waage, 1986; van Alphen and Vet, 1986; Duan and Messing, 2000; Sagarra et al., 2001; Mohamed et al., 2003). Our results concur with these findings as the most acceptable host, those reared on butternut, for ovipoistion by *A. pseudococci* female was also found to be the most suitable for its immature development. Parasitoid had of shortest days to mummification, coupled with the highest, percent of mummified nymphs, percent of eclosed parasitoid wasps, and sex ratio (proportion of female progeny) as well as shortest developmental time when reared on host maintained on butternut. On the other hand mealybugs which were less accepted (those reared weeping fig) were also least suitable in term of all evaluated parameters mentioned above. Similar result was reported by Souissi and Le Rü (1997) for the *Apo. lopezi* when reared on cassava mealybug, *P. manihoti* maintained on four different host plants. The authors reported that parasitoid number of mummies/female parasitoid, number of emerged parasitoid, wasp survival and development time varied considerably among mealybug host plants. In a separate study using the same tritrophic system as the above, Souissi et al. (1998) found that percent mummified nymphs as well as percent emerged parasitoids were significantly affect by *P. manihoti* host plants

In this study percent mummified nymph and percent adult parasitoid eclosion on weeping fig were not only least when compared to other host plants tested, but also the percent adult eclosion was only slightly above one third (38.6%) of the number of the mummified nymphs formed on this plant, suggesting that the majority of the parasitoid offspring were unable to complete their development in hosts maintained on weeping fig. This could be due to the immune reaction mounted by the host against the parasitoid immature stage. Although upon dissection no egg encapsulation was detected, however, other forms of cellular or hormonal defense may have been deployed by mealybugs reared on this plant.

The highest *A. pseudococci* sex ratio yielded from host reared on butternut indicates that the female of this parasitoid was able to assess the quality of the host and deposit more fertilized egg in the in the more suitable host (based on the size or nutritional quality of the host, or both). Alternative explanation could be that higher mortality of the female offspring in the inferior quality host, however, no dissection of uneclosed mummies was made to ascertain this.

Shorter developmental time is adaptive trait. In the field it shortens the duration of exposure of mummified mealybug containing parasitoid to predation, and hyperparasitism as the mummies are more vulnerable to adverse effect of these biotic factors. Shorter developmental time is also desirable trait for laboratory mass rearing of parasitoid intended for augmentative release. It had been documented that the duration of the parasitoid developmental time may vary with the variation of the host's nutritional history (Kouame and Mackauer, 1991; Souissi and Le Rü, 1997a). Our results agree with these previous finding as *A. pseudoccoci* showed significant variation in terms of developmental time when reared on mealybugs maintained on the five tested host plants. Analogous results of the effect of the host plant on the rearing host and on the duration of the parasitoid developmental time were also reported for other encyrtid species. For example, Souissi and Le Rü (1997a) reported that the duration of the development of *Apo. lopezi* varied greatly when reared on cassava mealybug *P. manihoti* maintained on four different host plants (Incoza, Zanaga, Faux-caoutchouc and Talinum). Also the developmental times of female *A. kamali* reared on mealybugs *M. hirsutus* (Homoptera: Pseudococcidae) maintained on Japanese pumpkin (*C. maxima* L. (Cucurbitaceae)), sprouted potato (*S. tuberosum* L. (Solanaceae)), and acorn squash (*C. pepo* L. (Cucurbitaceae)) was shorter by about 4 to 8 days than those reared on *M. hirsutus* maintained on chayote (*Sechium edule* (Jacq) Swartz (Cucurbitaceae)) and prickly pear (*Opuntia phaeacantha* Engelm. (Cactaceae)), and it was about 5 day shorter for males maintained on the former group of plants than those maintained on the latter (Serrano and Lapointe, 2002). However, the duration of the developmental time of the same species reared on the same host was not affected by host plant when reared on *H. rosa-sinensis* (Malvaceae), *H. sabdariffa* (Malvaceae), *S. tuberosum* (Solanaceae), and *C. pepo* (Persad and Khan, 2007). Difference in the duration of development of *A. pseudoccoci* reported in our study could have been caused by variation in the quality of mealybugs reared on the five host plant species tested. Also host size had a significant effect on parasitoid developmental time. A positive correlation between host size and parasitoid developmental time was reported in several host-parasitoid associations (e.g Ruberson et al., 1989; Vinson and Iwantsch, 1980), especially for idiobionts. However, for koinobionts there is no general pattern between parasitoid developmental time and host size (Godfray, 1994). In this study the developmental time of *A. pseudoccoci* was shortest on the large-sized hosts (those reared on butternut mealybug (Tanga et al., in press), and longest on small-sized hosts (those reared on weeping fig (Tanga et al., in press).

A positive correlation between host size and parasitoid adult size was documented as early as 1940 (Salt, 1940). Adult wasp size is a good indicator of parasitoid fitness (Jervis and Copland, 1996), as it determines the capacity of the parasitoid as a biological control agent (Godfray, 1994; Van Lenteren et al., 2002). For example, wasp size (based on the left hind tibial length) of the closely related species *Anagyrus kamali* (Hymenoptera: Encyrtidae), was found to be positively correlated with longevity, mating preference, fecundity, reproductive longevity, progeny emergence and sex-ratio (Sagarra, 2001). Parasitoid size is largely determined by that of its host (Barratt and Johonstone, 2001; López et al., 2009; Opp and Luck, 1986; Salt, 1940), as it dictates the amount of nutrients available for the developing larvae of the parasitoid. Host size in turn, is dependent on the food quality of its host plant (Barbosa et al., 1982). In this study, wasps of *A. pseudococci* were larger in size for both male and female when they were maintained on butternut mealybug.

Parasitoid longevity is one of the measures of a parasitoid's fitness (Waage and Ng, 1984). The reproductive success of the parasitoid is governed in part by the time the parasitoid can survive, especially for synovigenic parasitoids (Baggen and Gurr, 1998). The longer a female lives, the more hosts it parasitizes and the longer a male lives, the more females it can fertilize (Jervis and Copland, 1996). One of the most important factors that determine parasitoid longevity is the wasp size which is a function of host size. Other factors include the wasp's access to the host for oviposition. In the present study, host deprived *A. psuedoccoci* females, had the longest life span when reared on butternut and Jerusalem thorn (large size host) and it was shortest when the parasitoid was reared on mealybug on weeping fig (small size host). The effect on host plant on parasitoid longevity through effect of host quality was also reported for other related parasitoids. Souissi and Le Rü (1997a) reported that longevity of *Apo. lopezi* varied significantly with mealybug host plants. Similarly longevity of *A. kamali* was strongly influenced by the host plant species of it host *M. hirsutus* (Persad and Khan, 2007). However, when *A. psuedoccoci* females were offered hosts for oviposition, longevity was shortest on butternut while it was longest on weeping fig. This was mainly because females offered mealybugs maintained on butternut were actively ovipositing and this led to an average life time fecundity of 35.1 ± 0.6 eggs/female, while those offered hosts maintained on weeping fig mealybug had the least life time fecundity (16.2 ±1.6 eggs/female). This was because the females perceived this host to be of lower quality, resulting in egg resorption; the energy and materials of which wer used to increase female longevity. Increased longevity due to egg resorption was reported for other parasitoid species (Ramadan et al., 1995)

High reproductive potential is among the criteria used for selection of natural enemies (Overholt et al., 1997; van Lenteren, 1986). In our study the parasitoid achieved a greater intrinsic rate of natural increase (r_m), net reproductive rate (R_o), finite rate of increase (λ) on butternut. Also mean generation time (G) and population doubling time (T_d) were shortest on mealybugs maintained on the same host plant, while those reared on hosts maintained on weeping fig were of inferior quality with regards to all growth parameter evaluated. Similar findings of the effect of host plants on parasitoid's demographic parameters were also reported for *Apo. lopezi* (Souissi and Le Rü, 1997a).

This study showed that *A. psuedococci* reared on hosts maintained on butternut, mango, and Jerusalem thorn proved to be more suitable in mass rearing of this parasitoid as measured by percent parasitized nymph, percent mummified host, percent adult eclosion and sex ratio. The same host plants were found to be very suitable for the development of the host *R. iceryoides* (Tanga, et al., in press). However, mango, and Jerusalem thorn are leafy plants that require exposure to direct sunlight for their maintenance; therefore screen houses will be needed if the pest and the parasitoid are to be reared on these plants. On the other hand, butternut can be maintained in the laboratory and does not require large space for *R. iceryoides* as well as the parasitoid colony maintenance. Therefore, butternut is an ideal host for the mass production of the parasitoid and its subsequent releases for management of this pest.

CHAPTER SIX

Interaction between the arboreal weaver ant, *Oecophylla longinoda* (Hymenoptera: Formicidae), *Anagyrus pseudococci* Girault (Hymenoptera: Encyrtidae) and *Rastrococcus iceryoides* Green (Hemiptera: Pseudococcidae) under laboratory conditions

ABSTRACT

Anagyrus pseudococci Girault (Hymenoptera: Encyrtidae) is a well known indigenous parasitoid of the mango mealybug, *Rastrococcus iceryoides* Green (Homoptera: Pseudococcidae), in Kenya and Tanzania. The African weaver ant *Oecophylla longinoda* Latreille (Hymenoptera: Formicidae) forms a trophobiotic relationship with *R. iceryoides* and promotes the latter's infestations to unacceptable levels. The impact of ants attending the mealybug on the biological control activities of *A. pseudococci* was assessed in the laboratory. The percentage parasitism of *R. iceryoides* by *A. pseudococci* was significantly higher on ant-excluded trials (86.6 ± 1.31%) than on ant-attended trials (61.4 ± 4.67%). Although, mealybugs exhibited more vigorous defensive behaviours by walking away and flipping their abdomen, such behavioural defences were not effective against the persistent parasitoids. When *O. longinoda* workers were allowed access to butternuts containing mummified mealybugs parasitized by *A. pseudococci*, they were observed to remove mummies, which resulted in significantly reduced percentage of adult parasitoid eclosion. *Oecophylla longinoda* was also observed to show aggressive behaviour toward *A. pseudococci* and caused a significant adult parasitoid mortality (24.32 ± 3.32%). Disturbance by *O. longinoda* greatly affected the foraging activities and significantly reduced the oviposition success of *A. pseudococci*. Our findings, strongly suggest that *O. longinoda* has a negative effect on parasitoid efficacy, which could be a delimiting factor in field conditions in application of biological control agents. However, the interactions documented here require future investigations under field cage and open-field conditions, prior to release of parasitoids to suppress populations of *R. iceryoides*.

Keywords: *Rastrococcus iceryoides*; *Oecophylla longinoda*; *Anagyrus pseudococci*; Percent parasitism; mummy predation; Ant-parasitoid interactions; Mortality

6.1 Introduction

The mango mealybug, *Rastrococcus iceryoides* Green (Hemiptera: Pseudococcidae), is a major insect pest of mango but also known to attack 29 host plants from 16 families in Kenya and Tanzania (C.M.Tanga, unpublished data) and more than 80 known host plants from 35 families in Southeast Asia (Williams, 1989; Ben-Dov, 1994). Since its accidental introduction into Africa in the early 1990s (Williams, 1989; Luhanga and Gwinner, 1993), this insect has continued to cause serious damage to mango. Management of *R. iceryoides* has relied largely on repeated applications of insecticides but the use of chemical insecticides are not always effective for the management of several species of mealybug due to the heavy layers of waxy coating that shield their body (Kairo et al., 2000). The egg stage of *R. iceryoides* in particular and several other mealybug species are protected by thick waxy ovisac (M.C. Tanga, unpublished data; Meyerdirk et al., 1998), which most insecticide cannot penetrate (McKenzie, 1967). This combined with the extremely wide host range of several species of mealybugs makes it almost impossible to have a spraying program capable of bearing the cost and coping with the practicalities of treating the whole range of infested plants in an affected area (Sagarra and Peterkin, 1999).

Biological control is usually recommended for the management of mealybugs (Herren et al., 1987; Agricola et al., 1989; Neuenschwander, 1996). In a recent survey, we observed that *Anagyrus pseudococci* Girault (Hymenoptera: Encyrtidae) is a widely distributed indigenous primary parasitoid of *R. iceryoides* in Kenya and Tanzania (M.C. Tanga, unpublished data). The potential use of *A. pseudococci* in augmentative biological control of *R. iceryoides* is currently under evaluation at the International Centre of Insect Physiology and Ecology (*icipe*), Nairobi, Kenya. However, in addition to *A. pseudococci*, several species of predatory ant have been found associated with *R. iceryoides* with *Oecophylla longinoda* predominating (C.M. Tanga, unpublished data).

In agricultural and natural ecosystems, ants' interaction with the assiduously attended Hemipteran insects such as mealybugs reveals benefits such as access to a constant defensible and renewable carbohydrate energy-rich food source (Carroll and Janzen, 1973). In return, the ants render protection against parasitoids, predators and even their competitors (Steyn, 1954; Barlett, 1961; Bradley, 1973; Adenuga, 1975; Pierce and Mead, 1981; Hölldobler and Wilson, 1990; Jiggins et al., 1993), as well as sanitation and sometimes transport services to sedentary Hemiptera (Buckley, 1987; Lach, 2003). By affording protection to the mealybugs from natural enemies, the presence of certain species of ant can be detrimental to the impacts of biological control (Bartlett, 1961; Buckley, 1987; Itioke and Inoue, 1996; Martinez-Ferrer et al., 2003). For example, Whitehead (1957) and Myburgh et al. (1986) reported that ant foraging on plant canopies reduces natural enemy activity and promotes mealybug infestation and therefore, biological control of the insect pest is compromised. In California, Daane et al. (2007) found that *L. humile* promoted populations of *Pseudococcus viburni* (Signoret) while lowering populations of its parasitoids *Pseudaphycus maritimus* (Erhorn) accompanied by a serious reduction in its parasitoid populations. Buckley and Gullan (1991) reported a very low parasitism rates (< 10%) of coccids in the presence of *Oecophylla* and *Solenopsis* species and > 15% in the presence of the more aggressive *Tapinoma* and *Iridomyrmex* species. Itioka and Inoue (1996) in a comparative field investigation found a 94% decrease of the mealybug *Pseudococcus citriculus* Green (Hemiptera: Pseudococcidae) populations in a Satsuma orange orchard by natural enemies in the absence of the attendant ant, *Lasius niger* Linnaeus (Hymenoptera: Formicidae).

In most cases, the magnitude of ant protection differs depending on the parasitoid and ant species involved (Del-Claro and Oliveira, 2000). In fact, some parasitoids have developed escape strategies from ant species however others are so sensitive to the presence of ant that after an initial encounter, they are deterred not only by the ants but by any moving object including other parasitoids or the host itself, thereby greatly reducing their potential as biological control agents (Martinez-Ferrer et al., 2003). Therefore, it is important to understand the trophobiotic relationship between ants and their adopted Hemiptera (Buckley, 1987; Hölldobler and Wilson, 1990; Jiggins et al., 1993; Gibernau and Dejean, 2001); to guide implementation of management methods.

The importance of *O. longinoda* in suppressing fruit flies on mango in West Africa has been reported (Van Mele et al., 2007) but the role of this ant in the biological control of hemipteran pest in mango agro-ecosystem has not been quantified. Therefore, the purpose of this study was to examine, under laboratory conditions, if *O. longinoda* attending *R. iceryoides* influences the foraging behaviour, oviposition success and subsequent parasitism of *A. pseudococci*.

6.2 Materials and Methods

6.2.1 Insect colonies

6.2.1.1 Mealybug colonies

The colony was initiated from a cohort of 300 adult mealybugs collected from mango orchards in coastal Kenya and brought to the laboratory at the International Centre of Insect Physiology and Ecology (*icipe*), Nairobi, Kenya in February 2008. In the laboratory, the insect were reared on mature fruit of butternut squash purchased from a local grocery store, for about 20 generations before the start of the experiment. Before the onset of the experiments, butternuts surface sterile with 5% sodium hypochloride followed by three rinses in sterile distilled water to prevent fungal growth and later air dried for 24 h. For colony maintenance and to have insects of similar developmental stage, adults mealybugs were allowed to oviposit on 10-20 butternuts daily, after which the adults are removed and newly emerged nymphs of similar age are followed on the infested group of butternut fruits. Colonies are rejuvenated after every 6 month from fresh wild mealybug isolates from the field to ensure a broader genetic diversity in the laboratory population. Mealybugs were used at their third instar nymphs, which is the preferred developmental stage for *Anagyrus pseudococci* (Islam and Copland, 1997). The colonies were then maintained in the laboratory at 27 ± 1˚C, 12:12 (L: D) photoperiod and 50 - 80% RH.

6.2.1.2 *Oecophylla longinoda* Latr. colonies

Several ant nests consisting of workers (> 500 workers), queens and immature were collected from the *icipe* field station at Muhaka (04°19'24.8"S, 039°31'35.3"E, 30 m a. s. l) in the Coastal Province of Kenya. The nests were transported in transparent plastic containers (35 cm height by 29 cm top diameter by 20 cm bottom diameter), with an open lid (15 cm diameter screened with fine organdy-mesh) for ventilation. The original ant nests were reared onto potted *Ficus benjamina* seedlings in a screen house (2.8 m length by 1.8 m width by 2.2 m height). The plants were fertilized using N-P-K (nitrogen, potassium and phosphorus) fertilizers, to ensure suitable foliage development for nest-building and watered twice in a week. Only sister ant colonies were put together since different colonies are mutually antagonistic. The potted plants bearing the ant colonies were maintained onto a table (245 cm length x 78 cm width x 75 cm height) in the a screen house. Tangle foot glue (Tangle-trap; The Tanglefoot Company, Grand

Rapids, MI) was smeared around the feet and edges of the table to prevent attacked of *O. longinoda* by other predatory ant species. Fresh honey syrup in Petri dishes and living insect food sources (Dipterous larvae and adults, termites, lepidopteran adults, grasshoppers, worms etc) and high protein food sources (fish intestines) was provided regularly to augment the weaver ant population. The rearing conditions at the screen house were 22.3 ± 5.07 ˚C, 40 - 80% relative humidity (RH) and 12L: 12D photoperiod.

6.2.1.3 Parasitoid colonies

Mummified mealybugs collected from heavily infested mango orchard at Matuga (04° 11' 02.5" S, 039° 33' 38.4" E, 109 metres above sea level), Coast Province, Kenya were transported to the laboratory at *icipe*. They were monitored daily, and emerging *A. pseudococci* were collected for colony establishment using a stereomicroscope. *Anagyrus pseudococci* colony was initiated using 50 males and 100 females. The parasitoids were allowed to mate for 24-48 h after which the 100 mated female parasitoids were collected and transferred to Perspex cages (20 cm length by 20 cm width by 20 cm height) containing butternuts infested with third instar nymphs of *R. iceryoides*. The Perspex cage had an organza mesh glued to one side to allow for ventilation and the opposite side was fitted with a flipping door for introduction of food and water sources. Parasitoids were fed on droplets of pure honey on the ceiling of the cages and moist cotton wool as water sources. The parasitoids were maintained at 27 ± 1˚C, 50 – 80% RH and a 12:12 (L: D) photoperiod. In all cases newly emerged parasitoids were allowed to feed and mate before use in the trials. In this regard, five male parasitoids were given access to ten newly emerged females for 24 h and only mated three-day old females deprived of hosts prior to the experiment were used.

6.2.2 Effect of *O. longinoda* attendance on percentage parasitism of *R. iceryoides,* parasitoid eclosion and sex ratio

Butternut fruits infested with 100 third instar nymphs of *R. iceryoides* were used in this experiment. A total of 100 adult *O. longinoda* workers were transferred to the fruits and allowed to forage for 3 h on the mealybug-infested butternut. Thereafter, 10 three-day old fertilized female parasitoids were aspirated from the colony and gently introduced into the experimental cages. An ant-free cage with mealybugs and parasitoids only was used as control. After 24 h exposure, the ants and parasitoids were removed. Cages with mealybugs were then allowed to stand for 10 days, after which mummified mealybugs were checked daily and recorded. Percent mummified nymph was computed based on the initial number of exposed host (100 nymphs) while the percent parasitoid emergence was computed based on the number of the mummified nymphs for each treatment. Sex ratio (percent female) was computed as percentage emerging females over the total emerging wasps. The experiment was maintained at 27 ± 1˚C, 50 - 80% RH with a 12:12 (L: D) photoperiod. The experiment was replicated 10 times.

6.2.3 Interaction of *O. longinoda* with mummified mealybugs and the effect on adult parasitoid eclosion

The bottom portion of butternut fruits were cut into circular forms and placed into Petri dishes (8.6 cm diameter). Twenty, 10-day-old mummies containing *A. pseudococci* were placed on the butternut in the Petri dishes. Thereafter, the Petri dishes were randomly placed in the centre of transparent Persplex cages (20 cm length by 20 cm width by 20 cm height) prior to the experiment. The negative control consisted of sterilized sand grains (approximately 2 – 3 mm diameter). On days when experiments were conducted, five groups of ant populations that had been starved for 24 h in the laboratory were prepared (1, 5, 10, 15 and 20 ants). Prior to start of the experiment the different ant groups were introduced into the different cages and allowed to interact for a maximum of 30 min. The Petri dishes containing the mummies and sand grains were then gently introduced into the experimental arena and also allowed an interaction time of 5 min. Thereafter, observations were made at every 10 min interval for a total duration of 2 h to record the number of mummies removed from the Petri dishes by the ants. After each experiment, the ants were removed and the Petri dishes containing the remaining mummies kept separately until emergence of the adult parasitoids. Adult parasitoids that emerged were counted and expressed as a percentage of the initial number of mealybug mummies introduced onto each Petri dish at the start of the experiment. Five replicates of this experiment were conducted for each cohort of ant.

6.2.4 Assessment of *O. longinoda* aggression and escape strategy by *A. pseudococci*

Perspex cages containing butternut fruits were infested with 100 3rd instar nymphs. One hundred adult *O. longinoda* workers were then transferred to the cage for 3 h and thereafter 20 three-day old fertilized female parasitoids were introduced. Five minutes observations were made at 10 min interval for a total duration of 2 h. The following observations were recorded for *O. longinoda*: (1) *non-aggression*, in which the ant workers touched the female wasps with their antennae or their legs or passed the parasitoid within a closed distance without exhibiting any obvious responses; and (2) *aggression*, in which the ant workers attacked and seized the wasp with her mandibles.

Anagyrus pseudococci females responded to the above behaviour by the ant with one of four distinct behaviours: (1) *fly away*, in which the female fled, by flight, from the infested butternut on encounter with the ant; (2) *jump away*, on encounter with the ant without leaving the infested butternut; (3) *change of walking direction*, to avoid physical contact with the approaching ants; and (4) *ignoring* – the wasp continued its activities although at close contact with the ant. These various behavioural parameters of the ant and the parasitoids were recorded at the time intervals indicated above. The experiment was replicated 10 times.

6.2.5 Host handling time and oviposition success of *A. pseudococci* in the presence or absence of *O. longinoda*

Newly emerged wasps were sexed, mated and fed with honey and water as described above. In this experiment, only 72 h old host-deprived female wasps were used because they had a high oviposition pressure. The experiment consisted of ant-attended and ant-excluded treatments. Observation period for each trial began when a single mated female wasp was introduced into the cage containing butternut infested with 50 unparasitized third instar nymphs of *R. iceryoides* with or without ants. One hundred ants were used for each replicate. After the wasp was introduced into the experimental arena, qualitative descriptions of the time spent for each observational parameter during oviposition was recorded.

In this study, host handling time is defined as the sum of a female's examination of the mealybug, probing and oviposition time during the observation period in both ant-attended and ant-excluded treatments. For *A. pseudococci,* when a female wasp encountered a host, they exhibited a stereotypical host examination and ovipositional behaviour that has been well described (Hcidari and Jahan, 2000; Chong and Oetting, 2007). Following each host encounter, host handling was divided into four responses: (a) *host rejection* – occurs if the parasitoid moves away from the host without initiating probing (b) *host acceptance* – this is defined as a behavioural activity where foraging female wasp rotates her body to face away from the host, showing clearly that it had recognized the potential host; (c) *ovipositor probing and penetration*– occurs when the foraging female wasp flexed the tip of her abdomen such that the tip of the ovipositor touches the host body plus a fast and rhythmical insertion of ovipositor into the host.

During ovipositor probing and penetration, the host often exhibited a defensive response, which included raising and shaking its abdomen violently and rapidly in order to throw the parasitoid off its body or pulling its sucking mouth part and moving backward. This behavioural tendency exhibited by the host usually led to host rejection. Therefore, the number of mealybugs defending against oviposition by the parasitoids was also recorded in ant-attended and ant-excluded set up. (d) *oviposition* – characterized by a pumping movement of the abdomen and ending up with a strong and jerky withdrawal of the ovipositor (i.e., host bleed haemolymph exuding from the oviposition puncture).

The behavioural parameters were recorded for a period of 1 h after which the experiments were terminated. In previous preliminary studies, we did not find any difference between parasitized and unparasitized *R. iceryoides* with regard to their behaviour and development until mummification (C.M. Tanga, unpublished). Therefore, after oviposition was completed during each interactive phase, stung mealybugs (i.e. mealybug bleed haemolymph) were circled with a permanent marker pen and allowed to continue feeding on the butternut for 3 days. Thereafter, each mealybug from the different trials (ant-attended and ant-excluded treatments) were dissected in phosphate buffer solution (PBS) under a stereomicroscope and the number of parasitoid eggs present determined to confirm successful and unsuccessful oviposition. Each *A. pseudococci* female was observed only once, with a total of 15 female wasps in ant-attended and 15 females in ant-excluded treatments.

6.2.6 Statistical analysis

Data on the mean number of ovipositor penetration of host, successful and unsuccessful oviposition, searching time and the duration of behavioural sequence during host handling and oviposition in both ant-attended and ant-excluded treatments were subjected to Student's *t*-test. Percentage parasitism, percentage eclosion and sex ratio were subjected to arcsine transformation to correct for heterogeneity of variance before being subjected to *t* test. The frequency of each behavioural response of the parasitoid to escape attack by *O. longinoda* and proportion of mummified mealybug removed by *O. longinoda* at different time interval were arcsine transformed (Zar, 2009), prior to subjecting the data to multivariate analysis of variance for repeated measures using Proc GLM (SAS® 9.1, SAS Institute Inc., Cary, NC) (Ott et al., 2010). The percentage adult parasitoid eclosion was also arcsine transformed before subjecting the data to a one-way analysis of variance (ANOVA). The relationship between host handling time and successful oviposition in both ant-attended and ant-excluded experiments was examined by simple Pearson correlation tests. Values of $P \leq 0.05$ were used to indicate significance.

6.3 Results

6.3.1 The mean (± SE) number of ants and parasitoids on the mealybug-infested butternut squash

The number of *O. longinoda* on the mealybug-infested butternut in absence of the parasitoid ranged from 8 to 32 individuals per 1 min observation period and averaged 19.22 ± 0.41 ant workers. The number of ants on the infested butternut during 1 min period when the parasitoid was released ranged from 0 to 28 (9.96 ± 0.62 ant workers). The number of foraging ants on the mealybug- infested butternuts significantly decreased with time in the presence or absence of the parasitoid (Figure 6.1). The mean number of ants on the mealybug-infested butternut was significantly higher in the absence of *A. pseudococci* than when *A. pseudococci* was released ($t = 12.08$; $df = 23$; $P < 0.0001$). Individual ants were observed to remain attending the mealybug for longer period of time.

During the 1 min observation periods, the number of *A. pseudococci* on the mealybug-infested butternut in the presence of *O. longinoda* increased from 0 to 7 and averaged 2.83 ± 0.13 wasps. While in the ant-free cage, the number of *A. pseudococci* on the infested butternut ranged from 1 to 9 and averaged 5.39 ± 0.16 wasps during 1 min period of observation. The mean number of *A. pseudococci* foraging on the infested butternut differed significantly over the 2 h period in the presence and absence of *O. longinoda* ($t = 12.07$; $df = 23$; $P < 0.0001$). The number of foraging parasitoids on the mealybug-infested butternuts increased with time in the presence or absence of *O. longinoda*. Figure 6.2 illustrates the trends in the number of parasitoids on the mealybug-infested butternuts in the presence or absence of ants.

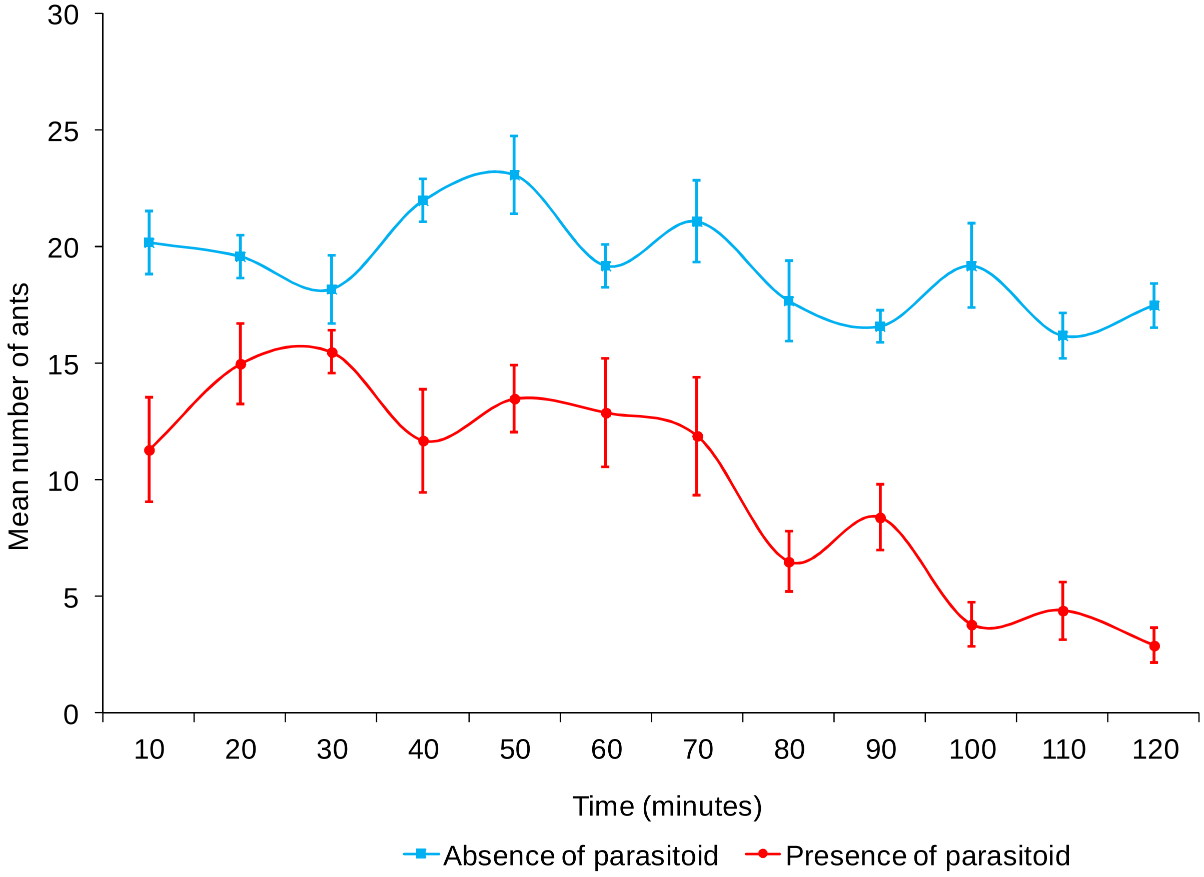


Figure 6. 1: The mean (± SE) number of *O. longinoda* observed on *R. iceryoides*-infested butternut during 1 min intervals over a 2 h observation period in the presence and absence of *A. pseudococci.*

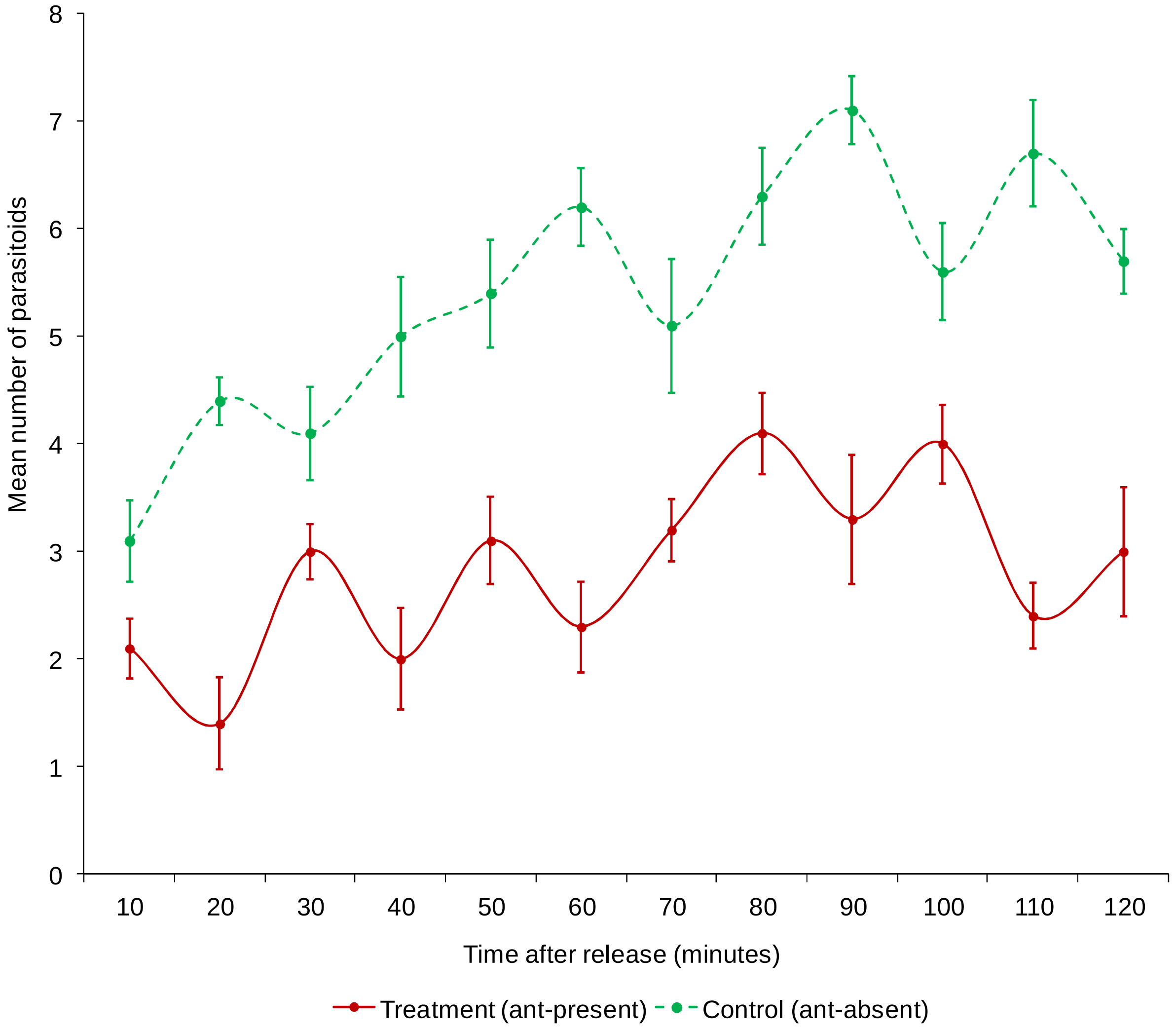


Figure 6. 2: The mean (± SE) number of *A. pseudococci* observed on *R. iceryoides*-infested butternut during 1 min intervals over a 2 h observation period in the presence and absence of *O. longinoda.*

6.3.2 Effect of *O. longinoda* attendance on percentage parasitism of *R. iceryoides,* parasitoid eclosion and sex ratio

The effect of ant disruption on percentage parasitism and parasitoid eclosion is given in Table 6.1. Parasitism of *R. iceryoides* by *A. pseudococci* in the ant-excluded treatment was 86.6 ± 1.3% and was significantly higher than the ant-attended treatment (61.4 ± 4.5%) (*t* = -5.58; *df*

= 18; *P* = 0.0001). There was a significant difference in the number of unparasitized mealybugs in the presence of the ants (36.8 ± 4.4%) than when ants were excluded (11.5 ± 1.3%) (*t* = 6.07; *df* = 18; *P* < 0.0001).

The rate of *A. pseudococci* emergence was significantly higher in ant-excluded treatment (94.5 ± 0.6%) than in *O. longinoda*-attended colonies (85.4 ± 2.3%) (*t* = - 3.34; *df* = 18; *P* = 0.0069). The overall offspring sex ratio in both treatments was female-biased but there was a significant difference in the offspring sex ratio between the ant-excluded treatment (71.7 ± 1.7%) and ant-attended treatment (62.2 ± 3.3%) (*t* = -2.54; *df* = 18; *P* = 0.0204).

Table 6. 1: Mean (± SE) percentage parasitism, adult eclosion and sex ratio of *A. pseudococci* after 24 h exposure period of third instar nymphs of *R. iceryoides* to *O. longinoda*

Treatment	Parasitized nymphs (%)	Non parasitized nymphs (%)	Adult eclosion (%)	Sex ratio (%)
Ant-present	61.4 ± 4.67b	36.8 ± 4.39a	85.42 ± 2.72b	62.2 ± 3.28b
Ant-absent	86.6 ± 1.31a	11.5 ± 1.26b	94.54 ± 0.55a	71.67 ± 1.71a

Means with different letters are significantly different at P ≤ 0.05.

6.3.3 Interaction of *O. longinoda* with mummified mealybugs and the effect on adult parasitoid eclosion

Ant encounters with mummified mealybugs were always followed by an antennal examination (Figure 6.3). During the 2 h observation period, the mean number of mummified mealybugs removed by the different ant groups was observed to increase with time (Figure 6.4). The highest number of mummies (7.8 ± 0.97) were removed when 20 ants were introduced into the experimental arena accounting for 39.0 ± 4.85% of total mummies removed in 2 h while the lowest was recorded when a single ant was introduced (0.2 ± 0.19 mummies) (Figure 6.4).

Significant difference was observed in the number of mummies removed by the different group of ants at different time interval ($F = 13.94$; $df = 4, 20$; $P < 0.0001$).

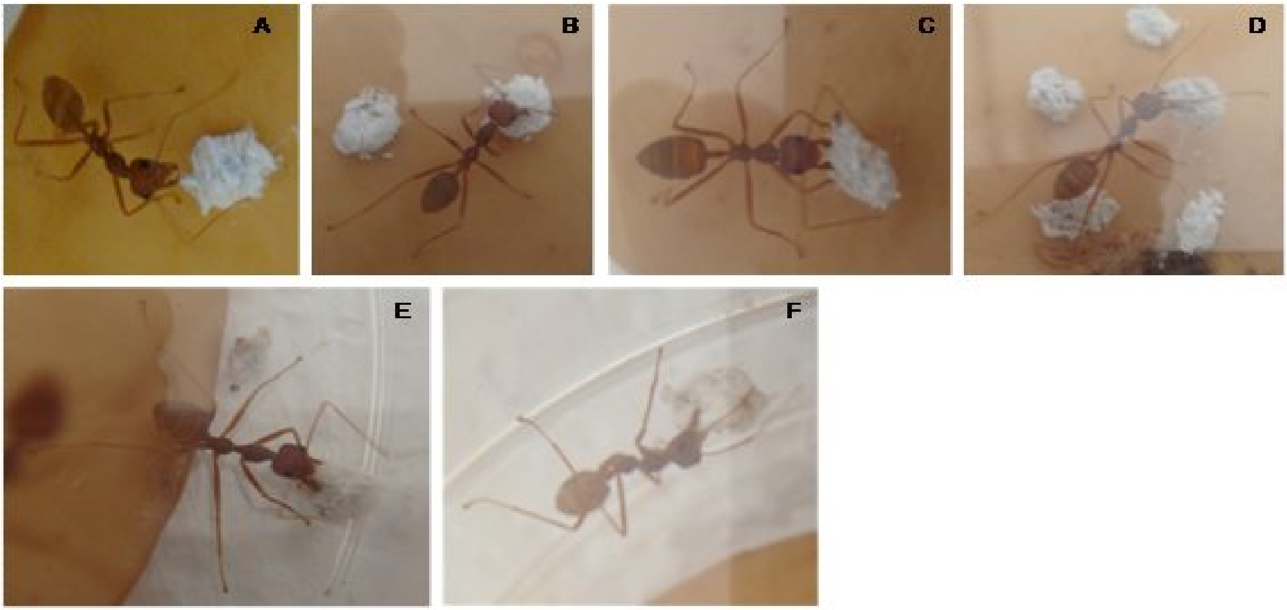


Figure 6. 3: Behavioural interactions between *O. longinoda* and mummified *R. iceryoides* in foraging cages.

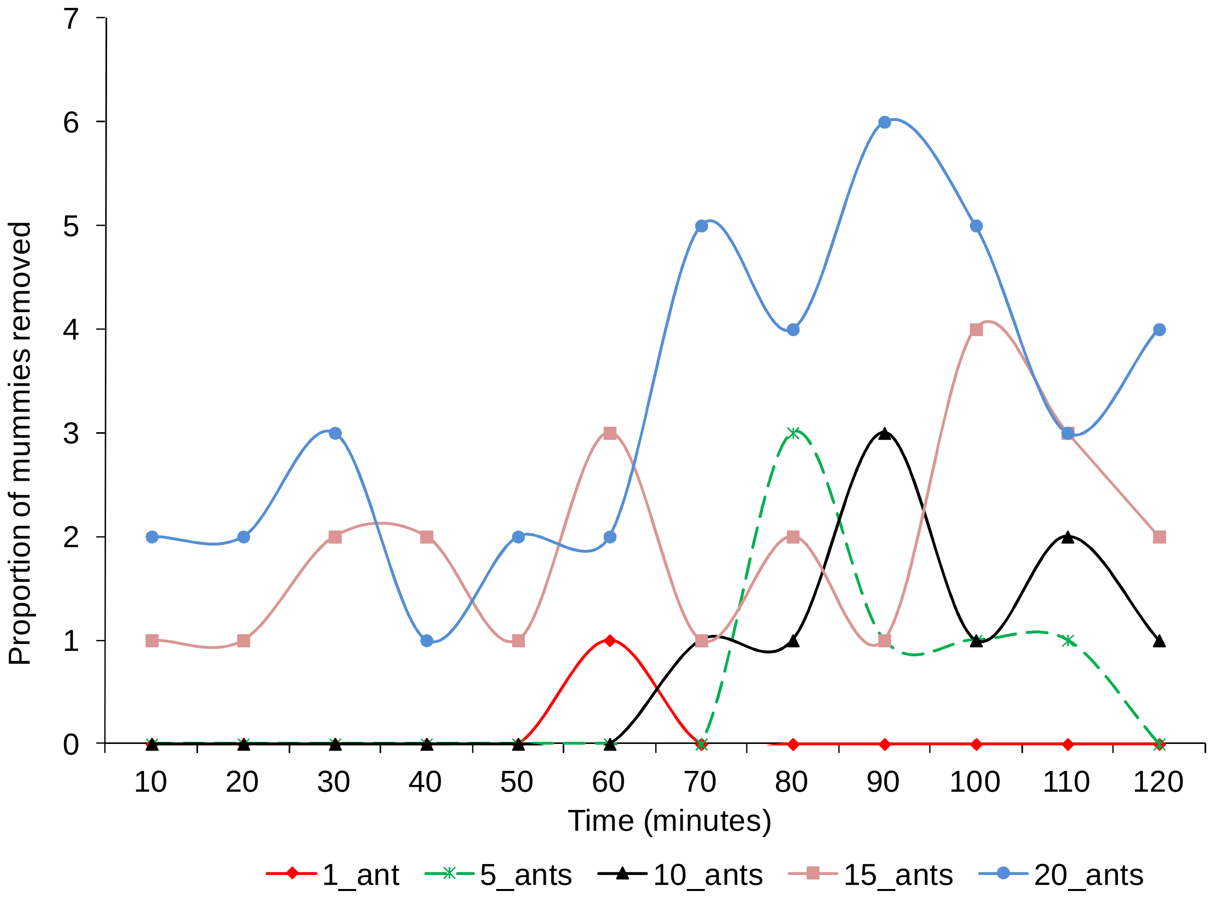


Figure 6. 4: The mean percentage (± S.E) of mummified mealybugs removed by *O. longinoda* at 10 min intervals on butternut during a 2 h observation period.

The highest percentage of adult parasitoid eclosion (95.0 ± 2.74%) was recorded when the mummies were exposed to a single ant treatment while the lowest was recorded when 20 ants were introduced (58.0 ± 4.06%) (Figure 6.5). There was significant difference in percentage adult parasitoid eclosion when mummified mealybugs were exposed to the different groups of ants (F = 9.94; df = 4, 20; P = 0.0001). However, no significant difference was observed in percentage adult parasitoid emergence when the mummies were exposed to 1, 5, and 10 ant's treatments, and between 10 and 15 ants (Figure 6.5).

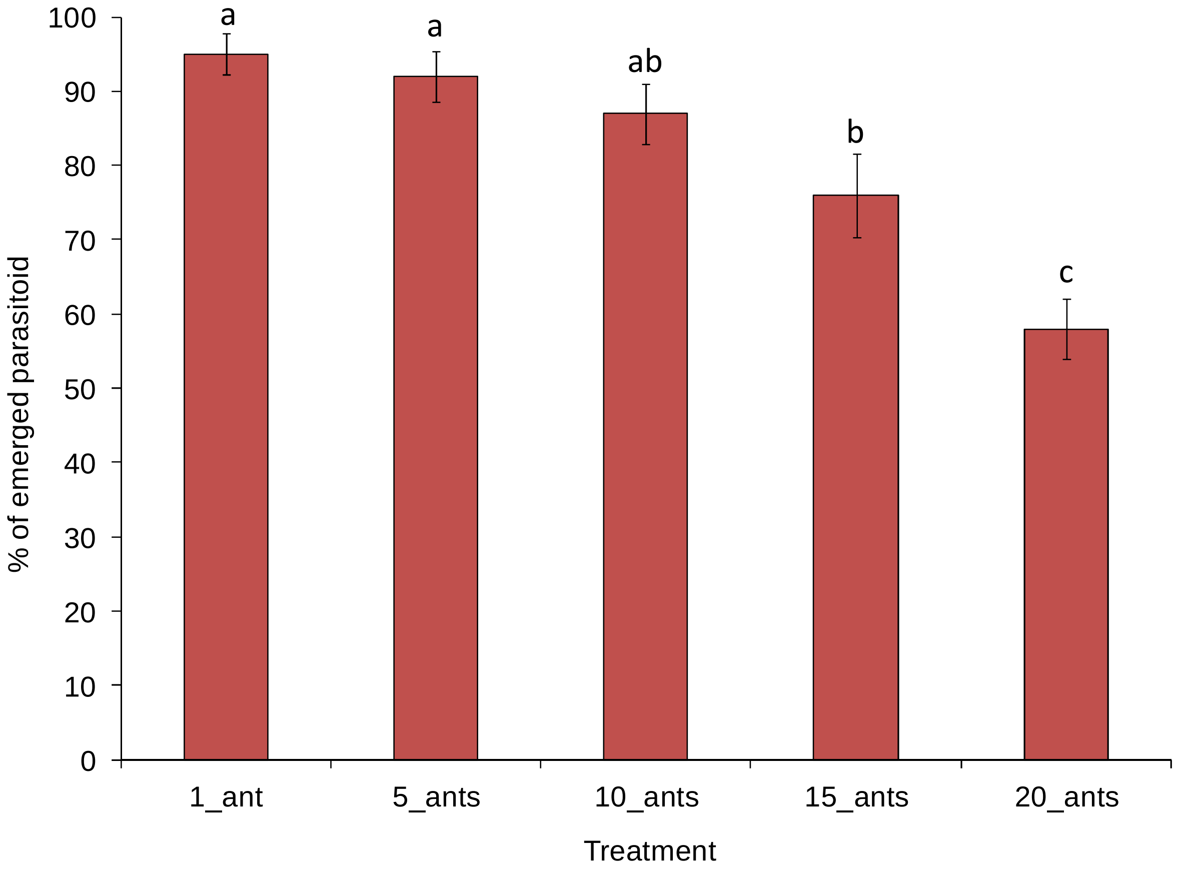


Figure 6. 5: Mean percentage (± S.E) adult parasitoid eclosion when mummified mealybugs were allowed access to different groups of *O. longinoda* workers during a 2 h period.

6.3.3.1 Assessment of *O. longinoda* aggression and escape strategy by *A. pseudococci*

When female parasitoids were attacked by *O. longinoda*, they either jump away (11.9 ± 1.95 to 36.2 ± 1.32% /observation) and continued searching elsewhere on the infested butternut or flew away (10.8 ± 1.13 to 30.5 ± 1.32% /observation) from the test arena (Figure 6.6). Most ovipositing parasitoids ignored (11.3 ± 1.13 to 22.9 ± 1.75% /observation) the ants and continued with their egg laying. Female parasitoids mainly avoided *O. longinoda* workers by retreating or by repeatedly changing their walking direction (27.1 ± 1.38 to 49.4 ± 1.39% /observation). The results indicates that there was a highly significant interaction between behavioural responses displayed by the parasitoids on encounter with an approaching ant during the 2 h observation period ($F = 8.13$; $df = 33, 396$; $P < 0.0001$).

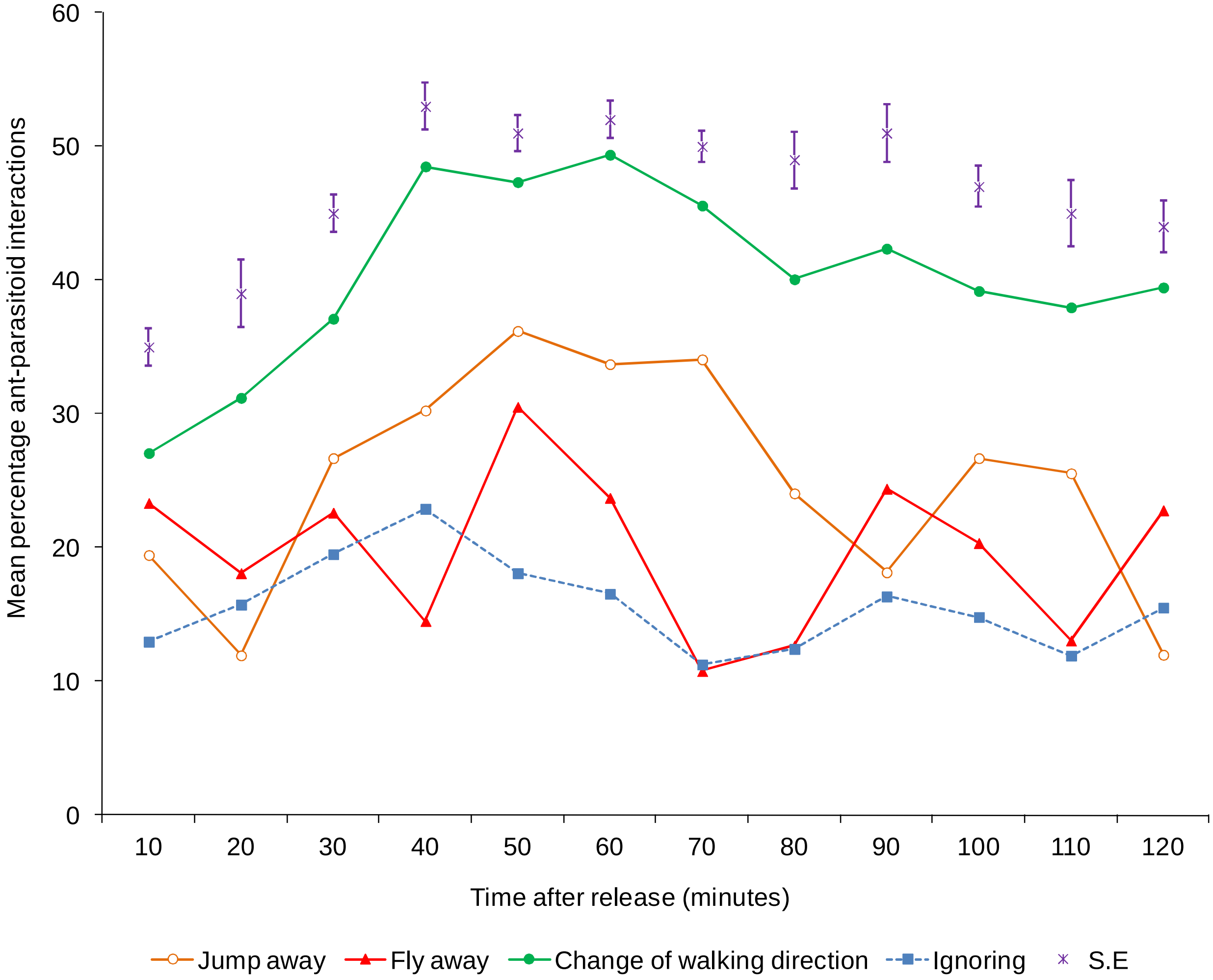


Figure 6. 6: The mean (± SE) percentage behavioural responses by *A. pseudococci* to evade encounters and attacks by *O. longinoda* during 5 min intervals on *R. iceryoides*-infested butternut during a 2 h observation period.

The encounters between ant and parasitoid appeared to be random rather than oriented search by the ant workers. Ants frequently showed aggressive behaviour towards *A. pseudococci* and when *A. pseudococci* was seized, it was sometimes released and picked up again immediately by the same or other ant workers (Figure 6.7). This repeated capturing and recapturing of the same parasitoids resulted in serious injury and sometimes death of the parasitoid. The percentage mortality of adult parasitoids was observed to decrease with time ranging from 1.0 ± 0.67 to 3.5 ± 0.68% (Figure 6.8). The mean percentage mortality of female wasps over the 2 h observation period was 24.32 ± 3.32%. However, during non-aggressive interactions with *O. longinoda*, the proportion of flights was considerably high. Response by ant workers was either touching the female wasps with their antennae or their legs or they passed the parasitoid within a closed distance without exhibiting any obvious responses (Figure 9).

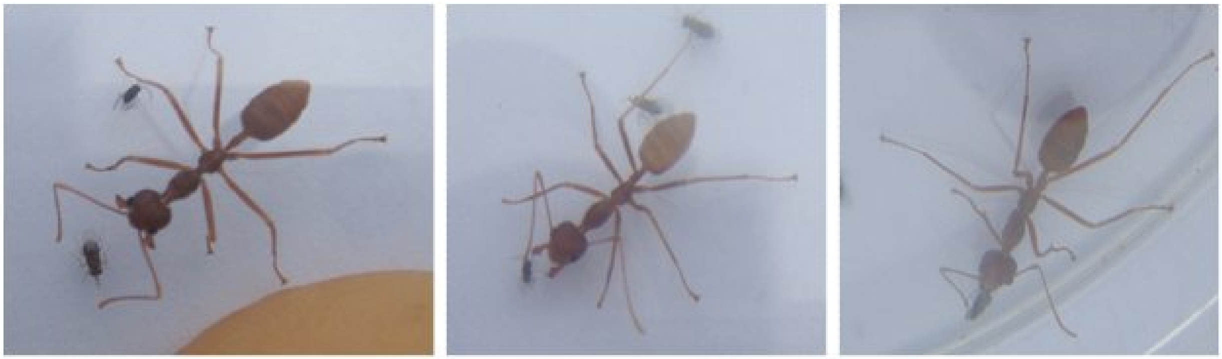


Figure 6. 7: Aggressive behaviour: *O. longinoda* worker in an aggressive posture, ready to attack and finally seized the female wasp with its mandibles.

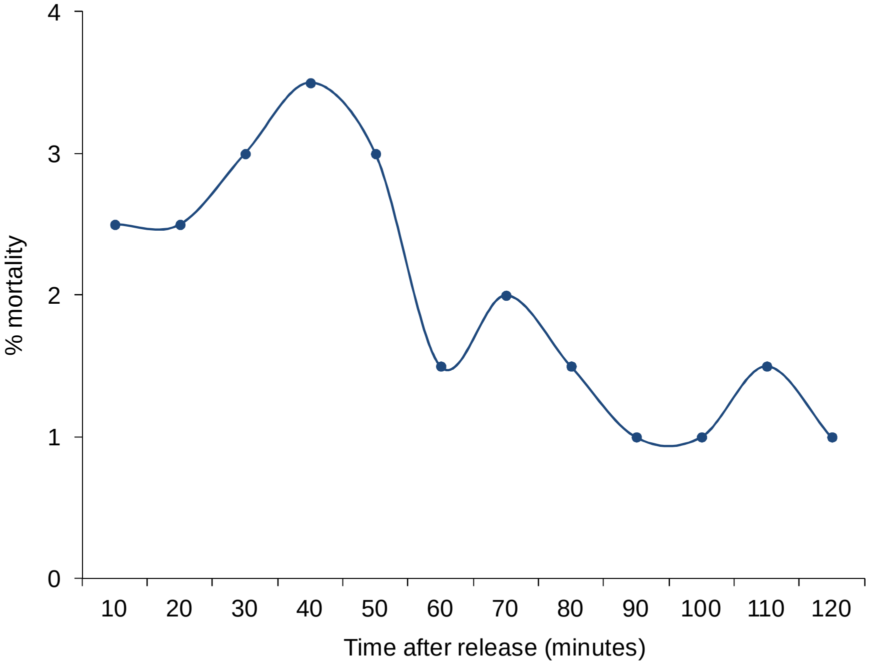


Figure 6. 8: The mean (± SE) percentage mortality of *A. pseudococci* observed in cages with *R. iceryoides*-infested butternut during a 2 h observation period in the presence of *O. longinoda*.

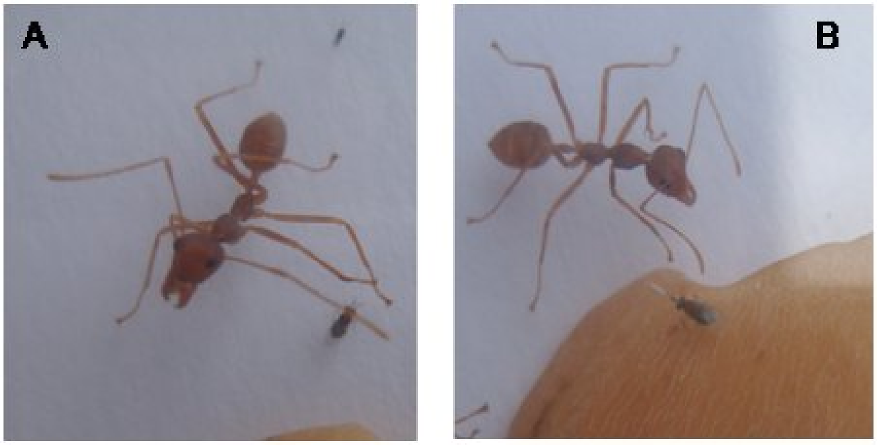


Figure 6. 9: Non-aggressive behaviour, in which the ant workers touched the female wasps with their legs (A) or their antennae (B) or passed the parasitoid within a closed distance without exhibiting any obvious responses.

6.3.3.2 Host handling time and oviposition success of *A. pseudococci* in the presence or absence of *O. longinoda*

The average searching time on ant-attended mealybug-infested butternut was significantly longer (440.9 ± 28.42 sec) compared to the time spent searching on ant-excluded treatment (334.5 ± 25.83 sec) ($t = 2.77$; $df = 38$; $P = 0.0086$). The duration beginning with initial host contact and ending with host rejection was significantly different between the ant-attended (7.53 ± 0.90 sec) and ant-excluded treatments (10.2 ± 0.77 sec) ($t = - 2.25$; $df = 28$; $P = 0.0323$). The observed time for host acceptance in ant-attended (12.73 ± 1.04 sec) and ant-excluded (16.0 ± 1.22 sec) treatments was not statistically different ($t = - 2.04$; $df = 28$; $P = 0.0514$). The duration of ovipositor probing and penetration was significantly different between ant-attended (28.07 ± 1.91 sec) and ant-excluded (21.73 ± 1.78 sec) treatments ($t = 2.43$; $df = 28$; $P = 0.022$). Oviposition duration was not significantly different between the ant-attended (31.53 ± 0.77 sec) and ant-excluded (32.80 ± 0.74 sec). *Anagyrus pseudococci* females required a mean of 79.53 ± 2.35 sec to complete all sequences of host examination and oviposition activities on a single host in ant-excluded treatment and 71.07 ± 1.99 sec in ant-attended treatment.

Anagyrus pseudococci females had significantly more contacts with the host when foraging in ant-excluded treatment (20.47 ± 1.66 contacts per hour) compared to ant-attended treatment (9.8 ± 0.91 contacts per hour) ($t = - 5.64$; $df = 28$; $P < 0.0001$). The mean number of host accepted per hour in ant-excluded treatment (13.4 ± 1.43) was significantly higher ($t = - 4.03$; $df = 28$; $P = 0.0006$) compared to ant-attended treatment (6.87 ± 0.75). Dissection of exposed *R. iceryoides* in ant-attended and ant-excluded treatments showed that *A. pseudococci* deposited a single egg with each oviposition bout, although it was not uncommon for the parasitoid to drill in different locations on the same host. *Anagyrus pseudococci* achieved significantly ($t = - 4.02$; $df = 28$; $P = 0.0006$) higher number of successful oviposition in ant-excluded (9.87 ± 1.25/h) than on ant-attended treatment (4.13 ± 0.70/h) (Table 6.2). The number of successful oviposition was significantly correlated with host handling time in ant-excluded treatment ($r = 0.8897$, $n = 15$, $P = 0.0392$) while there was no significant correlation in ant-attended treatment ($r = 0.2977$, $n = 15$, $P = 0.2881$).

Some mealybugs were rejected after several failed attempts by the parasitoid to insert the ovipositor, due to either host defence (Figure 6.10) or repeated failure to insert the ovipositor in an appropriate area of the host's body (Figure 6.11). The mean number of successful host defence was significantly (*t* = - 4.3; *df* = 28; *P* = 0.0002) higher in ant-excluded treatment (7.07 ± 0.77) compared to ant-attended treatment (2.93 ± 0.57).

Table 6. 2: The mean (± SE) number of ovipositor penetration of the host, successful and unsuccessful oviposition per h, when *A. pseudococci* females forage in the presence or absence of *O. longinoda*

Treatment	No. of ovipositor penetration/h	No. of successful oviposition/h	No. of unsuccessful oviposition/h
Ant present	5.87 ± 0.72b	4.13 ± 0.70b	1.73 ± 0.40a
Ant absent	11.27 ± 1.29a	9.87 ± 1.25a	1.40 ± 0.25b

Within column means followed by the same letter are not significantly different (P < 0.05).

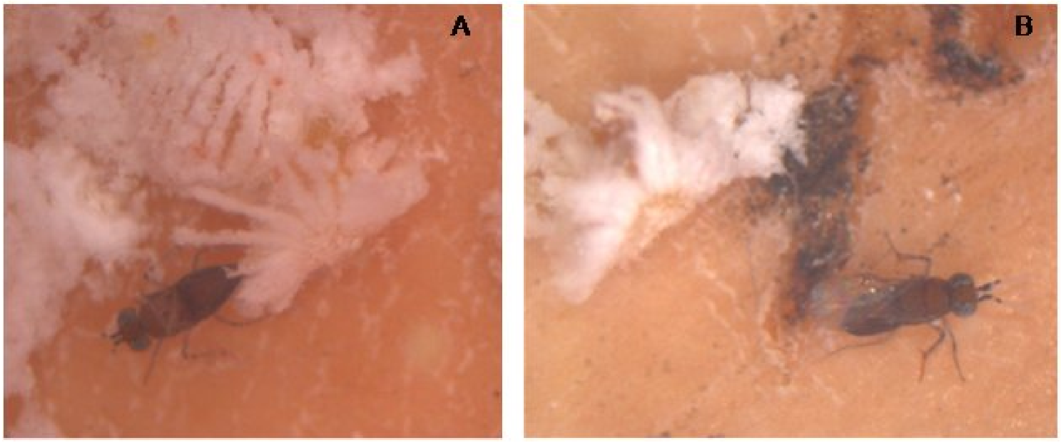


Figure 6. 10: (A) Third instar nymph of *R. iceryoides* exhibiting a more vigorous defense against an ovipositing female of *A. pseudococci*; (B) *A. pseudococci* terminates the oviposition process as it fails to subdue the host.

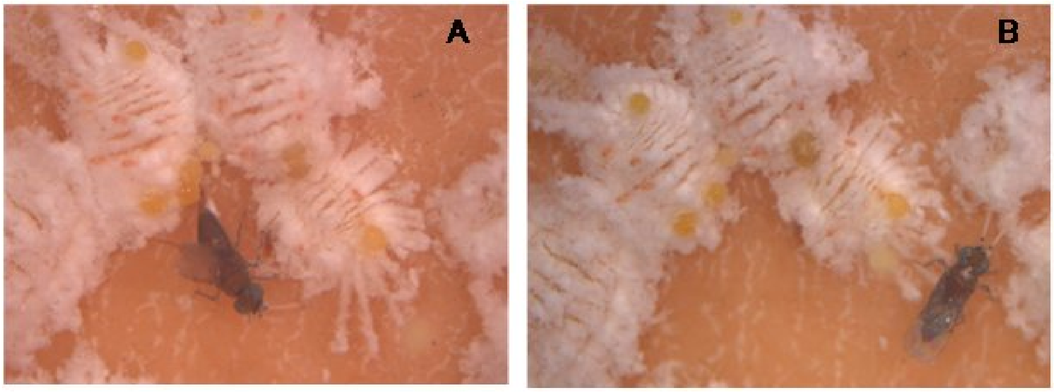


Figure 6. 11: (A) Repeated failure of *A. pseudococci* to insert the ovipositor in an appropriate area of the host's body as the host retreats; (B) Oviposition is terminated prematurely and *A. pseudococci* continued searching elsewhere.

6.4 Discussion

These experiments demonstrate that the mean number of *O. longinoda* workers on the mealybug-infested butternut decreased significantly when *A. pseudococci* was introduced into the arena. These findings are in accordance with the observations by Hölldobler and Edward (1978) who reported that short-range recruitment of intruders (i.e., parasitoids) can cause *O. longinoda* workers to shift to a more distinctly clumped pattern bringing more defenders into the vicinity. This complex recruitment and territorial behaviour displayed by *O. longinoda* is considered to be part of the adaptation of these relatively large ants to a strongly arboreal existence. Attendance by *O. longinoda* significantly increased the percentage mortality of the parasitoids and reduced resultant parasitism in the present study. This study demonstrated that although female parasitoids were seized and sometimes released immediately, the repeated capturing and recapturing of the same parasitoids by the same or other ant workers resulted to seriously injury and even death of the parasitoids. Contrary to the present study, Martinez-Ferrer et al. (2003) noted that larger ants do not easily recognized small natural enemies while Way, (1954) reported that the African weaver ant *O. longinoda* does not react to the presence of adult *Coccophagus nigritus* Compere (Hymenoptera: Aphelinidae), parasitoid of the scale insect *Saissetia zanzibarensis* Williams (Hemiptera: Coccidae).

However, given the invasive nature and wide distribution of *O. longinoda*, possibly perpetrated by suitable eco-climatic conditions from the coastal shorelines to 1220 meters above sea level (Way, 1954), its present a serious threat to biological control of Hemipteran pests like *R. iceryoides*, as they are capable of affecting parasitoids in orchards over large areas. In most orchards, they construct numerous temporary nests on tree canopies, presumably providing better protection to mealybugs (Way, 1954), thereby posing a serious threat to mealybug parasitoids.

This study observed that *Anagyrus pseudococci* foraged in a similar core sequence of oviposition behaviour as described in other encyrtids mealybug parasitoids (Boavida et al., 1995, Bokonon-Ganta et al., 1995, Karamaouna and Copland, 2000, Joyce et al., 2001). The female wasp always examined the encountered hosts. Similarly, the exotic parasitoid *Gyranusoidea tebygi* Noyes has been reported to examine every *Rastrococcus invadens* Williams encountered (Boavida et al., 1995). This behaviour suggests that host recognition is probably mediated through the presence of kairomone in the wax filaments of the mealybugs (Chong and Oetting, 2007). Female *A. pseudococci* spent approximately the same time in ant-excluded treatment per event of host examination as reported by Chong and Oetting (2007) for pre-reproductive adult female *Phenacoccus madeirensis* Green (Hemiptera: Pseudococcidae) (mean =17.4 s). Similarly, the time spent by *A. pseudococci* in examining *Planococcus citri* (Rossi) and *Pseudococcus affinis* (Maskell) (9.7 ± 0.37 to 13.56 ± 0.43 s) (Hcidari and Jahan, 2000) is within the range recorded in the present study.

The duration for ovipositor probing and penetration by *A. pseudococci* in this study ranged between 28 to 30 seconds in both ant-attended and ant-excluded treatments, which is slightly less than that reported by Chong and Oetting (2007) for *P. madeirensis* (45 seconds to 1 min). Ovipositor probing and penetration were the major factors that provoked host defense behaviour, although antennal examination could also provoke defensive behaviour to an extent. Some mealybugs were rejected after several failed attempts of the female wasp to insert the ovipositor, due to either host defense or repeated failure to insert the ovipositor in an appropriate area of the host's body. Islam and Copland (2000) reported that increased and frequent ovipositor probing was more prominent in parasitoids with high ovarian pressure. The average oviposition duration observed in this study was approximately 10 seconds compared to 30.5 ± 1.6 to 37.1 ± 2 seconds reported by Hcidari and Jahan (2000) for oviposition by *A. pseudococci* on *Planococcus citri* and *Pseudococcus affinis*. Joyce et al. (2001) reported an average oviposition time of 28 seconds for the solitary *Coccidoxenoides peregrinus* (Timberlake)

(Hymenoptera: Encyrtidae), parasitoid of *Planococcus ficus* (Signoret). In contrary, Chong and Oetting (2007) reported that the average time for each oviposition event by *Anagyrus* sp. nov. nr. *sinope* attacking first instar nymphs of *P. madeirensis* was 6 min (360 seconds), and increased to 15 min (900 seconds) for third-instar nymph females. The phenomenon of increased mean oviposition time in larger host has also been reported in the solitary *Gyranusoidea tebygi* Noyes (Hymenoptera: Encyrtidae) (Boavida et al., 1995) and *Anagyrus mangicola* Noyes (Hymenoptera: Encyrtidae) (Bokonon-Ganta et al., 1995).

The behaviour of a parasitoid in the presence of ants largely determines its own effectiveness as a biological control agent (Nixon, 1951). For example, parasitoids have been observed to abandon oviposition and keep away from mealybugs to avoid ants, limiting the number of eggs that could be laid into the host (Mgocheki and Addison, 2009). While some parasitoids have developed escape strategies from ants to improve their efficacy, others are so ant sensitive that after an encounter with ants, they are deterred not only by ants, but by any moving object including other parasitoids or the host, thereby greatly reducing their potential as biological control agents (Martinez-Ferrer et al., 2003; Flanders, 1958). It is apparent that *O. longinoda* did not only interfere with percentage parasitism of their adopted Hemiptera, but also reduce parasitoid abundance by causing direct mortality and consequently low reproductive success. Results from this investigation showed that *A. pseudococci* is very sensitive towards *O. longinoda*, as indicated by their low oviposition success which they achieved in the presence of ants. Similarly, Mgocheki and Addison (2009) also found that *Anagyrus* spp. were more sensitive to other ant species like *Anoplolepis steingroeveri*, *Crematogaster peringueyi* and *Linepithema humile*.

During our study we found that *A. pseudococci* has developed strategies to forage in ant-attended patches and to escape ant aggression based on behavioural responses, which were often combined with morphological adaptations to improve their efficacy. For example, the agile and quickly foraging behaviour of *A. pseudococci* is a typical representative of the avoidance type. The female wasp showed a striking high rate of avoidance behaviour, which prevented direct ant contacts and kept up a reasonable distance to the next ant worker. *Anagyrus pseudococci* females were observed to leave the mealybug-infested butternut immediately when coming in contact with *O. longinoda*. This sensitiveness toward any physical ant contact and following quick flight response, which is supported by an excellent jumping ability, ensured a low mortality risks in the presence of the very aggressive *O. longinoda.* Similarly, this corroborates with studies reported by several authors on the behavioural strategies of other encyrtid species interacting with ants (Novak, 1994; Völkl, 1995). The flight strategy of *A. pseudococci* on the other hand resulted in short residence times in the arena and significantly low numbers of host contacts in ant-attended treatments. Our investigation of *O. longinoda*-parasitoid interactions in the laboratory gives a better insight into the overall effectiveness of the escape strategies of *A. pseudococci* that might be experienced in the field.

In this study, the third instar nymphs of *R. iceryoides* exhibited vigorous defense against the parasitoid and this had a very important influence on their foraging behaviour. Antennal examination, ovipositor probing and oviposition were the major factors that provoked defensive behaviour of the mealybugs. Similarly, several studies have also reported that parasitoids attacking larger hosts always spend more time and energy in subduing the hosts and risk injury due to the host defense (Godfray, 1994, van Alphen and Jervis, 1996). Kairo and Murphy (1999) showed that the frequency of defensive behaviour exhibited by *Cinara cupressivora* Watson and Vogtlin (Hemiptera: Aphididae) increased with age and that the defenses of the third-instar nymphs acconted for 64% of the failed attempts by the parasitoid *Pauesia juniperorum* Stary (Hymenoptera: Braconidae). The frequency and success of the defensive behaviour of the galling wasp *Pontania proxima* Lepeletier (Hymenoptera: Tenthredinidae) against its parasitoid also increased with age of the larvae, causing the parasitoid to reduce parasitism efficiency and mean oviposition duration (Al-Saffar and Aldrich, 1998). Völkl and Mackauer (2000) and Wang and Keller (2000) reported that although pursued hosts have varying degrees of success in escaping parasitism, parasitoids on the other hand also have different methods and abilities in overcoming the hosts' defensive behaviour. The majority of encountered *R. iceryoides* were unable to deter attacks by *A. pseudococci*, as the wasp pursued the mealybug until it encountered another mealybug or succeeded in parasitizing the pursued host. This implies that *A. pseudococci* have evolved foraging and attack behaviours that are fine-tuned to the defensive capability of the intended hosts.

Therefore, mass release programs of *A. pseudococci* to control low mealybug infestation in ant infested mango orchards via inundative releases should be done with a lot of caution as this may not be effective depending on the ant species present. This is because, if *A. pseudococci* have evolved to avoid interference by *O. longinoda*, they probably have not successfully modified their oviposition behaviour to totally circumvent ants' aggression. In Kenya and Tanzania, eleven different ant species have been reported to be closely associated with *R. iceryoides*. These includes, *Anoplolepis custodiens* (Smith)*, Camponotus flavomarginatus* Mayr*, Crematogaster tricolor* st. rufimembrum Santschi*, Linepithema humile* Mayr, *Oecophylla longinoda* Latreille*, Pheidole megacephala* Fabricius*, Atopomyrmex mocquerysi* Bolton, *Lepisiota depressa* (Santschi), *Polyrhachis schistacea* (Gerstäcker), *Iridomyrmex purpureus* (F. Smith) and *Camponotus pennsylvanicus* De Geer (Tanga, unpublished data). Among these species, *P. megacephala, Camponotus* sp., and *Crematogaster* sp. have been implicated in heavily reducing parasitization of the cassava mealybug, *Phenacoccus manihoti* Matile-Ferrero, by the encyrtid parasitoid *Epidinocarsis lopezi* (De Santis) (Cudjoe et al., 1993). These results are important to growers who should be aware of the species of pest ants foraging in their orchards.

Based on these findings, ant control should be considered a priority when introducing any parasitoid as biological control agents of *R. iceryoides* as the ant species presence might not only affect parasitoid abundance but also reproductive success and possibly oviposition strategy of female parasitoid species. There is clearly a need to develop selective pesticide protein baits (Grafton-Cardwell and Reagan, 1999; James et al., 1996), sugar baited toxicants (Klotz et al., 1997; Klotz et al., 1998), chemical stem barriers (Addison, 2002; James et al., 1998), and repellent semio-chemicals (Shorey et al., 1992; Shorey et al., 1993; Sisk et al., 1996) to reduce ant numbers in mango orchard and therefore allow effective biological control of the mango mealybug. Further detailed future investigations with different ant species associated with *R. iceryoides* in the field is needed to ascertain this potential.

CHAPTER SEVEN

Effects of climatic factors on the occurrence and seasonal variation in populations of *Rastrococcus iceryoides* (Hemiptera: Pseudococcidae) and its associated natural enemies: implications for biological control

ABSTRACT

The fundamental aspect in developing a sound management strategy for a pest is the understanding of its seasonal fluctuation and that of the associated natural enemies. In this study the population dynamics of the alien invasive mango mealybug, *Rastrococcus iceryoides* (Hemiptera: Pseudococcidae) and its associated natural enemies were carried out in two major mango growing areas in coastal Tanzania during the period of December 2008 to June 2010. Destructive sampling, based on random selection of 80 leaves, 20 twigs (~10 cm) and 5 fruits, was carried out on a weekly and monthly basis during the mango and off mango season, respectively. The total number of *R. iceryoides* and the number of mummified mealybug were recorded for each plant part. Upon emergence, parasitoids were counted and identified. The number of predators was collected using beat-sheet techniques. The study revealed that populations of *R. iceryoides* population followed an annual cycle which is synchronized with the mango fruiting season, with a peak incidence occurring during the Northeast monsoon (December-February) at a temperature range of 23 to 33°C, relative humidity of 54 to 86% and total rainfall from 0 to 63 mm. The population trend of *R. iceryoides* is climate dependent and declined sharply following the onset of the heavy rains from March-May, and continues through the coldest and driest period of the year from June-October (Southwest monsoon). The mealybug population and its associated natural enemies were significantly and positively correlated with mean temperature, while it was significantly and negatively correlated with rainfall. The population of the parasitoid and predators were positively correlated with that of the host/prey, *R. iceryoides*. The study also revealed that there was high diversity of hyperparastoids, although their impact on the primary parasitoid was negligible. The findings of this study can be used to model the prediction of *R. iceryoides* population outbreaks and additionally to formulate an effective and sustainable pest management strategy in the agro-ecological system under consideration.

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Key words: Population dynamics, Mango, *Rastrococcus iceryoides*, natural enemies, weather factors, pest management

7.1 Introduction

The mango mealybug, *Rastrococcus iceryoides* Green (Hemiptera: Pseudococcidae), was accidentally introduced from Southern Asia into the African continent in the early 1990s (CABI, 2000). In Africa, *R. iceryoides* together with its close relative *R. invadens* are regarded as the two important exotic mealybug species native to Southern Asia that commonly infest mango. The latter devastated mango production in West and Central Africa but was managed through classical biological control with the introduction of the parasitoids, *Gyranusoidea tebygi* Noyes and *Anagyrus mangicola* Noyes (both Hymenoptera: Encyrtidae) from India (Noyes, 1988; Bokonon-Ganta and Neuenschwander, 1995). *Rastrococcus iceryoides* on the other hand is so far restricted to the East Africa region (mainly Tanzania and Coastal Kenya) and northern Malawi, where it has remained one of the most destructive sucking pests of fruit trees, especially mango as well as various ornamental plants (Tanga, unpublished data; Williams, 1989; Luhanga and Gwinner, 1993; CABI, 2000).

Surveys conducted in Kenya and Tanzania revealed that *R. iceryoides* has an extremely broad host range of about 29 cultivated and wild host plant species from 16 different families. Twenty-one of these host plants are new records for *R. iceryoides*, of which 18 are native to Africa (Chapter 3). The broad host range recorded in Africa strongly suggests that *R. iceryoides* is an emerging invasive polyphagous pest that is capable of rapidly expanding its host range. During the pest outbreak, the high infestation level lead to delays in flowering, fall of floral spikes and leaves, drying up of young fruit-lets, and slowing the growth of new branches due to the severe dieback effects on heavily infested plant parts (Tanga, unpublished data). It is also a nuisance by causing accumulation of large amounts of excreted honeydew that results in the formation of sooty mould, which causes drastic reduction in photosynthesis, leading to reduction in plant growth, flowering and fruiting as well as premature leaf drop. Members of the genus *Rastrococcus* have a potential to become major pests in newly invaded areas. For example, the mango losses caused by *R. invadens* were reported to be as high as 80% in Ghana (Entomological society of Nigeria, 1991), and range between 53% to 100% in Côte d'Ivoire (Hala et al., 2004). Chemical insecticides are among the conventional methods used in controlling *R. iceryoides* in Tanzania and Kenya. However, like other mealybug species *R. iceryoides* proved to be very hard to manage with the use of insecticides as they are covered with powdery hydrophobic wax that repels water-based insecticide solutions (Blumberg and Van Driesche, 2001; Derzelle et al., 2004). The inefficiency of the insecticides in controlling the pest coupled with its unaffordability for most small scale farmers has made mango cultivation uneconomical and has led to the abandonment of mango production in severely affected areas (Tanga et al. unpublished data).

The population fluctuation as well as the distribution of the mealybug pests depend largely upon the prevailing environmental factors (DeBach, 1949), as these pest are known to multiply tremendously during favourable weather conditions leading to population outbreaks (Amarasekare et al., 2008). Climatic conditions also influence natural enemy populations such as parasitoids and predators either directly or indirectly (Arif et al., 2006; Chaudhari et al., 1999). For developing an early warning weather based system for any pest in a specific agro-ecosystem, it is necessary to have basic information regarding population dynamics in relation to prevalent meteorological parameters (temperature, relative humidity and rainfall). This will help in determining appropriate times for intervention, and application of suitable methods of control.

Considering the fact that very little is known regarding the ecology of *R. iceryoides* as a recently introduced pest, and that the role of its indigenous natural enemies is also not documented, a thorough understanding of the interaction between meteorological parameters/pest/natural enemy dynamics is a prerequisite for a weather based pest forecasting model. Therefore, this study was carried out to determine the influence of weather parameters namely temperature, relative humidity and rainfall on population dynamic *R. iceryoides* and its associated natural enemies, which is of great significance in formulating efficient integrated pest management (IPM) strategies for sustainable agriculture.

7.2 Materials and Methods

7.2.1 Study sites

Tanzania is located in East Africa between latitude, 1° to 11°45′ S and longitude 29°21′ to 40°25′ E. The country is divided into four main climatic zones: (1) the coastal area and immediate hinterland, (2) the central plateau, (3) the semi-temperate highland areas, and (4) the high, moist lake regions. This study was carried out in the coastal area, which is characterised by tropical conditions, with temperatures averaging about 27°C, annual rainfall varying from 1000 to 1930 mm and high humidity. In this region the seasons are well defined; these are: northeast monsoon, from December to February (it is hot and comparatively dry), the long rains, from March to May, and the short rains, from November to December and the southwest monsoon from June to October (coldest and driest) (EON, 2011). Within the coastal area the sampling was carried out at two main mango growing localities; Kibaha (06˚ 43' 84" S; 038˚ 46' 07" E, 79 m above sea level) and Dar es Salaam (06˚ 45' 80" S; 039˚ 06' 25" E, 162 m a.s.l). The distance between the two localities is 38.66 km. At both orchard localities where sampling was done the farms were maintained under the same agricultural practices and had not been sprayed with insecticides for the previous two years.

7.2.2 Sampling procedures

The sampling methodology was a slight modification of that described by Pitan et al. (2000) and Bokonon-Ganta and Neuenschwander (1995). During the study period a destructive sampling was conducted weekly during mango fruiting season (October-February), while it was monthly during the mango off season (March - September) for a total period of 19 months (December, 2008-June, 2010). On each sampling date, a group of twenty mango trees were randomly selected at fixed distances and labelled to represent the whole orchard before commencing the sampling. For each sampling date, 80 leaves, 20 twigs (~10 cm long) and 5 fruits were selected at random for mealybug counts. To avoid taking mealybugs only from the upper portions of plants, the order in which plant parts were examined (bottom to top and vice versa) was reversed after each tree. During the survey, care was taken to make sure that no tree was sampled twice within the same month. The samples where then transported to the laboratory at the National Biological Control Program (NBCP), Kibaha. In the laboratory, and on the same day of the sampling, the individual numbers of each developmental stages (first, second, third nymphal instars, ovipositing and non-ovipositing female) were counted and recorded for each plant part. The total number of live individuals was used to represent the infestation level for each plant part and sampling date.

The different plant parts were checked for the presence of mummified *R. iceryoides* nymphs. The mummified mealybug nymphs were carefully removed from the plant parts using a fine hair brush and then placed into transparent plastic rearing containers (22.5 cm height x 20 cm top diameter x 15 cm bottom diameter). An opening (10 cm diameter) was made on the front side of the cage to which a sleeve, made from very fine organza material (about 0.1 mm mesh size) was fixed. The same material was fixed to the opposite opening (10 cm diameter) of the cage to allow for ventilation. A third opening (13 cm diameter) was made on the roof of the cage, which was also screened with the same material. Streaks of undiluted honey were applied to the roof of the cages and maintained in the laboratory at ambient temperatures of 26 – 28 ^{0}C, 70 ± 5 % RH, photoperiod of 12:12 (L : D) h,. The mummies were checked daily until the parasitoid wasp ceased to emerge. Mummified mealybugs from each sampling date and locality were maintained separately. Parasitoids that emerged from the mealybug mummies were counted and recorded. Thereafter, they were kept in vials containing 80% ethyl alcohol and labelled with their respective plant part and sampling date. All parasitoids that emerged were initially identified at Annamalai University, India and later confirmed at the National Collection of Insects, PPRI-Agricultural Research Council (ARC), Pretoria, South Africa. The level of parasitism was calculated as a percentage of the mummified mealybugs in the samples over the total number of mealybug for each plant part and sampling date separately.

Predators of *R. iceryoides* were sampled using the beat sheet technique (Wade et al., 2006), which involved beating 5-10 randomly selected branches of host plants over a 1m^2 cloth screen using a 60 cm long stick. The sampling was done in the morning (8:30-9:30 am). Predators that were dislodged onto the cloth were then counted and recorded before preserving in 80 % ethyl alcohol. Immature stages of predators were reared on mealybugs in perspex cages (15 cm height x 20 cm length x 15 cm width). An opening (10 cm diameter) was made on the front side of the cage to which a sleeve, made of organza material, was fixed. The same material was fixed to the opposite side of the cage to allow for ventilation. The rearing process was carried out in the laboratory set at the National Biological Control Program (NBCP), Kibaha, Tanzania until they developed to the adult stage and were later counted and recorded.

Records of the main climatic factors: daily minimum and maximum temperatures, minimum and maximum relative humidity and total rainfall were obtained from the nearest Meteorological and Agricultural Station in Kibaha. The daily value of these weather parameters were averaged to correspond with sampling dates.

7.2.3 Data analysis

Data are presented as means (± SE) per twig, leaf and fruit. A Poisson generalized linear model was implemented using generalized estimating equations to examine the effect of the weather parameters, namely, mean temperature, mean relative humidity and rainfall on the population dynamics of the mealybugs and the number of mummified mealybugs on the twigs, leaves and fruits. An AR-1 correlation structure was used for the generalized estimating equations (Zuur et al., 2009). Chi-square goodness of fit was used to test if the infestation level by the mealybugs, and the number of mummified mealybug were the same on the different plant parts. During the mango fruiting season, comparisons were made among the three plant parts (fruit, leaves and twigs), while during the mango off-season, comparisons were done only for the leaves and the twigs. The association between the mealybug and parasitoid populations on the different plant parts was assessed using correlation. All the analysis was performed in R 2.13.1 (R Development Core Team, 2011).

7.3 Results

7.3.1 Local variation of ambient temperature and relative humidity

During the entire study period, temperature and relative humidity were generally high, with an average minimum temperature of 23.34 °C and an average maximum of 31.93 °C. The average minimum relative humidity (RH) was 63.20 % and an average maximum of 83.82%. Total rainfall ranged between 0 mm and 259.2 mm throughout the study period. The mean values of temperature and relative humidity recorded during the study period from December 2008 to June 2010 is illustrated in Figure 7.1.

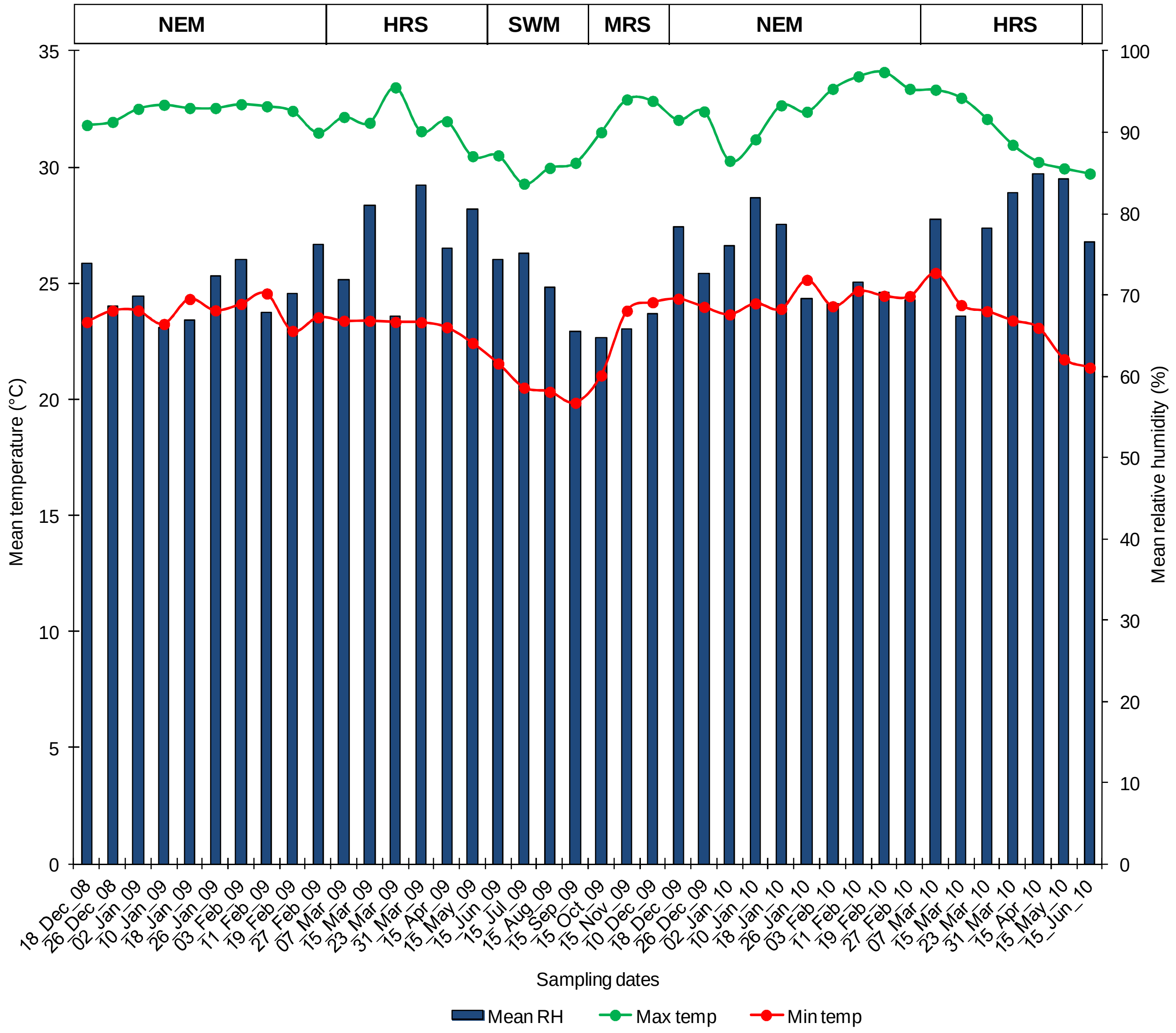


Figure 7. 1: Mean temperature and mean relative humidity in the Coast region of Tanzania from December 2008 to June 2010. NEM (December-March): Northeast monsoon (It is hot and comparatively dry); HRS (March-May): Heavy rainy season; SWM (June-October): Southwest monsoon (It is coldest and driest); MRS (November-December): Mild rainy season. Data obtained from Meteorological and Agricultural Station in Kibaha – Coast region, Tanzania.

7.3.2 Seasonal infestation levels by *R. iceryoides* on mango in Kibaha

The results of the survey showed that there was a great seasonal fluctuation in the population of *R. iceryoides* on the different mango plant parts with respect to rainfall (Figure 7.2). During the 2008/09 mango fruiting season the highest infestation levels on the fruit (171.2 ± 85.82 mealybugs/fruit) was recorded on the 26th of December 2008 (mean temp. of 27. 9°C, mean RH of 68.6%, and total rainfall of 4 mm). While the highest infestation on the twig (324.05 ± 82.26 mealybugs/twig) and the leaves (136.06 ± 17.89 mealybug/leaf) was recorded on 10th of January (mean temp. of 27.9°C, mean RH of 66%, and no rainfall). For the same mango fruiting season the lowest infestation level on the leaves (34.43 ± 8.03 mealybugs/leaf), and twigs (36.5 ± 9.54 mealybugs/twig) was recorded on 18th December 2008 (mean temp. of 27.6°C, mean RH of 73.8%, and rainfall of 44.6 mm), and 27th of February 2009 (mean temp. 27.5°C, mean RH of 76.19%, and rainfall of 6.1 mm), respectively. While the lowest infestation level on the fruit (0 mealybug/fruit) was recorded on 26th of January 2009 (mean temp. of 28.2°C, mean RH of 72.3%, and rainfall of 19.5 mm) and 19th of February 2009 (mean temp. of 27.7°C, mean RH of 70.2%, and total rainfall of 63.2 mm). During this mango fruiting season, the infestations by *R. iceryoides* varied significantly across the different plant parts (twigs, leaves and fruit) ($\chi2$ = 570.54; *df* = 2; *P* < 0.0001).

During the mango off-season from 07th of March to 15th August 2009, the highest infestation levels was recorded on 15th of March 2009 (mean temp. of 27.5°C, mean RH of 75.7%, and 35.2 mm of rainfall) and 15th of August 2009 (mean temp. of 25.2°C, mean RH of 70.9%, and 0.7 mm of rainfall) on the leaves (47.34 ± 10.36 mealybugs/leaf) and twigs (52.15 ± 19.49 mealybugs/twig), respectively. However, during the mango off-season there was no significant difference in the infestation levels between twigs and leaves ($\chi2$ = 0.147; *df* = 1; *P* = 0.7019).

During the 2009/10 mango fruiting season, the highest infestation levels on the leaves (88. 51 ± 13.65 mealybugs/leaf) and the fruit (178.4 ± 54.06 mealybug/fruit) was recorded on 10th of January 2010 (mean temp. 27.7°C, mean RH of 82%, and 23.9 mm of rainfall), while that on the twig (207.15 ± 54.55 mealybugs/twig) was on 18th January 2010 (mean temp. of 28.3°C, mean RH of 78.7%, and 16.3 mm of rainfall). On the other hand the lowest infestations was recorded on 15th of September 2009 (mean temp. of 25.0°C, mean RH of 65.6%, and 0.5 mm of rainfall for fruit (4.0 ± 2.67 mealybugs/fruit), and on 10th of December 2009 (mean temp. of 28.5°C, mean RH of 67.6%, and total rainfall of 114.9 mm) for both twigs (8.0 ± 2.96 mealybugs/twig) and leaves (11.49 ±2.92 mealybugs/leaf). Similar to the 2008/9 season, there was a significant difference in the infestation levels amongst the twigs, leaves and fruits ($\chi 2$ = 123.4; df = 2; $P < 0.0001$).

During the mango off-season, the highest infestation levels was recorded on 27th of February 2010 (mean temp. of 28.9°C, mean RH of 69.4%, and 2.6 mm of rainfall) for both twigs (34.8 ± 10.46 mealybugs/twig) and leaves (41.79 ± 9.17 mealybugs/leaf). While the lowest infestation was on 15th of June 2010 (mean temp. of 25.6°C, mean RH of 76.6%, and 63.8 mm of rainfall) for both twigs (2.55 ± 1.42 mealybugs/twig) and leaves (4.69 ± 1.85 mealybugs/leaf). However, there was a significant difference on the infestation levels observed on the twig and leaves during this period ($\chi 2 = 12.97$; $df = 1$; $P < 0.0003$).

7.3.3 Seasonal infestation levels by *R. iceryoides* on mango in Dar es Salaam

The population of *R. iceryoides* varied greatly with rainfall on all plant parts (Figure 7.3). During the 2008/09 mango fruiting season, the highest infestation levels by *R. iceryoides* was recorded on 03rd of January 2009 (mean temp. of 28.3°C, mean RH of 68.5%, and total rainfall of 0.2 mm) for the fruits (109.6 ± 45.60 mealybugs/fruit); while the infestation was highest on 11th of January 2009 (mean temp. of 27.9°C, mean RH of 66.6%, and no rainfall) for both twigs (148.55 ± 40.64 mealybugs/ twig) and leaves (82.55 ± 13.35 mealybug/leaf) (Figure 7.3). Thereafter, the infestation level continue to decline reaching the lowest level on 20th of February 2009 (mean temp. of 27.6°C, mean RH of 72.8%, and rainfall of 63.2 mm) on the twigs (10.25 ± 2.99 mealybugs/twig). Whereas, it was lowest on 08th March 2009 (mean temp. of 27.8°C, mean RH of 73.5%, and rainfall of 30.8 mm) on both leaves (10.89 ± 5.35 mealybugs/leaf) and fruits (4.6 ± 1.89 mealybugs/fruit) (Figure 7.3). During the 2008/09 fruiting season, *R. iceroides* infestation level varied significantly amongst the twigs, leaves and fruits ($\chi 2 = 36.63$; $df = 2$; $P < 0.0001$).

During the mango off-season, the highest infestation levels was recorded on 16th of March 2009 (mean temp. of 27.7°C, mean RH of 79.9%, and 36.7 mm of rainfall) and on 24th of March 2009 (mean temp. of 28.4°C, mean RH of 68.4%, and no rainfall), on the leaves (13.48 ±

5.41 mealybugs/leaf) and the twigs (16.05 ± 6.55 mealybugs/twig), respectively. On the other hand the lowest infestation was on 16th July 2009 (mean temp. of 25.0°C, RH of 74.4%, and rainfall of 16.5 mm) and 16th October, 2009 (mean temp. of 26.3°C, RH of 64.7%, and rainfall of 0.5 mm) for the twigs (3.65 ± 1.27 mealybugs/twig) and the leaves (5.24 ± 1.38 mealybugs/leaf), respectively. However, during this period there was no significant difference in the infestation levels amongst the different plant parts ($\chi2$ = 0.023; *df* = 1; *P* = 0.8802).

During the 2009/10 mango fruiting season, the highest infestation levels was recorded on 11th January 2010 (mean temp. of 27.4°C, mean RH of 81.5%, and 30.3 mm of rainfall) on the twigs (75.65 ± 21.22 mealybugs/twig). While it was highest on 27th of January 2010 (mean temp. 28.4°C, mean RH of 69.7%, and no rainfall) on the leaves (86.2 ± 14.05 mealybugs/leaf) and the fruits (82.6 ± 30.77 mealybugs/fruit). The infestation reached its minimum on 11th of December 2009 (mean temp. of 28.3°C, mean RH of 69.1, and total rainfall of 98.2 mm) on twigs (7.75 ± 3.61 mealybugs/twig) and the leaves (4.66 ± 2.04 mealybugs/leaf), and on 28th of February 2010 (mean temp. of 29.2°C, mean RH of 69.9%, and 6.1 mm of rainfall) on the fruits (5.2 ± 2.63 mealybugs/fruit) (Figure 3). There was a significant difference in the infestation levels amongst the twigs, leaves and fruits ($\chi2$ = 11.89; *df* = 2; *P* = 0.0026).

During the mango off-season of 2010, the highest infestation levels was recorded on 08th of March 2010 (mean temp. of 29.1%, mean RH of 78.4%, and 61.7 mm of rainfall) on twigs (18.1 ± 5.41 mealybugs/twig) and the leaves (21.24 ± 7.79 mealybugs/leaf). Thereafter, there was a gradual decline of the infestation level with the minimum being recorded on 16th of June 2010 (mean temp. of 25.8°C, mean RH of 76.5%, and 63.8 mm of rainfall) for both twigs (1.55 ± 0.95 mealybugs/twig) and leaves (3.48 ± 1.73 mealybugs/leaf). However, there was a significant difference on the infestation levels observed on the twigs and leaves during this period ($\chi2$ = 7.57; *df* = 1; *P* = 0.0059).

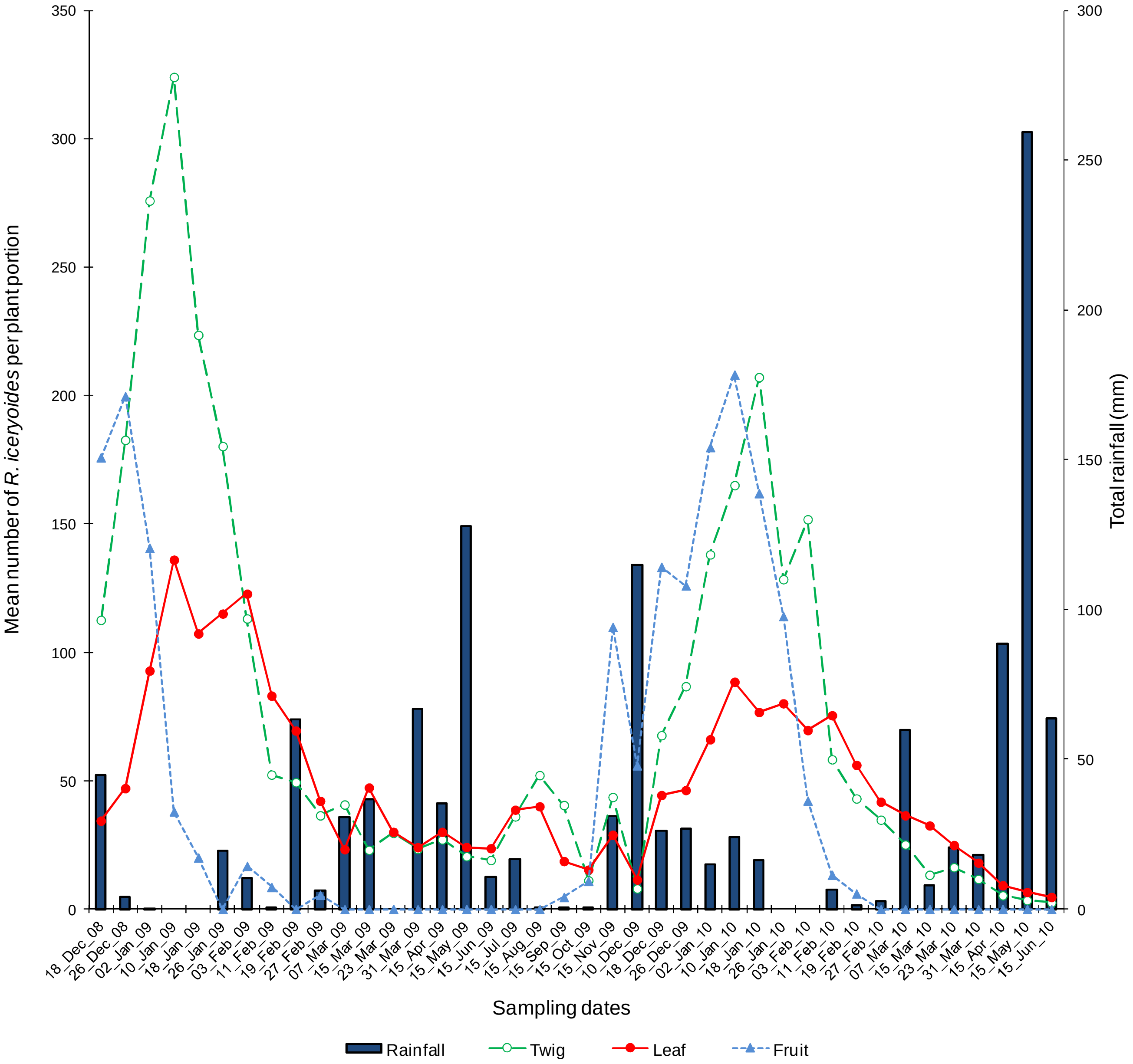


Figure 7. 2: Seasonal fluctuation of *R. iceryoides* on the twigs, leaves and fruit with corresponding rainfall (mm) from December 2008 to June 2010 in Kibaha

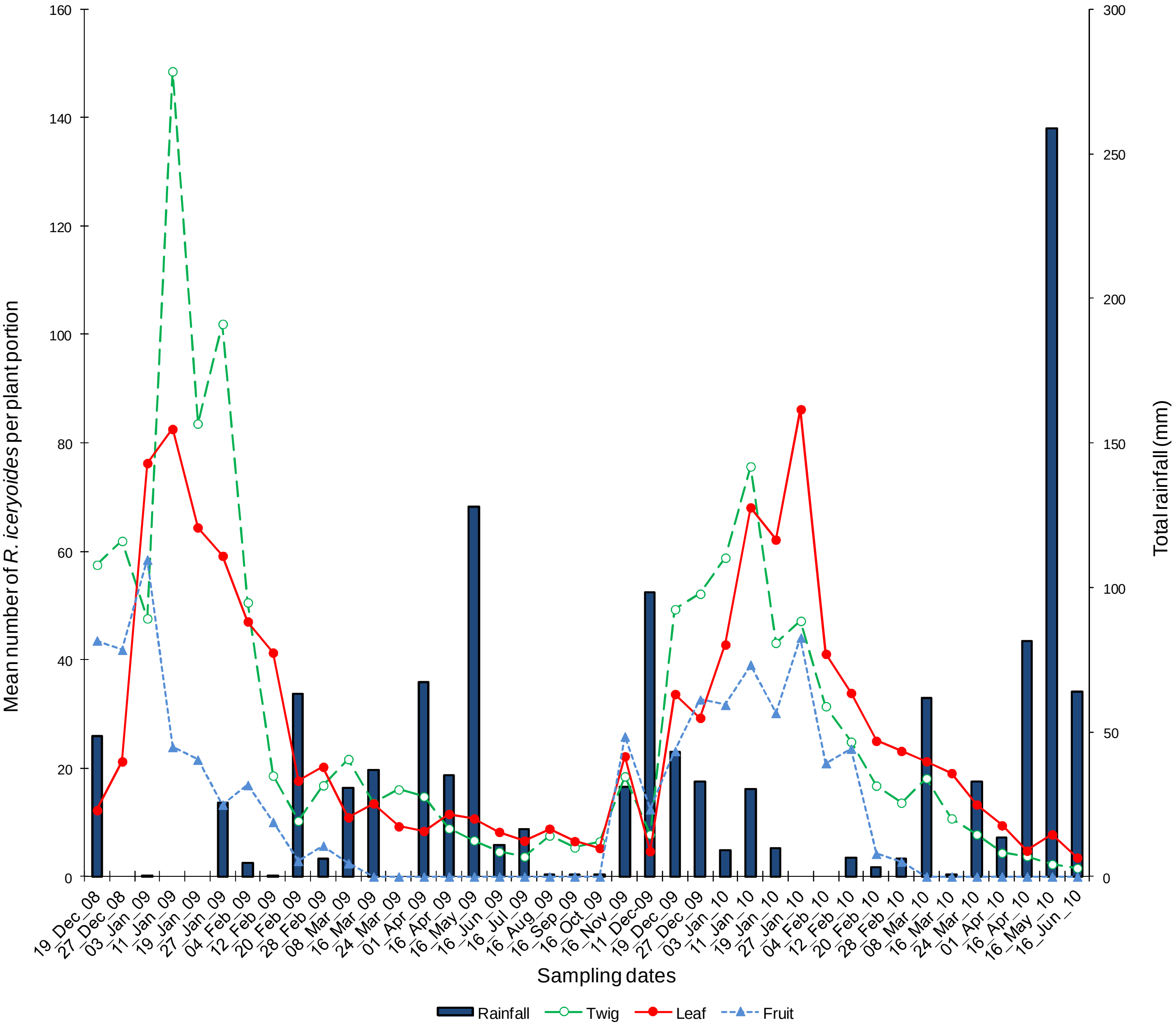


Figure 7. 3: Seasonal fluctuation of *R. iceryoides* on the twigs, leaves and fruit with corresponding rainfall (mm) from December 2008 to June 2010 in Dar es Salaam

7.3.4 Seasonal fluctuation of percentage parasitism of *R. iceryoides* in Kibaha

A total of 3421, 10357 and 261 mummified mealybugs, were recorded from the twigs, leaves and fruits, respectively, with respective mean percentage parasitism of 5.54 ± 0.18, 6.52 ± 0.12 and 3.44 ± 0.41%. The seasonal fluctuation trend in percentage parasitism did not follow that of the host (Figure 7.4). Percent parasitism was quite low at the beginning of the season with least percent parasitism being recorded on18th of December 2008 (mean temp. of 27.6°C, mean RH of 73.8%, and rainfall of 44.6 mm), for twigs (0.75±0.28%) and leaves (0.61± 0.18). While the least percent parasitism on the fruit (0.0 %) was recorded on 26th of January 2009 (mean temp. of 28.2°C, mean RH of 72.3%, and rain of 19.5 mm), and 19th of February, 2009 (mean temp. of 27.7 °C, mean RH of 70.2%, and rain of 63.2 mm). As the season advanced the percent parasitism increased slightly to reached its maximum on 18th of January 2009 (mean temp. of 28.4°C, mean RH of 66.9%, and zero rainfall), 3rd of February 2009 (mean temp. of 28.4°C, mean RH of 77.9%, and rainfall of 0.4 mm), and 11th of February 2009 (mean temp. of 26.6°C, mean RH of 70.19%, and rainfall of 63.2 mm), for the fruit (6.2±3.3%), twigs (4.4±1.3%) and leaves (3.1±1.0%) respectively. The percentage parasitism varied greatly ($\chi 2$ = 33.58; *df* = 2; *P* < 0.0001) amongst the different plant parts.

During the mango off-season period of 2009, the lowest percentage parasitism was recorded on 7th of March 2009 (mean temp of 27.8°C, RH of 71.8%, and rainfall of 30.8 mm), and 15th July 2009 (mean temp of 24.9°C, RH of 75.1%, and rainfall of 16.5 mm) for the leaves (0.31 ± 0.14%) and the twigs (0.67 ± 0.4), respectively. While the highest percent parasitism was recorded on 15th March, 2009 (mean temp. 27.7°C of, RH of 80.9%, and rainfall of 36.7 mm) and 15th July 2009, for twigs (2.49 ± 1.09%) and the leaves (1.95 ± 0.48%), respectively. However, during the mango off season there was no significant difference in the percentage parasitism between the twigs and leaves ($\chi 2$ = 0.36; *df* = 1; *P* = 0.5485).

During the 2009/2010 mango fruiting season, the lowest percent parasitism was recorded on 15th September, 2009 (mean temp. of 25.0°C, RH of 65.56%, and rainfall of 0.5 mm) and 26th December 2009 (mean temp. of 28.2°C, RH of 72.7%, and rainfall of 26.9 mm) for leaves (0.75± 0.26%) and twigs (0.89 ± 0.4%), respectively. However on the fruit no mummified *R. iceryoides* was recorded on 15th September, 2009 as well as on 19th February, 2010 (mean temp. of 29.3°C, RH of 70.3%, and rainfall of 1.2 mm). With the advancement of the mango season the percent parasitism increased to reach its maximum on 26th January, 2010 (mean temp. of 28.8°C, RH of 69.5%, and zero rainfall) for the fruits (4.39 ± 1.56%) and on 03rd February, 2010 (mean temp. of 28.7°C, RH of 68.9%, and zero rainfall), for both the twigs (4.52 ± 1.59%) and the leaves (4.57 ± 0.98%). . There was a significant difference in the percentage parasitism amongst the twigs, leaves and fruits ($\chi 2$ = 17.81; *df* = 2; *P* = 0.0001).

During the mango off-season 2010, the highest percent parasitism was recorded on 07th of March, 2010 (mean temp. of 29.4°C, RH of 79.4%, and rainfall of 59.9 mm) and 15th of March, 2010 (mean temp. of 28.5°C, RH of 67.4%, and rainfall of 8 mm) for twigs (5.64 ± 2.4), and leaves (1.99 ± 0.51), respectively. Thereafter, the percent parasitism started to decline to reach its minimum on 15th of June 2010 (mean temp. of 25.5°C, RH of 76.6%, and rainfall of 63.8 mm) for both twigs (0.2 ± 0.2%) and the leaves (0.45± 0.19%). Unlike that of the mango fruiting season, percent parasitism during this season was not influenced by the plant part from which the mummies were collected ($\chi 2$ =0.39; *df* = 1; *P* = 0.5316).

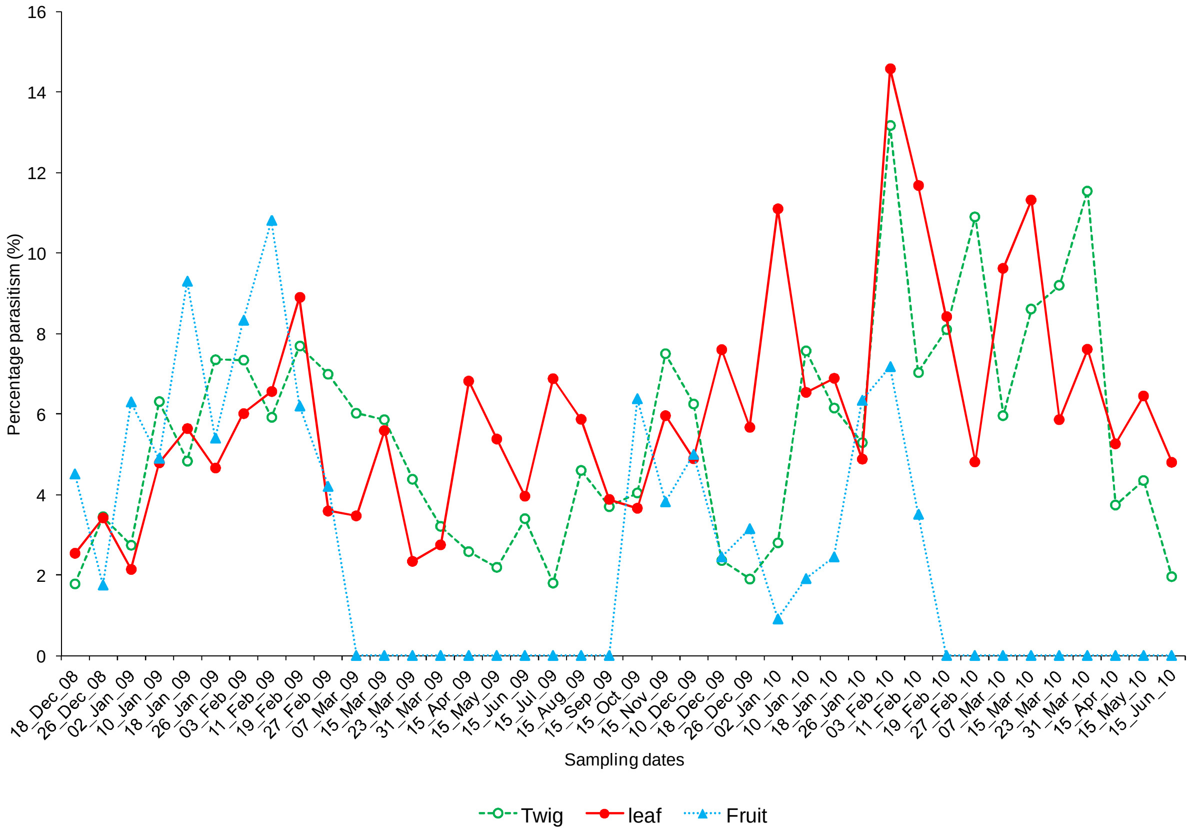


Figure 7. 4: Seasonal variation of percentage parasitism of *R. iceryoides* on twigs, leaves and fruits in Kibaha from December 2008 to June 2010.

7.3.5 Seasonal fluctuation of percentage parasitism of *R. iceryoides* in Dar es Salaam

A total of 1537, 6239 and 244 mummified mealybugs, were recorded on the twigs, leaves and fruits, respectively, with respective mean percentage parasitism of 6.44 ± 0.31, 7.14 ± 0.17 and 4.90 ± 0.6%. Like that in Kibaha, the seasonal fluctuation trend in percentage parasitism did not follow that of the host (Figure 7.5). Percent parasitism was very low at the beginning of the mango fruiting season with least percent parasitism being recorded on19th of December 2008 (mean temp of 27.6°C, mean RH of 73.9%, and rainfall of 48.6 mm), for leaves (1.13 ± 0.4%). While on the 27th December 2008 (mean temp of 27.9°C, mean RH of 67.8%, and zero rainfall), it was lowest on both twigs (1.16 ± 0.47%) and fruits (0.85 ± 0.69%). The percent parasitism increased, thereafter to record its maximum on 12th February 2009 (mean temp of 28.5 °C, mean RH of 67.9%, and rainfall of 0.4 mm), on 28th February 2009 (mean temp of 27.6 °C, mean RH of 74.1%, and rainfall of 6.1mm), and 08th March, 2009 (mean temp of 27.8 °C, mean RH of 73.5%, and rainfall of 30.8 mm) on fruits (5.78 ± 3.44%), leaves (3.91 ± 0.99%) and twigs (6.71 ± 2.24%), respectively. There was no significant difference in the percentage parasitism amongst the twigs, leaves and fruits ($\chi 2 = 4.90$; $df = 2$; $P = 0.0862$).

During the mango off-season of 2009, the highest percentage parasitism was recorded on 16th of March, 2009 (mean temp of 27.7°C, mean RH of 79.9%, and rainfall of 36.7mm), and 24th March 2009 (mean temp of 28.4°C, mean RH of 68.4%, and zero rainfall) for the twigs (4.99 ± 2.04%) and the leaves (2.24 ± 0.59%), respectively. While the lowest percent parasitism was recorded on 16th June 2009 (mean temp of 25.9°C, mean RH of 74.9% , and rainfall of 10.8 mm) and 16th July 2009 (mean temp of 25.0°C, mean RH of 74.4% and rainfall of 16.5 mm), for the twigs (0.58 ± 0.41%) and the leaves (0.60 ± 0.24%), respectively. There was no significant difference in the percentage parasitism recorded on the twigs and leaves ($\chi 2 = 0.09$; $df = 1$; $P = 0.7630$).

During the 2009/2010 mango fruiting season, the lowest percentage parasitism of *R. iceryoides* was recorded on 11th December 2009 (mean temp of 28.3°C, mean RH of 69.1% and rainfall of 98.2 mm), 19th December 2009 (mean temp of 28.5°C, mean RH of 75.1% and rainfall of 43.0 mm), and 27th December 2009 (mean temp of 28.0°C, mean RH of 73.8%, and rainfall of 32.8 mm) for leaves (0.37 ± 0.18%), fruits (0.84 ± 0.53%), and twigs (1.30 ± 0.59%), respectively. Thereafter, the percent parasitism increased with the maximum recorded on 27th January 2010 (mean temp of 24.2°C, mean RH of 69.7%, and rainfall of 28.4mm), 04th February 2010 (mean temp of 25.1°C, mean RH of 68.8%, and rainfall of 28.9 mm) and 20th of February 2010 (mean temp of 24.8°C, mean RH of 71.2%, and rainfall of 29.2 mm) on leaves (3.81 ± 0.63%), fruits (5.05 ± 2.28%), and twigs (5.08 ± 2.17%), respectively. During this period the parasitism was comparable amongst the different plant parts ($\chi 2 = 0.53$; $df = 2$; $P = 0.7665$).

During the mango off-season of 2009/10, the highest percentage parasitism of *R. iceryoides* was recorded on 16th March 2010 (mean temp of 29.5°C, mean RH of 66.8%, and rainfall of 0.7 mm), and 24th March 2010 (mean temp of 28.1°C, mean RH of 80.2%, and rainfall of 32.9 mm) for twigs (3.64 ± 1.72%) and leaves (1.30 ± 0.43%), respectively. Thereafter the percent parasitism declined with a minimum recorded on the twigs (0.60 ± 0.45%) and leaves (0.19 ± 0.1%) on 16th May 2010 (mean temp of 26.6°C, mean RH of 83.9%, and rainfall of 258.7 mm) and 16th June 2010 (mean temp of 25.8°C, mean RH of 76.5% and rainfall of 63.8 mm), respectively.

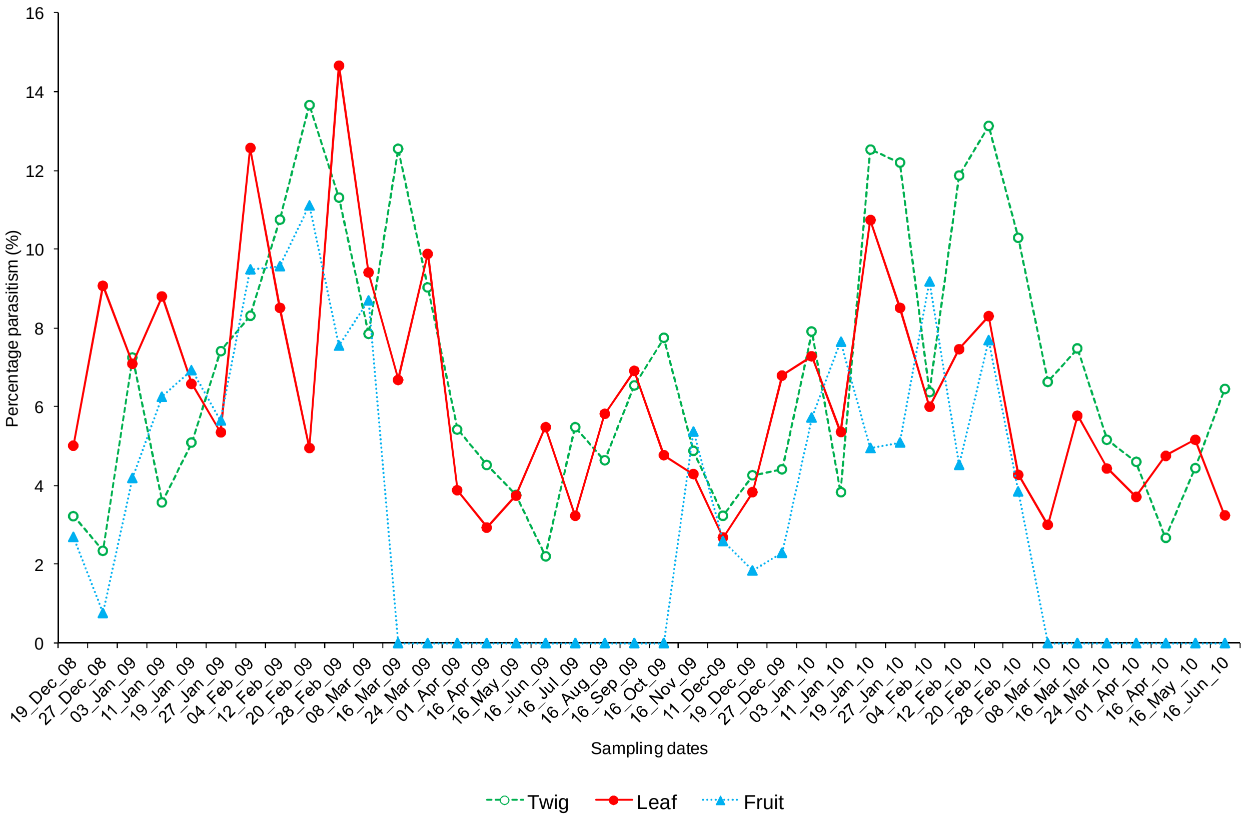


Figure 7. 5: Seasonal variation of percentage parasitism of *R. iceryoides* on twigs, leaves and fruits in Dar es Salaam from December 2008 to June 2010.

7.3.6 Seasonal population fluctuation of primary parasitoid species

In Kibaha, out of 14039 mummified mealybugs collected from the twigs, leaves and fruits during the study period, 7960 wasps emerged. Six primary parasitoid species were identified; accounting for 76.31% of total emergence. All the primary hymenopteran parasitoids were members of the family Encyrtidae. These were namely, *Anagyrus pseudococci* (Girault) accounting for 90.75%, followed by *Leptomastrix dactylopii* Howard (4.61%), *Anagyrus aegyptiacus* Moursi (1.84%), *Leptomastidea tecta* Prinsloo (1.14%), *Agarwalencyrtus citri* Agarwal (1.05%) and *Aenasius longiscapus* Compere (0.61%) (Figure 7. 6). The combined percentage parasitism by these parasitoids ranged from 0.13% on the 15th of June 2009 to 5.2% on the 07th of March 2009. There was a strong and positive association between the parasitoid population and that of their host, *R. iceryoides* on all plant parts ($r = 0.91$, $P < 0.0001$; $r = 0.87$, $P < 0.0001$; $r = 0.94$, $P < 0.0001$ for twigs, leaves, and fruits, respectively).

The highest number of the dominant parasitoid, *A. pseudococci* recovered from the collected mummies were 463 and 406 wasps for 2008/09 (on 10th January 2009, mean temp. of 27.9°C, mean RH of 66% and rainfall of 0 mm) and 2009/10 (on 10th January 2010, mean temp. of 27.7°C, mean RH of 82% and total rainfall of 23.9 mm) mango fruiting seasons, respectively (Figure 7. 6).

In Dar es Salaam, out of 8020 mummified mealybugs collected from the twigs, leaves and fruits during the study period, 3569 wasps emerged. The primary parasitoids accounted for 80.3% of the total emergence, while the remaining 19.7% were hyperparasitoids. The primary parasitoid species were *A. pseudococci* (94.04%), *L. dactylopii* (3.91%), *A. aegyptiacus* (1.19%), *L. tecta* (0.66%) and *A. longiscapus* (0.2%). The percentage parasitism throughout the entire study ranged from 0.47% on the 11th of December 2009 to 6.7% on the 19th of December 2008 (Figure 7.7). The population of the parasitoid was significantly and positively correlated with that of its host, *R. iceryoides* on all plant parts ($r = 0.89$, $P < 0.0001$; $r = 0.95$, $P < 0.0001$; $r = 0.94$, $P < 0.0001$ for twigs, leaves, and fruits, respectively).

The highest number of the dominant parasitoid, *A. pseudococci* was collected on the 19th of January 2009 (n = 274) at mean temperature of 28.5°C, mean RH of 67.01% and no rainfall; and on the 11th of January 2010 (n = 269) at mean temperature of 27.4°C, mean RH of 81.5% and total rainfall of 30.3 mm (Figure 7. 7).

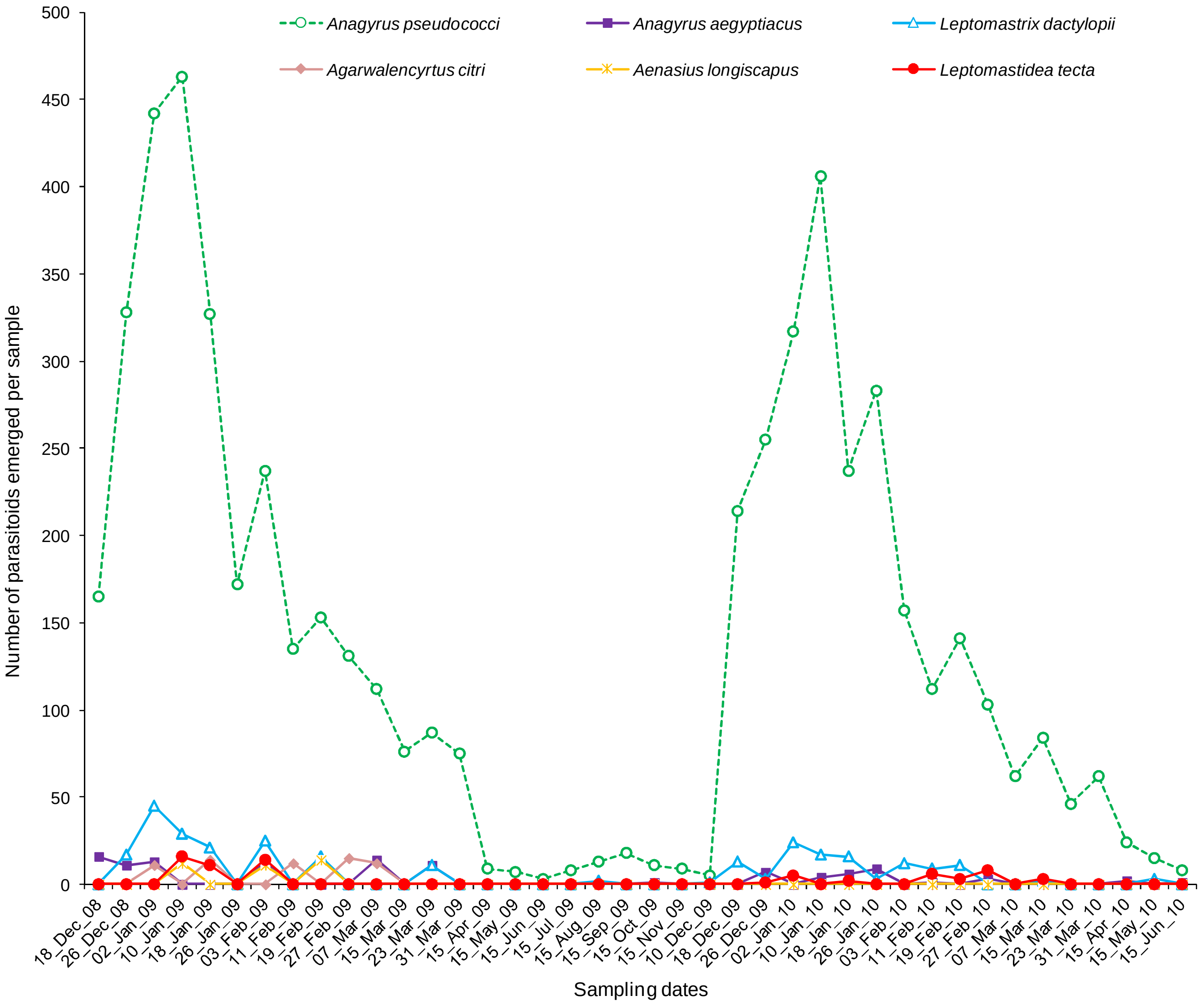


Figure 7. 6: Seasonal population variation of the primary parasitoid species from December 2008 to June 2010 in Kibaha.

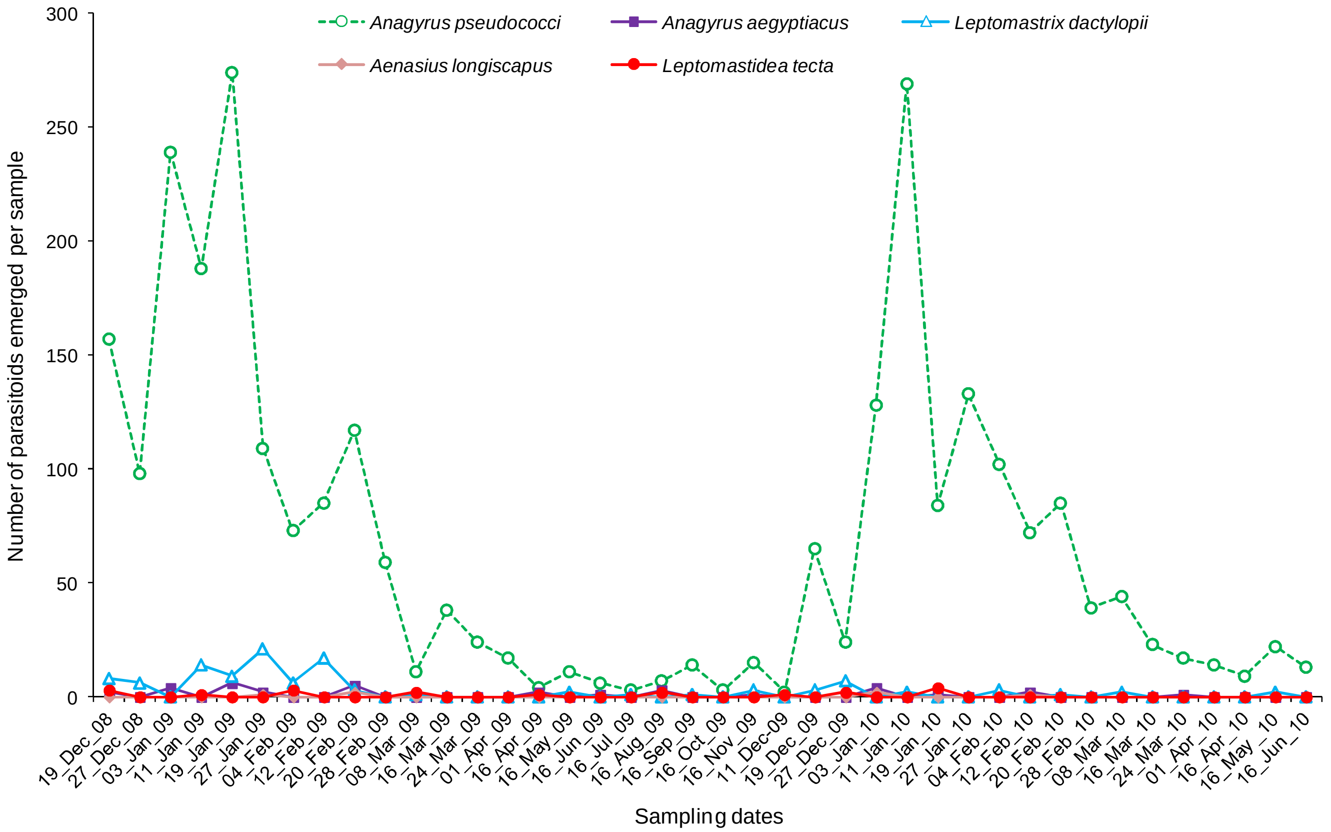


Figure 7. 7: Seasonal population variation of the primary parasitoid species from December 2008 to June 2010 in Dar es Salaam.

7.3.7 Seasonal population fluctuation of different predator species

In Kibaha, a total of nineteen species of predators were found preying on *R. iceryoides* throughout the study period. The major predators were six species of Coccinelidae: *Cryptolaemus montrouzieri* Mulsant (1.42%), *Hyperaspis amurensis* Weise (1.27%), *Hyperaspis bigeminata* Randall (12.11%), *Exochomus nigromaculatus* Goeze (3.47%), *Chilocorus renipustulatus* Scriba (15.72%), *Chilocorus nigrita* Fabricius (31.23%); one Lycaenidae (*Spalgis lemolea* Druce) (9.07%); one Drosophilidae (*Cacoxenus perspicax* Knab) (20.89%). Other minor predators included *Pyroderces badia* Hodges (Lepidoptera: Cosmopterigidae), *Hemerobius* sp. (Neuroptera: Hemerobiidae), *Cheiracanthium virescens* Sundevall (Arachnida: Clubionidae) and the rest were other coccinelids: *Rodolia limbata* Motschulsky, *Rodolia pumila* Weise, *Micraspis* sp., *Propylea dissecta* Mulsant, *Propylea 14-punctata* Schachbrett-Marienkäfer, *Telsimia nitida* Chapin, *Harmonia dimidiata* Fabricius and *Hyperaspis* sp. The population of the major predators was found to increase with the population build-up of *R. iceryoides*. The highest level of predator activity was recorded between December and February for each year (Figure 7. 8).

In Dar es Salaam, a total of 11 species of predators were recorded. In the order of their importance they were *C. nigrita* (30.32%), *H. bigeminata* (18.47%), *C. renipustulatus* (15.65%), *C. perspicax* (15.08%), *E. nigromaculatus*, *C. montrouzieri*, *H. amurensis*, *R. pumila*, *S. lemolea*, *Hyperaspis* sp. and *R. limbata*. Like in Kibaha, the population of the major predators increased in a density dependant manner together with that of their prey, *R. iceryoides*. The highest level of activities by the predators was observed between December and February for both years (Figure 7. 9).

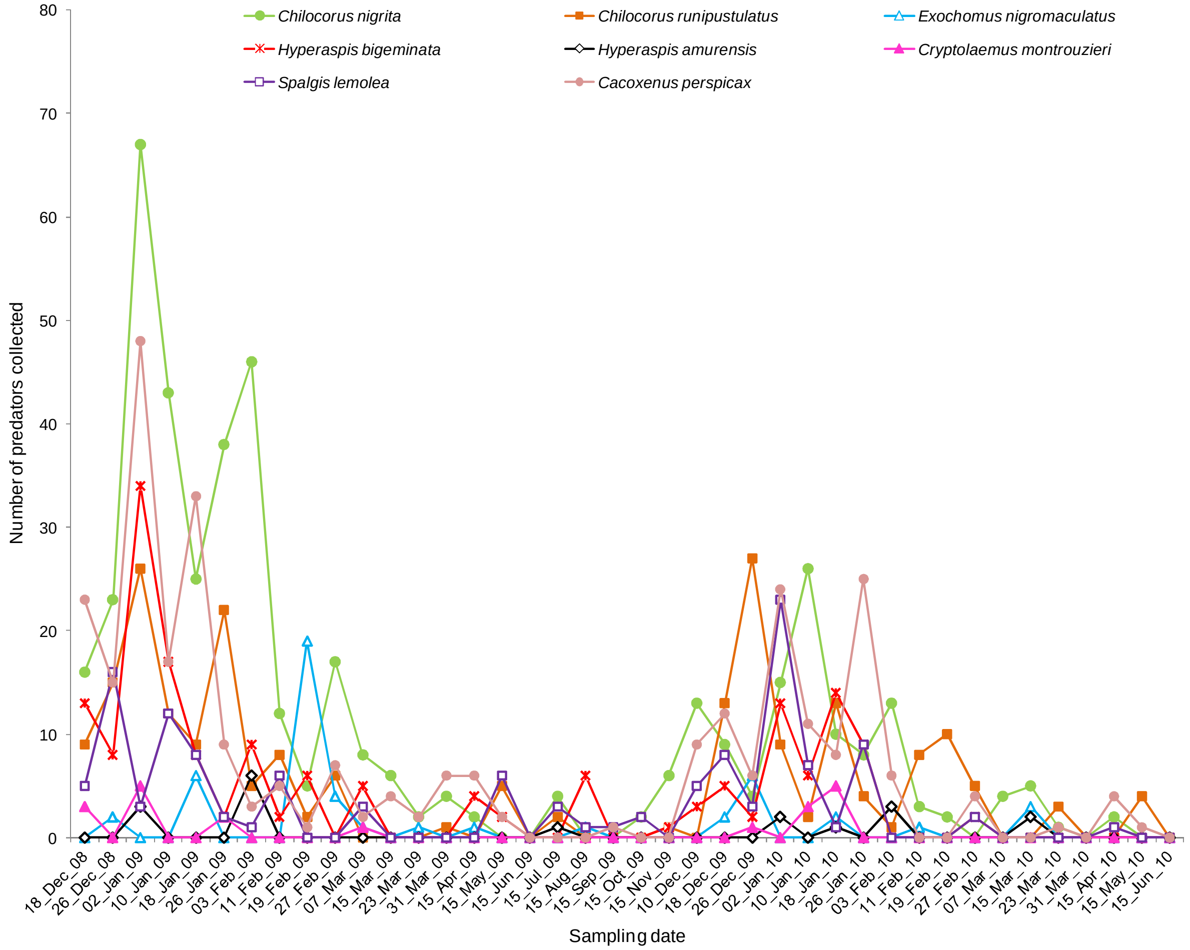


Figure 7. 8: Seasonal population variation of predator species on mango from December 2008 to June 2010 in Kibaha.

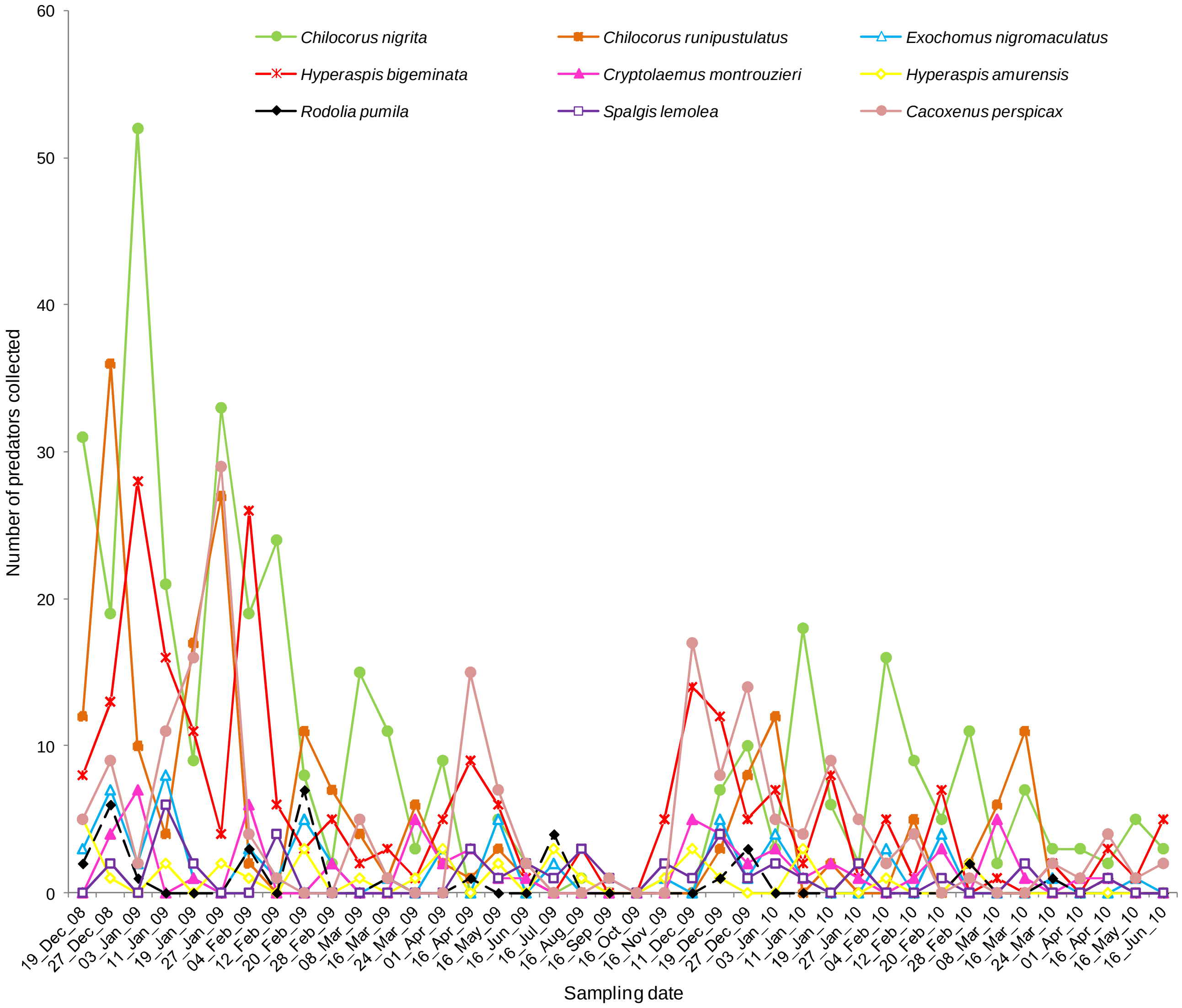


Figure 7. 9: Seasonal population variation of predator species on mango from December 2008 to June 2010 in Dar es Salaam.

7.3.8 Seasonal fluctuation of hyperparasitoid species population

In Kibaha, a total of 14 species of hyperparasitoids were found parasitizing the primary parasitoids of *R. iceryoides*. These hyperparasitoids belong to four families, namely Encrytidae (*Achrysopophagus aegyptiacus* Mercet, *Cheiloneurus carinatus* sp.nov, *Cheiloneurus angustifrons* sp.nov, *Cheiloneurus cyanonotus* Waterston and *Cheiloneurus latiscapus* Girault), Aphelinidae (*Coccophagus gilvus* Hayat, *Coccophagus pseudococci* Compere, *Coccophagus* *bivittatus* Compere, *Marietta leopardina* Motschulsky, *Coccophagus lycimnia* Walker and *Coccophagus nigricorpus* Shafee), Signiphoridae (*Chartocerus conjugalis* Mercet and *Chartocerus* sp.) and Eulophidae (*Tetrastichus* sp.). The most abundant hyperparasitoids were *C. conjugalis* (8.72%), *C. carinatus* (4.37%), *M. leopardina* (2.21%), *C. cyanonotus* (1.37%) and *C. latiscapus* (1.32%). The remaining nine hyperparasitoid species accounted for only 6% of the total wasps recovered. The highest number (n = 64) of the key hyperparasitoid, *C. conjugalis* was recorded on the 10th of January 2009 (mean temp. of 27.9°C, mean RH of 66%, and no rainfall) (Figure 7. 10). The number of the hyperparasitoid was positively correlated with that of the primary parasitoid ($r = 0.8343$; $P < 0.0001$).

In the Dar es Salaam study site, a total of 12 species of hyperparasitoids were recovered from the *R. iceryoides* mummies. These were from three families, Encrytidae (*A. aegyptiacus*, *C. carinatus*, *C. angustifrons*, *C. cyanonotus* and *C. latiscapus*); Aphelinidae (*C. gilvus*, *C. pseudococci*, *C. bivittatus*, *M. leopardina*, *C. lycimnia* and *C. nigricorpus*) and Signiphoridae (*C. conjugalis*). The most abundant hyperparasitoids were *C. conjugalis* (9.39%), followed by *C. carinatus* (2.66%), *M. leopardina* (2.63%), *C. pseudococci* (0.92%) and *C. angustifrons* (1.01%). The remaining seven hyperparasitoid species accounted for 3% of the total wasps recovered. The highest number of the dominant species, *C. conjugalis* (n = 46) was recorded on the 03rd of January 2009 (mean temp.of 28.3°C, mean RH of 68.5% and total rainfall of 0.2 mm (Figure 7. 11). Similar to the Kibaha scenario, the number of the hyperparasitoids were found to positively correlated with that of the primary parasitoid ($r = 0.6654$; $P < 0.0001$).

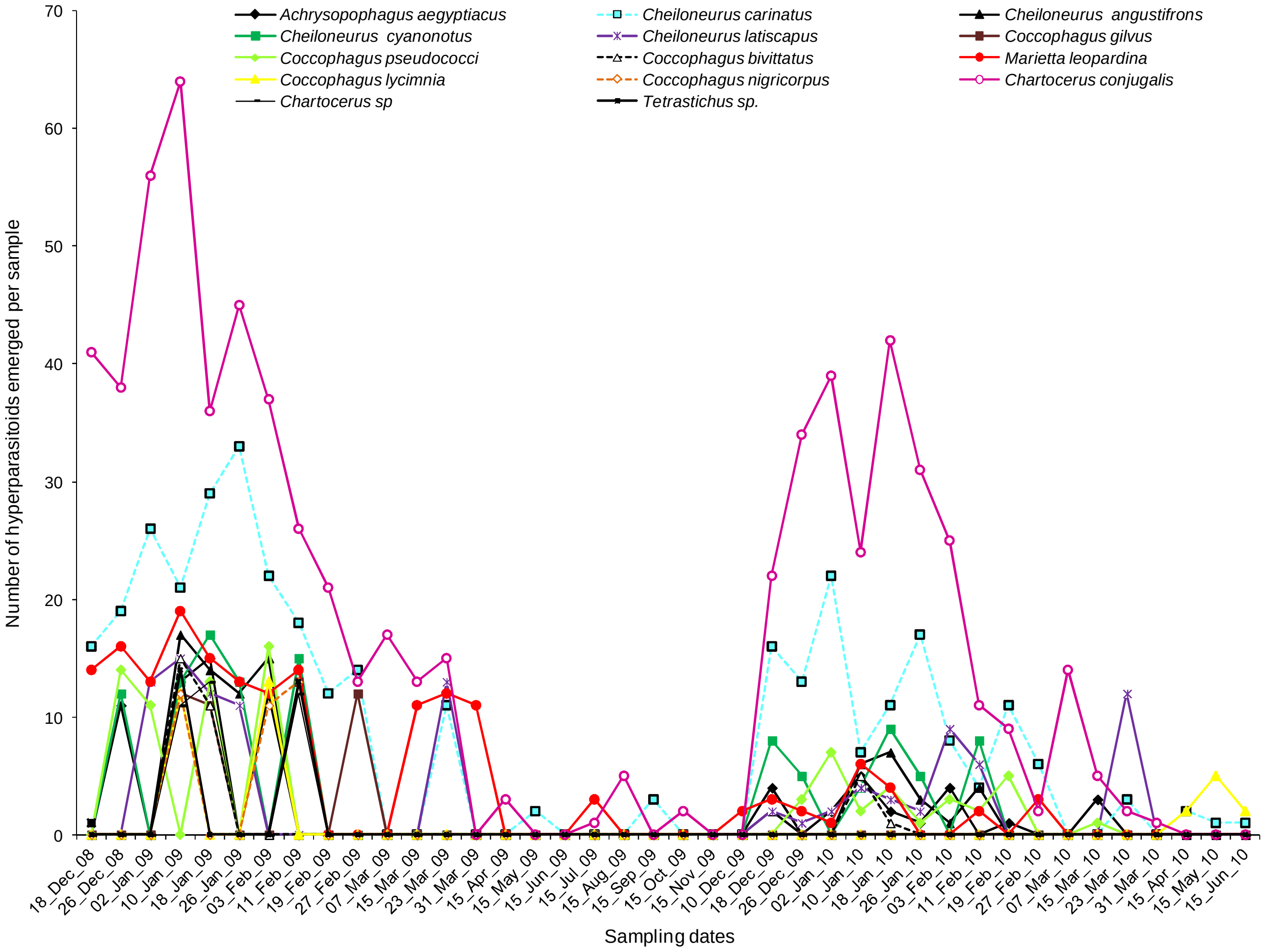


Figure 7. 10: Relative abundance of hyperparasitoids during December 2008 to June 2010 survey in Kibaha.

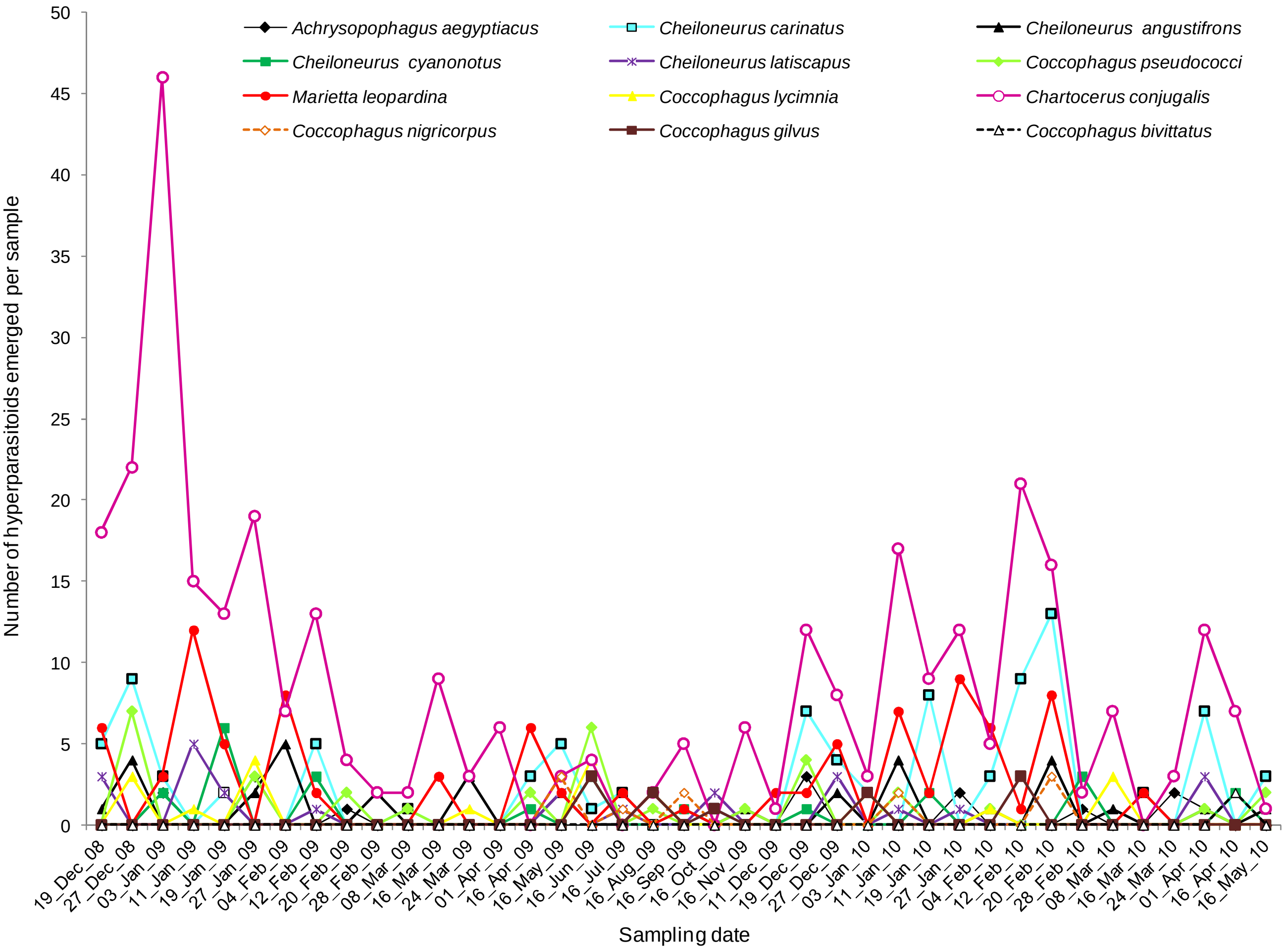


Figure 7. 11: Relative abundance of hyperparasitoids during December 2008 to June 2010 survey in Dar es Salaam.

7.3.10 Effect of weather variables on the infestation level and the number of mummified mealybugs on the different plant parts in Kibaha

The population dynamics of *R. iceryoides* on twigs and leaves was significantly and positively affected by the mean temperature (*Wald* = 4.21; *P* = 0.0402 and *Wald* = 15.33; *P* < 0.0001, for twigs and leaves, respectively). However, on the fruit the mealybug population dynamics was not affected by the temperature (*Wald* = 0.02; *P* = 0.8816). Similarly, rainfall had significant but negative effect on the population dynamics of *R. iceryoides* on twigs (*Wald* = 11.99; *P* = 0.0005) and leaves (*Wald* = 9.32; *P* = 0.0023), whereas the population dynamics on the fruit was not influenced by rainfall (*Wald* = 0.16; *P* = 0.6913). On the other hand mean relative humidity had no effect on population dynamics of the pest on the twigs (Wald = 0.01; P = 0.9218) and the leaves (*Wald* = 0.11; *P* = 0.7429). While, the pest population on the fruit was significantly and positively affected by the mean relative humidity (*Wald* = 11.33; *P* = 0.0008).

Like that of the host, the population dynamics of the parasitoids was significantly and positively influenced by the temperature on both twig (*Wald* = 9.99; *P* = 0.0016) and leaves (*Wald* = 11.02; *P* = 0.0009), while temperature had no effect on parasitoid population dynamics on fruit (*Wald* = 1.12; *P* = 0.2900). Likewise, rainfall had significant but negative effect on the population dynamics on the parasitoid on twigs (*Wald* = 11.57; *P* = 0.0007) and leaves (*Wald* = 4.48; *P* = 0.0342). However, the population dynamics of the parasitoid on the fruit was not influenced by rainfall (*Wald* = 0.07; *P* = 0.7900). On the other had mean relative humidity had no effect on population dynamics of the parasitoids on all plant part assessed (*Wald* = 0.07; *P* = 0.7893, *Wald* = 1.03; *P* = 0.3091 and *Wald* = 1.67; *P* = 0.2000 for twigs, leaves and fruit, respectively).

7.3.11 Effect of weather variables on the infestation level and the number of mummified mealybugs on the different plant parts in Dar es Salaam

Similar to that in Kibaha, the population dynamics of *R. iceryoides* on twigs and leaves was significantly and positively affected by the mean temperature (*Wald* = 4.81; *P* = 0.0280 and *Wald* = 10.31; *P* = 0.0013, for twigs and leaves, respectively). However, on the fruit the mealybug population dynamics was not affected by the temperature (*Wald* = 3.68; *P* = 0.0550). Also rainfall had significant but negative effect on the population dynamics of *R. iceryoides* on twigs (*Wald* = 5.51; *P* = 0.0190) and leaves (*Wald* = 5.51; *P* = 0.0189), while, the population dynamics on the fruit was not influenced by rainfall (*Wald* = 2.46; *P* = 0.1170). Mean relative humidity had no effect on population dynamics of the pest on the twigs, leaves and fruit (*Wald* = 0.01; *P* = 0.9210, *Wald* = 0.10; *P* = 0.7490, and *Wald* = 0.01; *P* = 0.9370, respectively).

The population dynamics of the parasitoids, like that of its host was also significantly and positively affected by the temperature on twig (*Wald* = 8.95; *P* = 0.0028) and leaves (*Wald* = 5.67; *P* = 0.0173), while temperature had no effect on parasitoid population dynamics on fruit (*Wald* = 1.19; *P* = 0.2762). Rainfall had a significant but negative effect on the population dynamics of the parasitoid on the twigs (*Wald* = 8.53; *P* = 0.0035), leaves (*Wald* = 8.49; *P* =

0.0035), and fruits (*Wald* = 10.14; *P* = 0.0015). Similar to that in Kibaha, relative humidity had no effect on population dynamics of the parasitoids on all plant part assessed (*Wald* = 0.75; *P* = 0.3851, *Wald* = 0.49; *P* = 0.4843 and *Wald* = 0.88; *P* = 0.3475, for twigs, leaves and fruits, respectively).

7.4 Discussion

The results of this study demonstrated that there was a clear and distinct seasonal pattern in population dynamics of the mango mealybug at the coastal area of Tanzania. The seasonal and annual fluctuations of *R. iceryoides* were very closely associated with the mango fruiting season. The population of *R. iceryoides* was found to be very low at the beginning of the mango season. Subsequently, the population built up as the mango season advanced, reaching its peak towards the mango harvesting season, which coincided with the dry season (northeast monsoon). Thereafter, the population declined to its lowest during the long rains (March to May), and cold and dry southwest monsoon (June-October). Boavida and Neuenschwander (1995) also reported a similar population dynamics pattern in relation to the rainfall for the related mealybug *R. invadens* in Benin. Further, the cassava mealybug, *Phenacoccus manihoti* Matile-Ferrero (Homoptera: Pseudococcidae), was also found to increase ten-fold during the dry season compared to the rainy season (Le Rü et al., 1991). Calatayud et al. (1994), attributed the increase of *P. manihoti* populations during the dry season to changes in the levels of secondary compounds which enhanced the plant resistance and that had a positive influence on the population dynamics of the pest. This argument may also explain the high mango infestation with *R. iceryoides* during the dry season, although, no chemical analysis was carried out to confirm this. However, in general, water stressed plants were more susceptible to mealybug infestations (Fabres and Le Rü, 1988; Gutierrez et al., 1993; Koricheva et al., 1998; Lunderstadt, 1998; Calatayud et al., 2000; Calatayud et al., 2002; Shrewsbury et al., 2004).

This study revealed that rainfall was significantly and negatively correlated, while temperature was significantly and positively correlated with *R. iceryoides* populations as well as its associated natural enemies on the various plant parts. Similar trends of association between these weather factors and *R. iceryoides* populations were reported by Suresh and Kavitha (2008). The authors found that relatively high temperatures and lack of rainfall were associated with an increase in mealybug populations. They also reported that for every unit increase in relative humidity and rainfall, there was a 0.05 unit population reduction in the *R. iceryoides* population on mango, in India. On the other hand, every unit of sunshine hours increased the population by 3.93 units. Heavy rains were observed to wash mealybugs off the mango plants down to the soil surface and led to considerable mealybug mortality; being one of the principal causes of the sharp decline of *R. iceryoides* population in that study. Similar findings were reported for other mealybug species such *Maconellicoccus hirsutus* (Green) (Mukherjee, 1919; Sriharan et al., 1979; Shree and Boraiah, 1988) and *Phenacoccus solenopsis* Tinsley (Suresh and Kavitha, 2008, Dhawan et al., 2009). In this study, besides rainfall, other factors such as availability of suitable host plants, and the action of natural enemies may have contributed to the population fluctuations of *R. iceryoides*. These factors were reported to have strong influences on the population dynamics of the congeneric pest, *R. invadens* (Pitan, 2000; Boavida and Neuenschwander, 1995). Besides its influence on the population dynamics, through washing down of the adults and crawlers, rainfall also promoted mango vegetative growth, which promoted new colonization sites for subsequent mealybug generations (Singh, 1968; Whiley, 1993; Boavida and Neuenschwander, 1995). For example, Boavida and Neuenschwander (1995) found that during flushing, large populations of young mealybug females, moved along different branches from older leaves or shoots to newer ones. This dispersal behaviour of mealybugs may explain the distinct mealybug population dynamics on the different plant parts (i.e., twigs, leaves and fruits). The highest mealybug infestation levels on the twigs and leaves was recorded on the 10th of January 2009 at a mean temperature of 27.97°C, mean relative humidity of 66% and no rainfall. This temperature has been reported to be within the optimal range for rapid proliferation of the population of the mealybug, *Paracoccus marginatus* Williams and Granara de Willink (Hemiptera: Pseudococcidae) (Chong et al., 2008). In an earlier study the author found that *P. marginatus* had the highest fecundity and longest life span at a temperature of 28.7°C and relative humidity of 65 ± 2% (Chong et al., 2003).

Parasitoid and predator populations were well synchronized with that of the host/prey, *R. iceryoides*. Despite the fact that these natural enemies seemed to be working in a density dependant manner in relation to the population of the pest, they played a minimal role in the seasonal dynamics of *R. iceryoides*. The individual and combined action of the parasitoid as well as that of predators were found to be very low on all plant parts in both Kibaha and Dar es Salam during the entire study period. This could be due to that fact that these parasitoid species, being indigenous to Africa, share no evolutionary history with that of the pest. On the other hand most predators encountered during the studies were generalists and therefore exerted very little pressure on the pest population; considering the fact that there were other sympatric mealybug species in the orchard (Tanga et al., unpuplished data), in addition to other pests.

Although several species of hyperparasitoids were recorded in this study, their abundance as well as percentage hyperparasitism was extremely low at both Kibaha and Dar es Salaam. Therefore, their impact on the population of the primary parasitoid was quite low. However, caution needs to be taken when introducing parasitoid species for biological control of the pest. Efforts are underway to introduce co-evolved parasitoids from the pest aboriginal home (India) but laboratory studies are needed to establish whether these hyperparasitoids might attack the introduced parasitoid species, and limit their efficiency in suppressing the pest. There is much evidence which showed that hyperparasitoids can influence the population dynamics and community structure of primary parasitoids enough to disrupt biological control of the host (Holler et al., 1993, Morris et al., 2001; Van Nouhuys and Hanski, 2000; Van Veen et al., 2001).

These results provide valuable information of the seasonal dynamics of the invasive pest *R. iceryoides*, and permits forecasting of possible outbreaks based on the prevailing weather conditions. Based on the light of the results of this study, it can also be concluded that the population of the indigenous parasitoids were not able to reach to a level that could suppress the exploding population of this pest. This calls for introduction of co-evoleved natural enemies from the pest's aboriginal home, India. Information generated during this study on the seasonal fluctuation pattern of *R. iceryoides*, could also be used to guide future augmentative releases of the most dominant indigenous parasitoid species, *A. pseudococci* or possible introduced parasitoid species. For example, the mass releases of the parasitoids during the off-mango season (March- Septemper), where the pest populations in mango orchards are quite low, but relatively high on the alternative host plants, such Jerusalem-thorn, *P. aculeata* and pigeon pea *C. cajan* (Tanga, unpublished data), will help parasitoid population build-up ahead of the heavy infestations by *R. iceryoides* during the mango fruiting season (October-February). Therefore, contributing to the suppression of the pest and enhancing the livelihood of the growers.

CHAPTER EIGHT

Exploratory survey for natural enemies of *Rastrococcus iceryoides* Green (Hemiptera: Pseudococcidae) in India and climate matching to guide their introduction into Africa

ABSTRACT

The mango mealybug, *Rastrococcus iceryoides* Green (Hemiptera: Pseudococcidae) is one of the most destructive mealybug pest on several horticultural crops in Africa, mostly Tanzania, Kenya and Malawi where it continues to expand its host range. Although several indigenous parasitoid species have been found to attack the pest in both Kenya and Tanzania, their combined effort is unable to suppress the exploding populations of the mealybug pest below economically damaging levels. Being an alien invasive pest, classical biological control is mostly likely the optimal option for suppressing the population outbreaks in Africa. This prompted an exploratory survey in the aboriginal home of the pest across fifteen major horticultural production districts in the state of Tamil Nadu, India to identify efficient coevolved natural enemies for introduction into Africa. Based on the exploratory survey data, two correlative approaches to the challenge of ecological niche modeling (genetic algorithm and maximum entropy) were used to identify climatically suitable areas in Africa that are agro-meteorologically similar to the aboriginal home of the pest, based on associations between known occurrence records and a set of environmental predictor variables. Our results showed that *R. iceryoides* is widely distributed throughout the state of Tamil Nadu and was recorded from ten cultivated and non-cultivated host plant species from 8 different families with extremely low infestation levels. The combined percent parasitism based on the proportion of mummified mealybugs ranged between 16.67 to 91.3% on the different host plant parts. A total of eleven parasitoid species were recovered from *R. iceryoides*, out of which eight species are new records. *Praleurocerus viridis* Agarwal (Hymenoptera: Encyrtidae) and *Anagyrus chryos* Noyes & Hayat (Hymenoptera: Encyrtidae) were the dominant and most widely distributed species with maximum percentage parasitism of 43 and 41, respectively. In addition to the parasitoids, 10 predators from 7 families were recorded. The results of this work suggested that the concerted action of the native natural enemies was highly successful and quite effective in suppressing *R. iceryoides* populations. The two models yielded similar estimates, largely corresponding to Equatorial climate classes with temperature seasonality contributing the most in the ecological model with the highest predicted suitability for the pest and parasitoids. The maximum entropy approach was somewhat more conservative in its evaluation of suitability, depending on thresholds for presence/absence that are selected, largely excluding areas with distinct dry seasons; the genetic algorithm models, in contrast, indicate that climate class as partly suitable. The models parameters derived from India fitted well with the introduced distributional range of the pest in Africa and strongly suggest that the humid tropical coastlines of Kenya and Tanzania are climatically suitable for introduction. In addition to informing risk assessments for accidental introductions of the invasive pest, this prediction can also be used to focus monitoring activities.

Keywords: Mango mealybug, *Rastrococcus iceryoides*, foreign exploration, natural enemies, climate matching, Classical biological control, GARP, Maxent

8.1 Introduction

The invasive mango mealybug, *R. iceryoides* (Green) (Homoptera: Pseudococcidae), is a pest of major economic importance in East Africa (Williams, 1989). This insect was first reported in the early 1990s damaging fruit, twigs and foliage of mango in Tanzania. It has since spread inland to northern Malawian boarder and Coastal Kenya and has become a major pest of mango in these countries as well (Neuenschwander, 1993; Tanga, unpublished data). Heavily infested orchards in Tanzania and Kenya were observed to experience damage ranging from 30% to complete crop failure and fruits that suffered feeding damage are either unmarketable or downgraded in packing houses. Feeding damage on the plant results in defoliation, early dropping of the inflorescence and young fruits as well as severe dieback effect of affected young branches. As with other mealybug species, severe infestation on fresh mango fruits can cause a significant reduction in weight and size (Tobih et al., 2002; Pitan et al., 2002; Tanga et al., unpublished data).

The current control methods by majority of the growers to suppress *R. iceryoides* is cutting down and burning of infested trees in addition to aggressive broad insecticidal canopy sprays (Tanga et al., unpublished data; Association of Mango Growers Association of Tanzania, pers. comm.). Although, chemical control is largely practiced in Tanzania and Kenya, it is ineffective because of the waxy coating of mealybugs. The introduction of strict maximum residue level by the EU has further compounded the overuse of chemical pesticide application. The increase and ineffective use of chemical insecticide is likely to jeopardize resistance development as has been reported for other mealybug species (Myburgh and Siebert, 1964; Flaherty et al., 1982).

Biological control with natural enemies is most recommended of mealybug management (Norgaard, 1988; Agounké and Fischer, 1993; Kairo et al., 2000; Moore, 2004; Roltsch et al., 2006; Garcia-Valente et al., 2009). In a recent survey (Chapter three), although up to six parasitoid species (*Anagyrus pseudococci* Girault, *Anagyrus aegyptiacus* Moursi, *Leptomastrix dactylopii* Howard, *Agarwalencyrtus citri* Agarwal, *Aenasius longiscapus* Compere and *Leptomastidea tecta* Prinsloo) were recovered from *R. iceryoides* (some representing new associations) only *A. pseudococci* was able to cause up to 21% parasitism. Parasitism by the other parasitoid species encountered during the survey did not exceed 1%. Despite the relatively high levels of parasitism by *A. pseudococci*, the ability of the parasitoid to regulate the population of *R. iceryoides* in Tanzania and Kenya has been inadequate. In its native home range (Southern Asia), Tandon and Lal (1978), Narasimham and Chako, (1988), and Tandon and Srivastava (1980) reported that *R. iceryoides* is attacked by several parasitoid species (*Tetrastichus* sp., *Allotropa* sp., *Anagyrus* sp. nr *inopus* Noyes & Hayat, *Praleurocerus viridis* (Agarwal), *Coccophagus* sp., *Promuscidea unfasciativentris* Girault and *Chartocerus* sp) with parasitism rates exceeding 40%. This high level of parasitism renders the pest to be of no economic significance in that region. Interestingly, in spite of the rising importance and high levels of damage by *R. iceryoides* to Agriculture in Africa no natural enemies have been introduced so far into Africa to manage the pest.

Biological control through the introduction of natural enemies has been practiced for over 100 years and it is believed to be the only long-term sustainable solution to the problems caused by exotic pests. However, much remains to be discovered about the factors contributing to the success or failure of introductions. About two thirds of natural enemies introduced have failed to establish (Hall and Ehler, 1979; Stiling, 1990), and only about half of the introductions that established against arthropod pests provided some level of economic control. These failures have often been attributed to a lack of climatic matching between area-of-origin and areas-of-establishment (Clausen, 1978; Stiling, 1993; Goolsby et al., 2005). It has therefore been proposed that biological control needs to make the transition from an empirical method to climatic predictive science (Greathead, 1986; Ehler, 1990; Hoddle and Syrett, 2002). This is because climate is an important factor in the bio-ecology of insect natural enemies and a useful predictor of potential establishment and spread in areas of introduction (Cammell and Knight, 1992; Gevrey and Worner, 2006; Kiritani, 2006; Tuda et al., 2006; Guo et al., 2006; Hance et al., 2007). In this regard, is it now widely recommended that natural enemies should be collected from climates that closely match the environment into which they will be introduced (Stiling, 1993; Sutherst, 2003; Hoelmer and Kirk, 2005). Current reviews of invasive species biology have emphasized the great complexities involved in species' occupancy of new distributional areas (Carlton, 1996; NAS, 2002). However, advances in the emerging field of ecological niche modeling have opened the possibility of using species' ecological characteristics as evaluated on native distributional areas to predict potential distributional areas in other regions (Higgins et al., 1999; Skov, 2000; Zalba et al., 2000; Hoffmann, 2001), given the precept that species' ecological niche characteristics tend to remain fairly constant (Peterson, 2003) although some examples of plasticity have been documented (Maron et al., 2004). Predictive modeling of species geographic distributions based on the environmental variables of sites of known occurrence constitutes are now widely used to predict potential establishment and distributional ranges of introduced natural enemies (Yom-Tov and Kadmon, 1998; Peterson et al., 1999; Welk et al., 2002; Scott et al., 2002; Peterson and Shaw, 2003).

This investigation establishes the patterns of *R. iceryoides* distribution, host plant relationships and its associated coevolved natural enemies in southern Indian State of Tamil Nadu with the aim of selecting candidate natural enemies that could be introduced into Africa for the management of the pest. Ecological niche modeling was applied to better understand the global range of expansion of *R. iceryoides* and suitable climatic conditions and localities for introduction and potential for establishment of the natural enemies in Africa.

8.2 Materials and Methods

8.2.1 Sampling sites

India is the largest mango producer in the world accounting for about 40% of the global production (Hanemann, 2006). Within the country, Tamil Nadu is considered as one of the most important mango production states both in terms of quantity and commercial utilization (Mehta and George, 2003; Tharanathan et al., 2006) with 385 fruit processing units (Raj, 2008**).** The sampling sites were chosen based on this previous knowledge of mango and other horticultural production. The field surveys were conducted in 15 major mango growing districts of Tamil Nadu State.

8.2.2 Plant collection, handling and assessment of infestation

The survey methodology was a slight modification from that described by Pitan et al. (2000) and Bokonon-Ganta and Neuenschwander (1995). The procedure was based on an unbiased choice of sample locations along footpaths and jeep trails in major mango production localities of the state of Tamil Nadu. Sampling was carried out in cultivated fields, backyard gardens, woodlands, high-ways, intra-city roads, motorable village roads, forested areas and protected reserves. At each location, a 6-10 km transect was set up with sampling points at 0 km, 2 km, 4 km, 6 km, 8 km and 10 km from the most northerly point of the transect. At each of the sampling points along the transect host plants were selected using random bearings from which 80 leaves, 20 (10-cm length) twigs and five fruits were selected for mealybug counts. Sampling along the transect leading away from the locations was discontinued after several stops without *R. iceryoides* infestation (Bokonon-Ganta and Neuenschwander, 1995). The GPS (global poistioning system) readings were recorded for all the sampled points within each area surveyed. Plant parts were individually transferred to paper bags and transported to the laboratory in cool boxes. In the laboratory, the total number of *R. iceryoides* per sampled plants parts were counted with the aid of a head lens and or stereomicroscope, and recorded. The severity of mealybug infestation was scored for each locality and host plant from the infested foliage, twigs, and fruits following the scale developed by Tobih et al. (2002) for *R. invadens*. Infestation by *R. iceryoides* was also expressed as the total number of female mealybugs per plant part sampled for each locality.

From the field collected mealybug, five to ten adult mealybug samples were randomly selected and slide-mounted at the Departments of Agricultural Entomology, Centre for Plant Protection Studies (CPPS), Tamil Nadu Agricultural University (TNAU) using the methodology of Watson and Kubiriba (2005), for further confirmation of their identity. Voucher specimens for collected mealybug samples were deposited at CPPS, Tamil Nadu Agricultural University Unit. Samples of leaf and or twig and fruit (for small fruit) from unknown plant species were collected, pressed and bagged. The collected plant samples were identified using a field key to the trees and lianas of the evergreen forest of India (Bole and Vaghani, 1986; Pascal and Ramesh, 1997). Photographs were also taken of each plant and or fruit sampled to aid in plant identification and voucher specimens of all collections of the plant species are maintained in the above institution. Plant nomenclature used conforms to the International Plant Names Index database (IPNI, 2005) and the Missouri Botanical Garden database W^3 TROPICOS (MBOT, 2006).

8.2.3 Parasitoid and predator recovery from field collected mealybug samples

After the census of mealybugs on infested plant parts, live and mummified specimen were transferred into plastic paper bags with well ventilated tiny openings made using entomological pins # 000 (length 38 mm, 0.25 mm diameter) or transparent plastic rearing containers (22.5 cm height x 20 cm top diameter x 15 cm bottom diameter). An opening (10 cm diameter) was made on the front side of the cage to which a sleeve, made from very fine organza material (about 0.1 mm mesh size) was fixed. The same material was fixed to the opposite opening (10 cm diameter) of the cage to allow for ventilation. A third opening (13 cm diameter) was made on the roof of the cage, which was also screened with the same material. Streaks of undiluted honey were applied to the roof of the cages and maintained in the laboratory at 70 ± 5% RH, photoperiod of 12:12 (L: D) h and ambient temperatures (26-28^{0}C) until parasitoid emergence. Mummies with emergence holes were discarded after counting. Mummified mealybugs from each infested host plant species and locality were maintained separately. Parasitoids that emerged from the mealybug cultures were collected daily and counted. All parasitoids that emerged were initially identified by Dr. Sagadai Manickavasagam at Annamalai University, India and later confirmed at the Department of Zoology, Aligarh Muslim University, India by Dr. Hayat Mohammad. Voucher specimens of parasitoids of the present study were deposited in the collections of Project Directorate of Biological Control (PDBC), Aligarh Muslim University (AMU), Bangalore; Departments of Agricultural Entomology, Centre for Plant Protection Studies (CPPS), Tamil Nadu Agricultural University (TNAU) and in Annamalai University, Faculty of Agriculture.

At each sampling date and site, predators of *R. iceryoides* were sampled by beating 10 randomly selected branches of each host plants over a 1 – m^2 cloth screen using a 60 cm long stick. The sampling was done during the early hours of the morning of 8:30-9:30 am. The predators that were dislodged onto the cloth were then recorded and preserved in 70 % ethyl alcohol. Immature stages of predators were reared on mealybugs in transparent plastic rearing containers (22 cm length x 15 cm width x 15 cm height) with an opening (10 cm diameter) made on the front side of the plastic container to which a sleeve, made of organza material was fixed. The set up was maintained at 26-28˚C, 40 - 80% relative humidity (RH), under a photoperiod of 12L: 12D in the laboratory at the Departments of Agricultural Entomology, Tamil Nadu

Agricultural University (TNAU), until they developed to the adult stage and later counted. The interactions between ant and mealybug populations in the field were randomly assessed by means of visual inspection.

8.2.4 Statistical Analysis

Data for field surveys are presented according to plant species, family, location, infestation levels, combined percentage parasitism based on the proportion of mummified mealybug and the number of emerged adult parasitoids. Infestation by *R. iceryoides* was expressed as the total number of mealybugs of all developmental stages per plant part sampled for each locality. Parasitism was expressed as percentage of mummified mealybugs collected or as percentage of emerged parasitoid species to the total number of hosts in the samples for each host plant per locality. The data on mealybug incidences and percentage parasitism were compared across plant parts by subjecting the data to Wilcoxon-Mann-Whitney two sample signed rank test and Kruskal-Wallis test to detect differences (Conover, 1999). All computations were performed using SAS 9.1 software (SAS Institute, 2010).

8.2.5 Climate matching models and computation of climate suitability

The basic concept underlying species occurrence modelling is the definition of the climate niche: each species occurs within specific ranges of environmental variables, enabling individuals to survive and reproduce (Austin, 2002). The most common strategy for estimating the potential geographic distribution of a species is to characterize the environmental conditions that are suitable for that species. The spatial distribution of environments that are suitable for a species can then be estimated across a given study region. A wide variety of modeling techniques have been developed for this purpose, including generalized linear models, generalized additive models, bioclimatic envelopes, habitat suitability indices, and the genetic algorithm for rule-set prediction (GARP). In this study, two correlative approaches namely Maxent (maximum entropy modeling; Phillips et al., 2006), and the OM-GARP (genetic algorithm for rule-set prediction; Stockwell and Noble, 1992) were used. These algorithms produce predictions from incomplete information by estimating the most uniform distribution of points of occurrence across the area of study. The evaluation of performance of the models is also carried out to determine the significance of each individual climate variable and provides a measure of the accuracy of the model. This procedure allows for the selection of the final set of significant variables. Calculation of the basic climate niche for each species is an adequate framework to understand the climate-derived drivers of climate suitability.

8.2.5.1 Occurrence data of *R. iceryoides*

Native records of *R. iceryoides* in the state of Tamil Nadu, India and non-native distribution in Africa (particularly Kenya and Tanzania) are summarized in Figure 8.1, resulting from independent surveys conducted by the authors in Kenya, Tanzania and India (C.M. Tanga et al., unpublished data). All records are based upon specimens clearly identified as *R. iceryoides* and differentiated from other taxa within the genus *Rastrococcus*. This list is far from being exhaustive, in the sense that it comprises only data from our survey excluding all extensively unpublished data. The non-native data enable quantitative tests of the predictive ability of the ecological niche models regarding the geographic potential of the species. Only occurrence data originating from the species' native distribution observed during our survey was used to generate the ecological niche modeling.

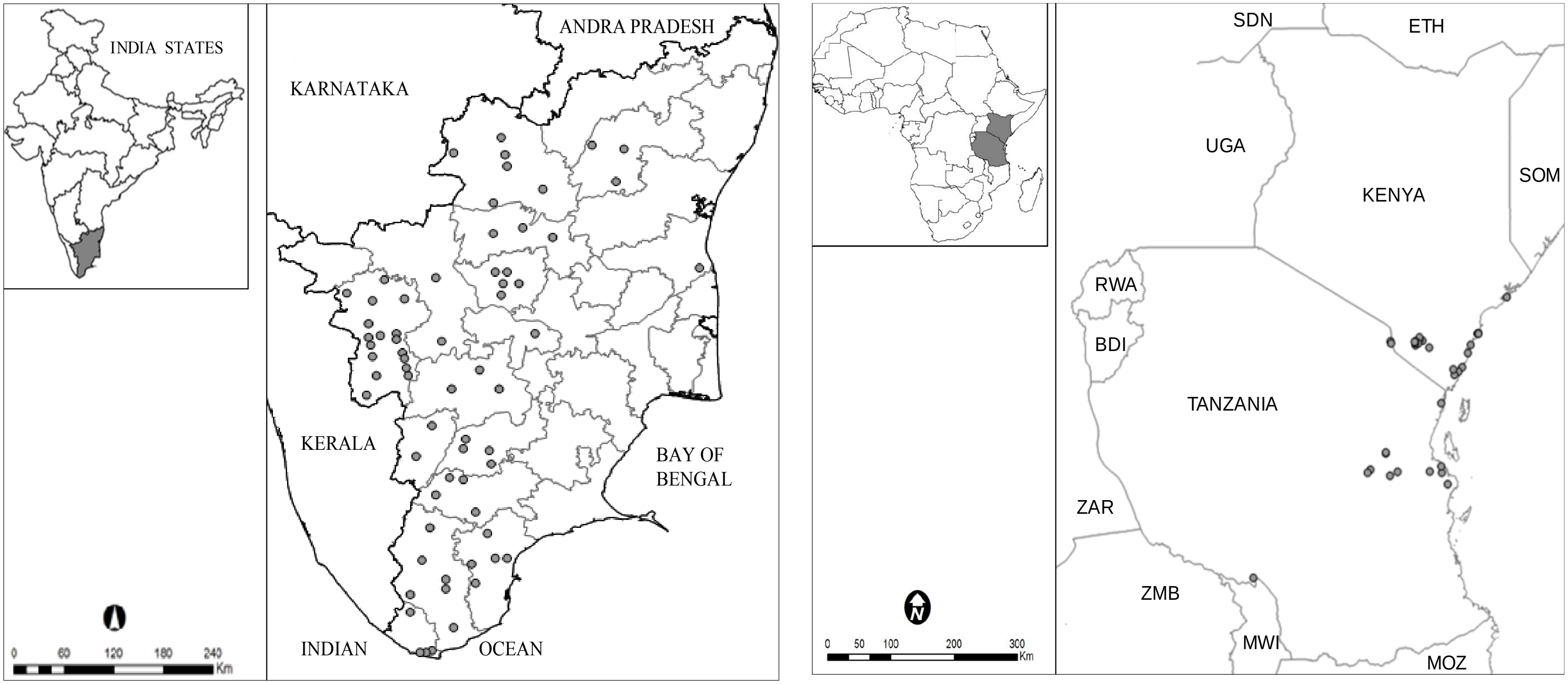

INDIA STATES
0 60 120 180 240 Km
KARNATAKA
ANDRA PRADESH
KERALA
BAY OF BENGAL
INDIAN
OCEAN
0 50 100 200 300 Km
N
SDN
ETH
UGA
KENYA
SOM
RWA
BDI
TANZANIA
ZAR
ZMB
MWI
MOZ

Figure 8. 1: Native records of *R. iceryoides* in the state of Tamil Nadu, India. and which were used in GARP and Maxent to determine climatically similar areas of India, Africa and the world. Non-native records in Africa (Kenya and Tanzania).

8.2.5.2 Environmental data

A set of 19 aggregated bioclimatic variables averaged over a 50-year time period from 1950–2000 at 2.5 arc-minutes spatial resolution were downloaded from www.worldclim.org. These particular climate dimensions were chosen to represent environmental dimensions relevant to distributions and survival of small arthropods (Fletcher, 1989; Vargas et al., 1987; Vera et al., 2002), of which mealybugs are inclusive. No vegetation or land cover data layers were used owing to the heterogeneous nature of habitats, including man-made horticultural environments that can potentially be occupied by *R. iceryoides*. Although host range can provide useful information with regard to species recognition, this information remains incomplete for *R. iceryoides*, particularly as regards the non-native range.

8.2.5.3 Ecological niche modeling (ENM)

The approach used was based on the idea of modeling species' ecological niches, which are considered to constitute long-term stable constraints on species' potential geographic distributions (Peterson et al., 1999; Peterson, 2003; Raxworthy et al., 2003; Martinez-Meyer et al., 2004; Wiens and Graham, 2005). Ecological niches are herein defined as the set of conditions under which a species is able to maintain populations without immigration (Grinnell, 1917; Grinnell, 1924). This condition is assumed here although the species is an extraordinary poorly known one, in particular in its non-native range in Africa. Our approach consisted of four steps: (1) model ecological niche requirements of the species based on known occurrences on its native distributional area during our survey; (2) project niche models to global scales to identify areas fitting the niche profile, (3) test the accuracy of native and invaded range predictions, and (4) project the niche model globally to identify additional areas putatively susceptible to invasion. The global projection was based on a niche model trained using all the native and non-native range records.

The inferential tools used for the ecological niche modeling were GARP and Maxent, both on default settings. These two techniques provided contrasting results in recent comparisons of niche modeling techniques (Elith et al., 2006; Peterson et al., 2007; Peterson et al., 2008). GARP uses an evolutionary-computing approach to carry out a flexible and powerful search for non-random associations between environmental variables and known occurrences of species.

GARP has been used widely (Peterson, 2001; Peterson, 2005; Anderson et al., 2002; Anderson et al., 2003; Stockwell and Peterson, 2002). Specifically, available occurrence points are sub-sampled to create two suites of points: half of the available points are set aside as extrinsic testing points; the remaining points are then re-sampled with replacement to create a population of 1250 presence points; an equivalent number of points is re-sampled from the population of grid squares ('pixels') from which the species has not been recorded ('pseu-doabsence data'). These 2500 points are divided equally into training (for creating models) and intrinsic testing (for evaluating model quality) data sets. Models are composed of a set of conditional rules developed through an iterative process of rule selection, evaluation, testing, and incorporation or rejection. First, a method is chosen from a set of possibilities (e.g. logistic regression, bioclimatic rules, range rules etc), and applied to the training data set. Then, rules 'evolve' by a number of means (mimicking DNA evolution: point mutations, deletions, crossing over, etc.) to maximize predictive accuracy. After each modification, rule quality is evaluated based on the intrinsic testing data; change in predictive accuracy from one iteration to the next is used to evaluate whether a particular rule should be incorporated into the final rule-set. The algorithm runs either 1,000 iterations or until addition of new rules has no effect on predictive accuracy. The final rule-set (the ecological niche model) is then projected worldwide to identify a potential geographic distribution. In general, all analyses were run on default settings, and the best-subsets procedure (Anderson et al., 2003; Rice et al., 2003) was used to choose a subset of models for further consideration, which were then summed to produce a single grid summarizing model agreement in predicting presence vs. absence. This grid was converted to a binary prediction of presence vs. absence by choosing the lowest threshold at which the species was known to occur (Pearson et al., 2007). The result was a set of binary grids summarizing the geographic extents of the environmental niche calculated by GARP for the species.

Maxent makes use of presence records and a set of background values (pseudo-absences) drawn from the entire study region. However, habitat suitability models are calculated only with presence data and predict the species' fundamental niche (i.e., the full range of abiotic conditions within which the species is viable), such that the model output is the probability of a particular habitat to be suitable for the species' survival (Brito and Crespo, 2002). The maximum entropy algorithm (Phillips et al., 2006) for species distributions modeling is one of the most accurate and globally used ecological niche models (Hernandez et al., 2006). Maxent besides generating accurate models, provides an output which identifies the role of each environmental variable in the prediction model. Maxent is a generative approach, rather than discriminative, which can be an inherent advantage when the amount of training data is limited. Maxent estimates the ecological niche of a species by determining the distribution of maximum entropy, subject to the constraint that the expected value of each environmental variable under this estimated distribution matches its empirical average (Phillips et al., 2006). We used default parameters in Maxent (version 3.1) to produce models: feature selection automatic, regularization multiplier at unity, maximum iterations 500, convergence threshold 10^{-5} and random test percentage at zero. The result is a set of probabilities that sum to unity across the entire study area; to make values more manageable, these suitability indices are usually presented as logistic transformations of cumulative probabilities (Phillips et al., 2006), with values ranging 0–100 (low to high suitability).

Spatial predictions of presence and absence can include two types of error, omission (predicted absence in areas of actual presence) and commission (predicted presence in areas of actual absence: Fielding and Bell, 1997). Because GARP is a random-walk procedure, it does not produce unique solutions; consequently, we followed best-practices approaches to identifying optimal subsets of resulting replicate models (Anderson et al., 2003). In particular, we developed 100 replicate models; of these models, we retained the 20 with lowest extrinsic omission error rates and then retained the ten models with intermediate extrinsic commission error (i.e. we discarded the ten models with area predicted present showing greatest deviations from the overall median area predicted present across all low omission models). This 'best subset' of models was summed pixel by pixel to produce final predictions of potential distributions in the form of grids with values ranging from 0 (all models agree in predicting absence) to 10 (all models agree in predicting presence). Since the two modeling techniques produce different sorts of output with very different frequency distributions, correct choice of thresholds becomes critical in interpreting the resulting maps (Peterson et al., 2007). As such, we used the lowest training presence threshold approach (LTPT) of Pearson et al. (2007); specifically, we inspected the native-range occurrence information relative to the raw outputs from GARP and Maxent. We determined the lowest predictive level at which any training presence point was predicted and used that level as a minimum criterion for prediction of presence (vs. absence) in non-native regions.

8.2.5.4 Model testing

To evaluate the model predictions, we offer two sets of tests. First, we developed initial models across the native range region based on a subset of available data, in which ten randomly chosen points were set aside (for testing) prior to model development; this procedure was repeated twice, with different random subsamples. Statistical significance of these predictions was assessed using the cumulative binomial probability approach described below. Second, we assessed the predictive ability in Africa (using African records) for a model that was calibrated using all records from the native region. Because our goal was predicting global invasive potential, we tested model predictivity with the null hypothesis that the observed coincidence between prediction and test points was no better than chance expectations.

The most common mode of evaluating niche models in recent literature is via the area under the curve in a receiver operating characteristic (ROC) analysis (e.g., Elith et al., 2006). ROC analysis, however, is not appropriate to the present situation for two reasons: (i) ROCs require absence data, which are not available in the present case; and (ii) ROCs weight type 1 and type 2 errors equally, but the focus on invasive potential would weight omission error more heavily than commission error (Soberon and Peterson, 2005; Peterson et al., 2008). However, we use an adaptation of the ROC curve approach as a means of assessing predictive ability visually, plotting omission on an inverse scale (=‘sensitivity’) against proportion of area predicted present (an estimator of 1–specificity: Phillips et al., 2006; Peterson et al., 2008).

8.3 Results

8.3.1 Distribution

In the state of Tamil Nadu, out of the 15 districts sampled, *R. iceryoides* was recorded from all the localities of the districts, but with varying degree of infestation (Table 8.1). Among all the locations sampled, infestation was heaviest on mango in Kundal village found in the district of Kanyakumari with 9.5 ± 3.0 mealybugs/leaf, 45.4 ± 15.2 mealybugs/twig and 73.8 ± 50.1 mealybugs/fruit, followed by Pechiparai in the same district with 2.0 ± 0.8 mealybugs/leaf and 22.4 ± 7.1 mealybugs/twig. The lowest infestation level on mango was recorded in the district of Erode with 0.1 ± 0.1 mealybugs/leaf and 0.8 ± 0.7 mealybugs/twig.

8.3.2 Host-plants

During the survey, *R. iceryoides* was recorded from ten cultivated and wild host plants belonging to eight different families with extremely low levels of infestation (Table 8.1). The host plants included *Mangifera indica* L. [Anacardiaceae], *Manilkara zapota* L. [Sapotaceae], *Tectona grandis* L. [Verbenaceae], *Ficus benghalensis* L. [Moraceae], *Gossypium hirsutum* L. [Malvaceae], *Gossypium gossypioides* Ulbr. [Malvaceae], *Pongamia pinnata* L. [Fabaceae], *Psidium guajava* L. [Myrtaceae], *Cajanus cajan* (L) Millsp. [Fabaceae], *Ceiba pentandra* L. [Bombacaceae]. The highest infestation levels were recorded on the twigs (45.4 ± 15.2 mealybugs/twig), leaves (9.5 ± 3.0 mealybugs/leaf) and fruits (73.8 ± 50.1 mealybugs/fruit) of *M. indica* in Kundal village, Kanyakumari district. *Psidium guajava* was the second most infested cultivated host plant (1.69 ± 0.80 mealybugs/leaf and 4.60 ± 2.17 mealybugs/twig), followed by *M. zapota* (1.96 ± 1.05 mealybugs/leaf and 3.90 ± 2.26 mealybugs/twig) in Coimbatore.

However, there was no significant difference in the infestation levels between leaves and twigs of the different host plants sampled during the survey except for mango in Paiyur (Mann-Whitney test, $Z = -2.1632$; $df = 1$; $P = 0.0305$). On mango, the fruits recorded the highest number of mealybugs although no significant differences was observed when compared to those on the twigs and leaves (Kruskal-Wallis test, $\chi^2 = 2.7027$; $df = 2$; $P = 0.2589$) (Table 8.1). In Coimbatore, where the second highest level of infestation on mango was recorded, the mealybug population on the twigs were higher than on the leaves but not significantly different (Mann-Whitney test, $Z = 0.2196$; $df = 1$; $P = 0.8262$). The most important wild host plant was *F. benghalensis* with infestation levels of 2.0 ± 1.07 mealybugs/leaf and 2.7 ± 1.3 mealybugs/twig. However, the number of mealybug on the twig of *F. benghalensis* was not significantly different from that on the leaves (Mann-Whitney test, $Z = -0.2299$; $df = 1$; $P = 0.2187$) (Table 8.1).

Other mealybug species were also encountered, although at negligible level on mango, these include: *R. invadens* (William) found in two localities only (Coimbatore and Madurai) from two host plants (*M. indica* and *M. zapota*); *Drosicha mangiferae* (Green) found in Paiyur only; *Icerya aegyptiaca* (Douglas) and *Icerya seychellarum* (Westwood), which were restricted to Coimbator; *Planococcus citri* (Risso), *Ferrisia virgata* (Cockerell) and *Paracoccus marginetus* (Williams and Granara de Willink) [important on *Carica papaya* Linn. (Family Caricaceae)], *Plumeria alba* Linn. (Family: Apocynaceae), *Solanum torvum* Swartz (Family: Solanaceae) and *P. guajava* (Family: Myrtaceae)].

Table 8. 1: Distribution, host plants and infestation levels of *R. iceryoides* in the state of Tamil Nadu, India

						Statistics		
District/Locality	Host plants	Plant family	Mean no. of mealybugs/leaf	Mean no. of mealybugs/twig	Mean no. of mealybugs/fruit	*Z/χ2*	*df*	*P*
Coimbatore	*Mangifera indica* Linn.	Anacardiaceae	3.70 ± 1.74	9.15 ± 4.43	-	0.2196	1	0.8262
	Manilkara zapota Linn.	Sapotaceae	1.96 ± 1.05	3.90 ± 2.26	-	-0.6124	1	0.5403
	Tectona grandis Linn.	Verbenaceae	0.68 ± 0.36	2.0 ± 1.12	-	0.5238	1	0.6004
	Ficus benghalensis Linn.	Moraceae	2.03 ± 1.07	2.7 ± 1.34	-	-0.2299	1	0.2187
	Gossypium hirsutum Linn.	Malvaceae	0.25 ± 0.16	0.30 ± 0.21	-	-0.9172	1	0.3590
	Gossypium gossypioides Ulbr.	Malvaceae	0.38 ± 0.20	0.90 ± 0.50	-	1.1616	1	0.2454
	Pongamia pinnata Linn.	Fabaceae	1.09 ± 0.59	1.85 ± 1.12	-	-0.5490	1	0.5830
	Psidium guajava Linn.	Myrtaceae	1.69 ± 0.80	4.60 ± 2.17	-	-0.2861	1	0.7748
	Cajanus cajan (L) Millsp.	Fabaceae	0.29 ± 0.22	0.70 ± 0.42	-	0.3568	1	0.7213
Salem								
Erummaiputti	*Mangifera indica* Linn.	Anacardiaceae	2.78 ± 1.33	7.10 ± 3.23	-	-0.3571	1	0.7210
Dharmapuri								
Paiyur	*Mangifera indica* Linn.	Anacardiaceae	3.21 ± 1.24	1.40 ± 0.98	-	-2.1632	1	0.0305
	Ceiba pentandra Linn.	Bombacaceae	0.54 ± 0.27	0.60 ± 0.34	-	-1.4759	1	0.1400
Periyapatti	*Mangifera indica* Linn.	Anacardiaceae	4.61 ± 1.96	5.75 ± 2.43	-	-1.1216	1	0.2620
	Cajanus cajan (L) Millsp.	Fabaceae	0.86 ± 0.43	0.80 ± 0.52	-	-1.8371	1	0.0662
Kariyamangakam	*Mangifera indica* Linn.	Anacardiaceae	1.39 ± 0.63	3.25 ± 1.79	-	-0.5170	1	0.6052
Madurai								
Othakadei	*Mangifera indica* Linn.	Anacardiaceae	1.08 ± 0.52	1.15 ± 0.52	-	-1.3038	1	0.1923
	Ceiba pentandra Linn.	Bombacaceae	0.35 ± 0.20	0.35 ± 0.18	-	1.7530	1	0.0796
Valeyapatti	*Mangifera indica* Linn.	Anacardiaceae	0.64 ± 0.36	0.75 ± 0.32	-	1.9347	1	0.0530

Plants parts samples based on 80 leaves, 20 twigs of 10 cm length and 5 fruits; - = plant part were either not infested and omitted from analysis or not available during sampling.

Table 8.1 continues. Distribution, host plants and infestation levels of *R. iceryoides* in the state of Tamil Nadu, India

District/Locality	Host plants	Plant family	Mean no. of mealybugs/leaf	Mean no. of mealybugs/twig	Mean no. of mealybugs/fruit	Statistics		
						Z/χ2	*df*	*P*
Virudhunagar								
Rajapalayam	*Mangifera indica* Linn.	Anacardiaceae	0.81 ± 0.35	0.75 ± 0.44	-	-1.9246	1	0.0543
Theni								
Periyakulam	*Mangifera indica* Linn.	Anacardiaceae	0.43 ± 0.29	0.95 ± 0.50	-	0.3721	1	0.7098
Kanyakumari								
Pechiparai	*Mangifera indica* Linn.	Anacardiaceae	2.03 ± 0.80	22.35 ± 7.09	-	-1.6340	1	0.1022
Kundal	*Mangifera indica* Linn.	Anacardiaceae	9.46 ± 2.99	45.4 ± 15.23	73.8 ± 50.11	2.7027	2	0.2589
Erode	*Mangifera indica* Linn.	Anacardiaceae	0.13 ± 0.07	0.75 ± 0.56	-	0.1800	1	0.8571
	Ceiba pentandra Linn.	Bombacaceae	0.58 ± 0.26	1.60 ± 0.73	-	-0.2873	1	0.7739
Cuddalore	*Manilkara zapota* Linn.	Sapotaceae	0.50 ± 0.24	1.60 ± 0.76	-	0.3214	1	0.7479
Tirunelveli	*Mangifera indica* Linn.	Anacardiaceae	0.29 ± 0.15	1.40 ± 0.74	-	0.4920	1	0.6228
Tiruvannamalai	*Gossypium hirsutum* Linn.	Malvaceae	0.11 ± 0.07	1.0 ± 0.50	-	-0.3018	1	0.7628
Tuticorin	*Gossypium gossypioides* Ulbr.	Malvaceae	0.01 ± 0.01	0.95 ± 0.48	-	-1.0607	1	0.2888
Karur	*Psidium guajava* Linn.	Myrtaceae	0.43 ± 0.25	0.60 ± 0.42	-	-0.8839	1	0.3768
Dindigul	*Mangifera indica* Linn.	Anacardiaceae	0.23 ± 0.10	1.35 ± 0.81	-	1.4473	1	0.1478
Namakkal	*Mangifera indica* Linn.	Anacardiaceae	0.24 ± 0.12	0.65 ± 0.35	-	0.4871	1	0.6262

Plants parts samples based on 80 leaves, 20 twigs of 10 cm length and 5 fruits; - = plant part were either not infested and omitted from analysis or not available during sampling.

8.3.3 Parasitoids associated with *R. iceryoides* in the state of Tamil Nadu, India

Out of 5950 *R. iceryoides* collected from the eight host plant species across the 15 districts, 3788 mealybugs were parasitized and yielded a parasitism rate of 63.66%. Out of the total number of mummified mealybug collected from the different host plants, 3167 mummified mealybugs were from mango accounting for 83.61% of total mummified mealybugs. Combined parasitism rate based on proportion of mummified mealybugs varied across host plants as well as host plant parts (Table 8.2). The highest combined percentage parasitism was recorded on mango in Othakadei, Madurai district with 88.37% on the leaves and 91.3% on the twigs. The lowest combined percentage parasitism was recorded on *P. guajava* in Karur district with 20.59% on the leaves and 16.67% on the twigs. Combined percentage parasitism on the different plant parts across the different host plants generally showed insignificant relationships between leaves and the twigs except on mango in Paiyur, Dharmapuri district ($Z = 2.0888$; $df = 1$; $P = 0.0367$) and on *F. benghalensis* in Coimbatore ($Z = -2.3368$; $df = 1$; $P = 0.0195$). The parasitoid community was composed of eleven primary parasitoid species recovered from *R. iceryoides*, out of which seven are new records (Table 8.3). These parasitoid species were from the family Encyrtidae: *Praleurocerus viridis* Agarwal, *Anagyrus chryos* Noyes & Hayat, *Parechthrodryinus excelsus* Hayat, *Ericydnus paludatus* Haliday, *Neoplatycerus tachikawai* Subba Rao, *Agarwalencyrtus ajmerensis* Fatima & Shafee, *Aenasius advena* Compere, *Leptomastidea minyas* Noyes & Hayat, *Carabunia bicoloripes* Hayat, *Aphycus sapporoensis* Compere & Annecke and *Anagyrus mirzia* Agarwal & Alam (Figure 8.2). *Praleurocerus viridis* and *A. chryos* were the most abundant and widely distributed species during the survey accounted for 34.05 and 28.17% of total emerged parasitoids, respectively. The remaining nine parasitoid species comprised only 14.35% of the total wasps recovered. The percentage parasitism of the different parasitoid species varied considerably among the different host plant species (Table 8.3). For example, *P. viridis* achieved a maximum percentage parasitism of 43% on twigs of *C. pentandra* in Othakadei, Madurai district while *A. chryos* achieved a maximum percentage parasitism of 41% on leaves of the mango plant in Periyakulam, Theni district. The percentage parasitism of *P. viridis* and *A. chryos* recorded on mango fruit in Kundal village, Kanyakumari district ranged between 16 – 30%.

Two hyperparasitoid species were also recovered from *R. iceryoides* during the survey: *Coccophagus ceroplastae* (Howard) (Hymenoptera: Aphelinidae) and *Coccidoctonus terebratus*

(Hayat, Alam & Agarwal) (Hymenoptera: Encyrtidae) (Figure 8.3), with the later restricted to Salem only. Both hyperparasitoids accounted for 12.83% of total parasitoids recovered from *R. iceryoides* during the survey. *Coccophagus ceroplastae* was the most dominant hyperparasitoids accounting for a hyperparasitism rate of 2.7 - 11.11% on leaves and 0 - 13.33% on twigs across the different host plants sampled during the survey (Table 8.3). However, percentage hyperparasitism was sporadic and low on all sampled localities.

Other parasitoids recovered during the survey from different mealybug species included, a dipteran parasitoid species, *Cryptochaetum iceryae* (Williston) (Diptera: Cryptochaetidae) from *I. seychellarum* in Coimbatore. This parasitic fly was observed from 6 parasitized out of the 8 samples collected from the field accounting for 75% parasitism of *I. seychellarum*. *Aenasius advena* Compere (Hymenoptera: Encyrtidae) was recovered from *P. citri* and *F. virgata*. *Gyranusoidea tebygi* Noyes and *Anagyrus mangicola* Noyes (Hymenoptera: Encyrtidae) were recovered from *R. invadens* in Coimbatore and Madurai districts. No parasitoids were recovered from *Drosicha mangiferae* and *Icerya aegyptiaca.*

Table 8. 2: Combined percentage parasitism based on the number of mummified *R. iceryoides* in the state of Tamil Nadu, India

		Percentage parasitism			Statistics		
District/Locality	Host plants	Leaves	Twigs	Fruits	Z/χ^2	*df*	*P*
Coimbatore	*Mangifera indica* Linn.	73.99 (296)	74.86 (183)	-	-0.4518	1	0.6514
	Manilkara zapota Linn.	40.76 (157)	43.59 (78)	-	0.2745	1	0.7837
	Tectona grandis Linn.	55.56 (54)	55.0 (40)	-	-0.1235	1	0.9017
	Ficus benghalensis Linn.	57.41 (162)	20.37 (54)	-	-2.3368	1	0.0195
	Gossypium hirsutum Linn.	25.0 (20)	50.0 (6)	-	0.5401	1	0.5892
	Gossypium gossypioides Ulbr.	30.0 (30)	44.44 (18)	-	-0.5843	1	0.5590
	Pongamia pinnata Linn.	47.13 (87)	70.27 (37)	-	1.3858	1	0.1658
	Psidium guajava Linn.	59.26 (135)	33.7 (92)	-	-1.3571	1	0.1747
	Cajanus cajan (L) Millsp.	34.78 (23)	35.71 (14)	-	0.1768	1	0.8597
Salem							
Erummaiputti	*Mangifera indica* Linn.	82.88 (222)	78.87 (142)	-	-0.7153	1	0.4744
Dharmapuri							
Paiyur	*Mangifera indica* Linn.	77.04 (257)	89.29 (28)	-	2.0888	1	0.0367
	Ceiba pentandra Linn.	34.88 (43)	41.67 (12)	-	0.1246	1	0.9009
Periyapatti	*Mangifera indica* Linn.	81.3 (369)	85.22 (115)	-	0.5898	1	0.5553
	Cajanus cajan (L) Millsp.	49.28 (69)	68.75 (16)	-	0.3737	1	0.7086
Kariyamangakam	*Mangifera indica* Linn.	87.39 (111)	78.46 (65)	-	-0.2932	1	0.7694
Madurai							
Othakadei	*Mangifera indica* Linn.	88.37 (86)	91.3 (23)	-	1.0746	1	0.2826
	Ceiba pentandra Linn.	25.0 (28)	57.14 (7)	-	-1.1616	1	0.2454
Valeyapatti	*Mangifera indica* Linn.	82.35 (51)	60.0 (15)	-	0.6464	1	0.5180
Virudhunagar							
Rajapalayam	*Mangifera indica* Linn.	72.31 (65)	80.0 (15)	-	1.8403	1	0.0657
Theni							
Periyakulam	*Mangifera indica* Linn.	82.35 (34)	84.21 (19)	-	-0.1246	1	0.9009
Kanyakumari							
Pechiparai	*Mangifera indica* Linn.	12.41 (162)	35.54 (447)	-	-1.5218	1	0.1281
Kundal	*Mangifera indica* Linn.	23.82 (757)	09.38 (908)	18.44 (369)	2.7836	2	0.2486
Erode	*Ceiba pentandra* Linn.	41.3 (46)	34.38 (32)	-	-0.8643	1	0.3874
Cuddalore	*Manilkara zapota* Linn.	37.5 (40)	53.13 (32)	-	1.2928	1	0.1961
Tuticorin	*Gossypium gossypioides* Ulbr.	-	21.05 (19)	-	-	-	-
Karur	*Psidium guajava* Linn.	20.59 (34)	16.67 (12)	-	-0.1852	1	0.8530

- = infested plant parts were either not infested or not available at the time of sampling. Numbers in parentheses represent the actual number of *R. iceryoides* collected per plant part during the survey.

Table 8. 3: Parasitoid complex associated with *R. iceryoides* on different host plants in the state of Tamil Nadu, India

			Percentage parasitism			Statistics		
District/Locality	Parasitoid species	Plant species	Leaves	Twigs	Fruits	Z/χ^2	*df*	*P*
Coimbatore	*Praleurocerus viridis* Agarwal	*Mangifera indica* Linn.	26.69 (296)	27.32 (183)	-	-0.3660	1	0.7144
	Anagyrus chryos Noyes & Hayat	*Mangifera indica* Linn.	21.28 (296)	16.39 (183)	-	-1.3229	1	0.1859
	*******Coccophagus ceroplastae* Howard	*Mangifera indica* Linn.	7.09 (296)	6.01 (183)	-	-0.2208	1	0.8253
	Parechthrodryinus excelsus Hayat	*Mangifera indica* Linn.	2.36 (296)	2.19 (183)	-	1.0849	1	0.2780
	Ericydnus paludatus Haliday	*Mangifera indica* Linn.	1.01 (296)	2.19 (183)	-	1.4187	1	0.1560
	Neoplatycerus tachikawai Subba Rao	*Mangifera indica* Linn.	2.7 (296)	3.28 (183)	-	1.0008	1	0.3169
	Agarwalencyrtus ajmerensis Fatima & Shafee	*Mangifera indica* Linn.	2.7 (296)	0	-	-1.6985	1	0.0894
	Aenasius advena Compere	*Mangifera indica* Linn.	0	4.37 (183)	-	2.2768	1	0.0228
	Praleurocerus viridis Agarwal	*Manilkara zapota* Linn.	26.11 (157)	26.92 (78)	-	1.1023	1	0.2703
	Anagyrus chryos Noyes & Hayat	*Manilkara zapota* Linn.	19.75 (157)	25.64 (78)	-	0.1225	1	0.9025
	***Coccophagus ceroplastae* Howard	*Manilkara zapota* Linn.	10.19 (157)	8.97 (78)	-	0.4229	1	0.6723
	Parechthrodryinus excelsus Hayat	*Manilkara zapota* Linn.	5.10 (157)	10.26 (78)	-	1.3703	1	0.1706
	Neoplatycerus tachikawai Subba Rao	*Manilkara zapota* Linn.	8.28 (157)	5.13 (78)	-	-0.2460	1	0.8057
	Praleurocerus viridis Agarwal	*Tectona grandis* Linn.	27.78 (54)	15.0 (78)	-	-0.4920	1	0.6228
	Anagyrus chryos Noyes & Hayat	*Tectona grandis* Linn.	20.37 (54)	25.0 (78)	-	0.1246	1	0.9009
	***Coccophagus ceroplastae* Howard	*Tectona grandis* Linn.	9.26 (54)	7.5 (78)	-	-0.6228	1	0.5334
	Parechthrodryinus excelsus Hayat	*Tectona grandis* Linn.	5.56 (54)	12.5 (78)	-	0.6708	1	0.5023
	Ericydnus paludatus Haliday	*Tectona grandis* Linn.	7.41 (54)	2.5 (78)	-	-0.5821	1	0.5605
	Praleurocerus viridis Agarwal	*Ficus benghalensis* Linn.	25.31 (162)	16.67 (54)	-	-0.2299	1	0.2187
	Anagyrus chryos Noyes & Hayat	*Ficus benghalensis* Linn.	20.99 (162)	31.48 (54)	-	1.3472	1	0.1779
	***Coccophagus ceroplastae* Howard	*Ficus benghalensis* Linn.	8.64 (162)	7.41 (54)	-	0.4229	1	0.6723
	Praleurocerus viridis Agarwal	*Psidium guajava* Linn.	28.89 (135)	25.0 (92)	-	0.4346	1	0.6639
	Anagyrus chryos Noyes & Hayat	*Psidium guajava* Linn.	16.30 (135)	20.65 (92)	-	0.1451	1	0.8847
	***Coccophagus ceroplastae* Howard	*Psidium guajava* Linn.	11.11 (135)	9.78 (92)	-	-0.5143	1	0.6070

- = infested plant parts were either not infested or not available at the time of sampling. Numbers in parentheses represent the actual number of mealybug collected per plant part during the survey. ** = Hyperparasitoids emerged from the mummified *R. iceryoides*

Table 8.3 continues. Parasitoid complex associated with *R. iceryoides* on different host plants in the state of Tamil Nadu, India

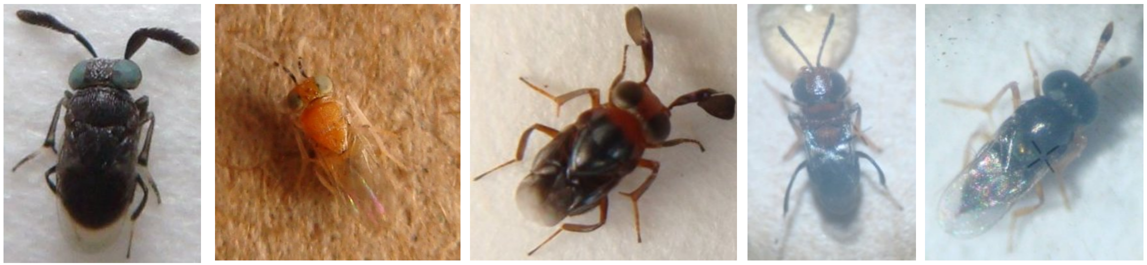
			Percentage parasitism			Statistics		
District/Locality	Parasitoid species	Plant species	Leaves	Twigs	Fruits	Z/χ^2	*df*	*P*
Salem								
Erummaiputti	*Praleurocerus viridis* Agarwal	*Mangifera indica* Linn.	20.27 (222)	20.42 (142)	-	-0.2861	1	0.7748
	Anagyrus chryos Noyes & Hayat	*Mangifera indica* Linn.	15.77 (222)	19.01 (142)	-	1.4486	1	0.1474
	***Coccophagus ceroplastae* Howard	*Mangifera indica* Linn.	2.70 (222)	2.11 (142)	-	-0.0814	1	0.9351
	***Coccidoctonus terebratus* Hayat, Alam & Agarwal	*Mangifera indica* Linn.	17.12 (222)	17.61 (142)	-	-1.5000	1	0.1336
	Ericydnus paludatus Haliday	*Mangifera indica* Linn.	2.70 (222)	2.11 (142)	-	-0.0814	1	0.9351
	Leptomastidea minyas Noyes & Hayat	*Mangifera indica* Linn.	2.70 (222)	3.52 (142)	-	0.6222	1	0.5338
Dharmapuri								
Paiyur	*Praleurocerus viridis* Agarwal	*Mangifera indica* Linn.	15.18 (257)	21.43 (28)	-	0.0783	1	0.9388
	Anagyrus chryos Noyes & Hayat	*Mangifera indica* Linn.	24.13 (257)	28.57 (28)	-	-0.5363	1	0.5918
	Anagyrus mirzia Agarwal & Alam	*Mangifera indica* Linn.	3.89 (257)	0	-	-1.9291	1	0.0537
	***Coccophagus ceroplastae* Howard	*Mangifera indica* Linn.	5.84 (257)	0	-	-1.6714	1	0.0016
	Praleurocerus viridis Agarwal	*Ceiba pentandra* Linn.	18.60 (43)	16.67 (12)	-	-0.4025	1	0.6873
	Anagyrus chryos Noyes & Hayat	*Ceiba pentandra* Linn.	20.93 (43)	16.67 (12)	-	-0.1246	1	0.9009
	***Coccophagus ceroplastae* Howard	*Ceiba pentandra* Linn.	6.98 (43)	0	-	-1.1739	1	0.0008
Periyapatti	*Praleurocerus viridis* Agarwal	*Mangifera indica* Linn.	21.14 (369)	13.91 (115)	-	-1.3577	1	0.1746
	Anagyrus chryos Noyes & Hayat	*Mangifera indica* Linn.	23.04 (369)	21.74 (115)	-	-0.0589	1	0.953
	***Coccophagus ceroplastae* Howard	*Mangifera indica* Linn.	8.67 (369)	10.43 (115)	-	0.6505	1	0.5154
	Praleurocerus viridis Agarwal	*Cajanus cajan* (L) Millsp.	11.59 (69)	6.25 (16)	-	-0.6708	1	0.5023
	Anagyrus chryos Noyes & Hayat	*Cajanus cajan* (L) Millsp.	18.84 (69)	25.0 (16)	-	0.4229	1	0.6723
	***Coccophagus ceroplastae* Howard	*Cajanus cajan* (L) Millsp.	8.70 (69)	6.25 (16)	-	-0.9391	1	0.3477
Kariyamangakam	*Praleurocerus viridis* Agarwal	*Mangifera indica* Linn.	17.12 (111)	24.62 (65)	-	0.3765	1	0.7066
	Anagyrus chryos Noyes & Hayat	*Mangifera indica* Linn.	20.72 (111)	20.0 (65)	-	0.1474	1	0.8828
	***Coccophagus ceroplastae* Howard	*Mangifera indica* Linn.	8.11 (111)	9.23 (65)	-	-0.6159	1	0.5380

- = infested plant parts were either not infested or not available at the time of sampling. Numbers in parentheses represent the actual number of mealybug collected per plant part during the survey. ** = Hyperparasitoids emerged from the mummified *R. iceryoides*

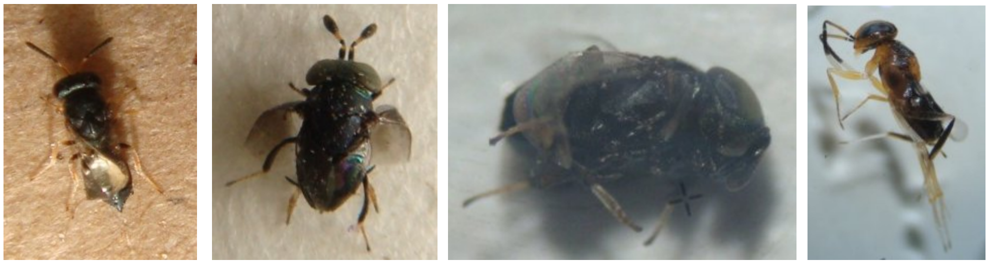
Table 8.3 continues. Parasitoid complex associated with *R. iceryoides* on different host plants in the state of Tamil Nadu, India

			Percentage parasitism			Statistics		
District/Locality	Parasitoid species	Plant species	Leaves	Twigs	Fruits	Z/χ^2	*df*	*P*
Madurai								
Othakadei	*Praleurocerus viridis* Agarwal	*Mangifera indica* Linn.	24.42 (86)	13.04 (23)	-	-1.0104	1	0.3123
	Anagyrus chryos Noyes & Hayat	*Mangifera indica* Linn.	24.42 (86)	21.74 (23)	-	-0.1641	1	0.8696
	***Coccophagus ceroplastae* Howard	*Mangifera indica* Linn.	6.98 (86)	13.04 (23)	-	0.1811	1	0.8563
	Praleurocerus viridis Agarwal	*Ceiba pentandra* Linn.	10.71 (28)	42.86 (7)	-	-0.4613	1	0.0046
	Anagyrus chryos Noyes & Hayat	*Ceiba pentandra* Linn.	39.29 (28)	28.57 (7)	-	1.1983	1	0.2308
	***Coccophagus ceroplastae* Howard	*Ceiba pentandra* Linn.	10.71 (28)	0	-	1.3229	1	0.0001
Valeyapatti	*Praleurocerus viridis* Agarwal	*Mangifera indica* Linn.	27.45 (51)	13.33 (15)	-	1.2478	1	0.2121
	Anagyrus chryos Noyes & Hayat	*Mangifera indica* Linn.	31.37 (51)	20 (15)	-	1.5884	1	0.1122
	***Coccophagus ceroplastae* Howard	*Mangifera indica* Linn.	9.80 (51)	13.33 (15)	-	0.0995	1	0.9207
Virudhunagar								
Rajapalayam	*Praleurocerus viridis* Agarwal	*Mangifera indica* Linn.	16.92 (65)	33.33 (15)	-	0.3300	1	0.0211
	Anagyrus chryos Noyes & Hayat	*Mangifera indica* Linn.	23 (65)	20 (15)	-	-0.5413	1	0.5883
	***Coccophagus ceroplastae* Howard	*Mangifera indica* Linn.	9.23 (65)	13.33 (15)	-	0.1114	1	0.9113
	Ericydnus paludatus Haliday	*Mangifera indica* Linn.	4.62 (65)	0	-	-1.4427	1	0.0319
Theni								
Periyakulam	*Praleurocerus viridis* Agarwal	*Mangifera indica* Linn.	41.18 (34)	42.11 (19)	-	0.1303	1	0.8963
	Aenasius advena Compere	*Mangifera indica* Linn.	11.74 (34)	10.53 (19)	-	0.2697	1	0.7874
Kanyakumari								
Pechiparai	*Praleurocerus viridis* Agarwal	*Mangifera indica* Linn.	12.93 (162)	10.16 (447)	-	0.9131	1	0.3612
	Anagyrus chryos Noyes & Hayat	*Mangifera indica* Linn.	5.90 (162)	8.72 (447)	-	-0.1409	1	0.2539
	Aphycus sapporoensis Compere & Annecke	*Mangifera indica* Linn.	2.35 (162)	1.23 (447)	-	0.2311	1	0.3661
	***Coccophagus ceroplastae* Howard	*Mangifera indica* Linn.	3.26 (162)	5.29 (447)	-	-0.6900	1	0.4902
	Leptomastidea minyas Noyes & Hayat	*Mangifera indica* Linn.	1.09 (162)	2.79 (447)	-	0.5620	1	0.5741
Kundal	*Praleurocerus viridis* Agarwal	*Mangifera indica* Linn.	6.95 (757)	1.92 (908)	8.28 (369)	2.7977	2	0.2469
	Anagyrus chryos Noyes & Hayat	*Mangifera indica* Linn.	4.62 (757)	3.10 (908)	4.16 (369)	4.9536	2	0.0840
	***Coccophagus ceroplastae* Howard	*Mangifera indica* Linn.	5.14 (757)	2.59 (908)	2.13 (369)	22.7534	2	< 0.0001
	Leptomastidea minyas Noyes & Hayat	*Mangifera indica* Linn.	1.06 (757)	0.33 (908)	0	1.3729	2	0.5034
	Carabunia bicoloripes Hayat	*Mangifera indica* Linn.	0.79 (757)	0.33 (908)	0	0.7290	2	0.6946

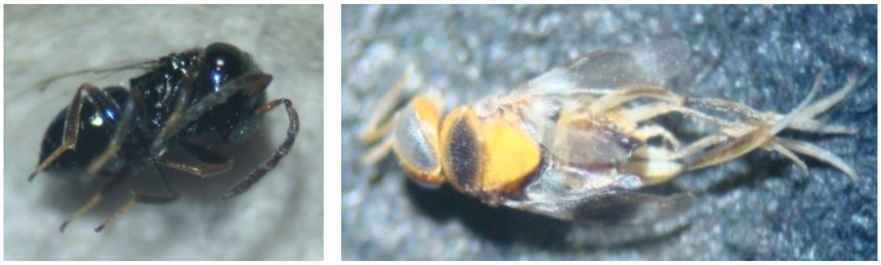
- = infested plant parts were either not infested or not available at the time of sampling. Numbers in parentheses represent the actual number of mealybug collected per plant part during the survey. ** = Hyperparasitoids emerged from the mummified *R. iceryoides*.



Praleurocerus viridis *Anagyrus chryos* *Neoplatycerus tachikawai* *Anagyrus mirzia* *Parechthrodryinus excelsus*

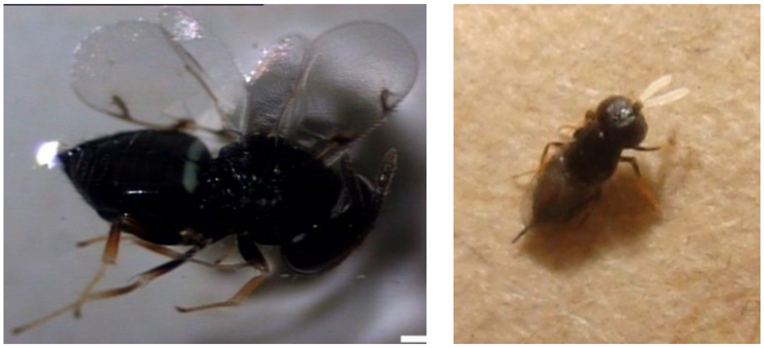


Ericydnus paludatus *Aenasius advena* *Agarwalencyrtus ajmerensis* *Leptomastidea minyas*



Carabunia bicoloripes *Aphycus sapporoensis*

Figure 8. 2: Catalogue of indigenous primary parasitoids recovered from *R. iceryoides* in the state of Tamil Nadu, India.



Coccophagus ceroplastae *Coccidoctonus terebratus*

Figure 8. 3: Catalogue of hyperparasitoids recovered from mummified *R. iceryoides* in the state of Tamil Nadu, India.

8.3.4 Predators associated with *R. iceryoides* in the state of Tamil Nadu, India

In addition to the parasitoids, 10 predators from 7 families were recorded. These included, *Spalgis epius* (Westwood), (Lepidoptera: Lycaenidae), several species of coccinelids namely: *Crytolaemus montrouzieri* Mulsant, *Hyperaspis maindroni* Sicard, *Scymnus coccivora* Ayyar and *Chilocorus nigritus* Fabricius. Among these, the predaceous beetle, *C. nigritus* was the most abundant and widespread species. Brown lacewing, *Hemerobius* sp and green lacewing, *Chrysopa* sp., were also found feeding on *R. iceryoides*. Furthermore, dipteran predators from 3 different families were collected: *Leucopis* sp. (Chamaemyiidae: Diptera), *Cacoxenus perspicax* Knab (Drosophilidae: Diptera), Hover fly-larvae (Syrphidae: Diptera), implying they could be rapacious predators of *R. iceryoides*. The predaceous drosophilid, *C. perspicax* was the most abundant in this group and was commonly encountered in high density among mealybug colonies.

8.3.5 Ant species associated with *R. iceryoides* in the state of Tamil Nadu, India

Five major ant species were observed tending *R. iceryoides* in the field but their distribution was greatly varied from one location to the other: *Camponotus* (*Myrmosericus*) *rufipes* Fabricius, *Camponotus barbaricus* Emery, *Monomorium pharaonis* Linnaeus, *Crematogaster cerasi* Fitch and *Oecophylla longinoda* Latreille. The most remarkable interaction of these ant species was observed with *C. rufipes*, transporting *R. iceryoides* from mango tree top to the roots where they were nesting and actively attacking the larva of *S. epius* preying on ovipositing female of *R. iceryoides*.

8.3.6 Climatically suitable and similar regions in Southern Asia, Africa and the Americas

The projected distribution of *R. iceryoides* in Southern Asia conforms well to its known published distribution, which encompasses Indonesia, and Sri Lanka except in Bangladesh, Malaysia, Singapore and China. Apart from the above mentioned countries, new occurrence points climatically suitable for *R. iceryoides* and its natural enemies was successfully predicted by the two models in Bangkok, Cambodia, Thailand and Philippines (Figure 8.4). GARP predicted higher suitability further inland from the coastlines, while Maxent indicated suitability more restricted to isolated pockets in these parts when high threshold values are taken into account only. By definition all of the input locations derived from the distribution records had perfect match with themselves, therefore regions with highest climatic similarity values in the GARP model (Figure 8.4) corresponded perfectly with the distribution records. The climatic similarity of points in the State of Tamil Nadu decreased with distance from the input points. The rate at which the similarity decreased appeared to follow logical topo-climatic gradients.

Projecting niche models to Africa (Figure 8.5) again yielded similar predictions between the two methods, with Maxent again appearing more conservative. Both models predicted high suitability in the East African coastal regions and marginal suitability in the Equatorial rain forest belt. Invaded areas for the purpose of model validation were in East Africa, where most reported non-native mealybug populations are located (Figure 8.5). The coincidence of known records of this species in Africa with the area predicted by the projection of the native-range model was excellent, again statistically significantly more coincident than random models (χ^2 tests, all $P < 10^{-6}$).

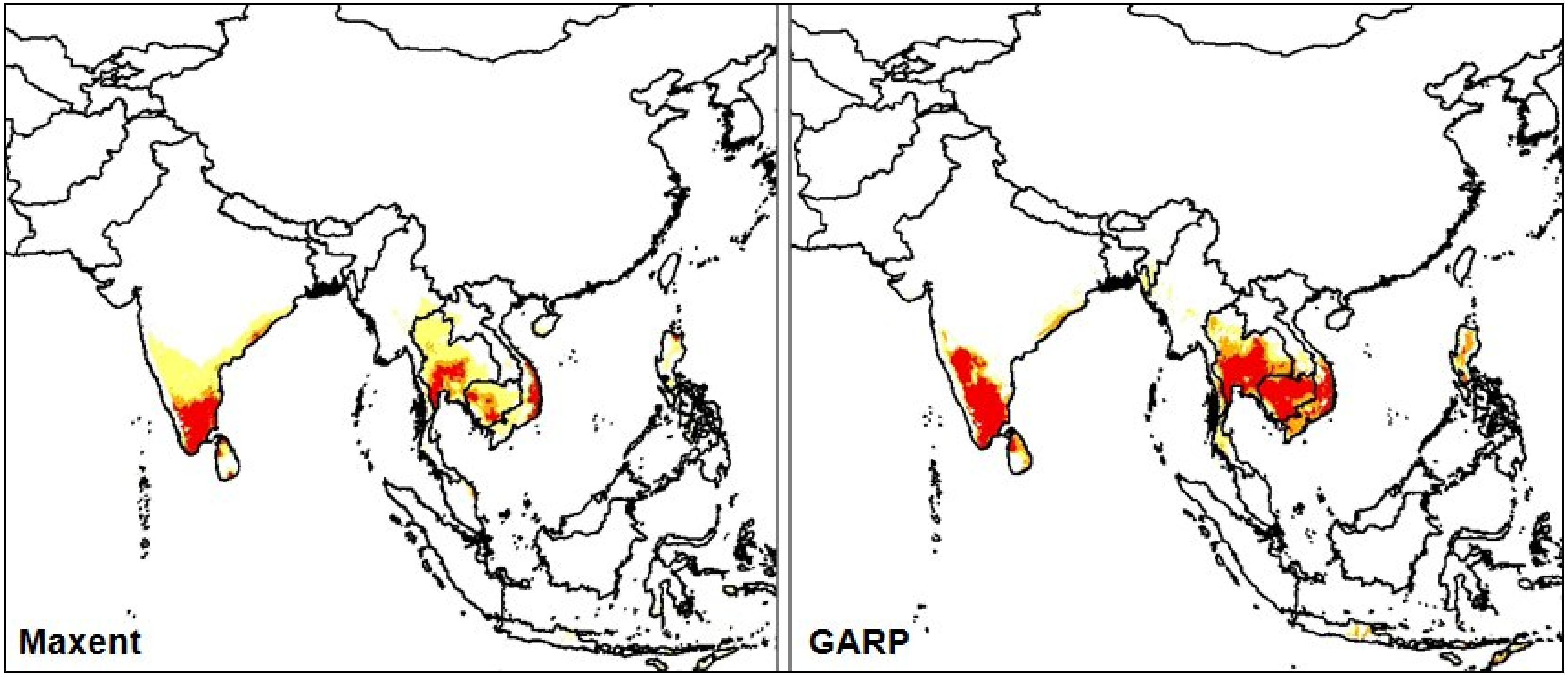


Figure 8. 4: Climatic suitability for *R. iceryoides* in its native range, Southern Asia, using genetic algorithm for rule-set prediction (GARP) and maximum entropy method (Maxent). White, predicted no suitability, as indicated by the LTPT thresholding; shades of orange indicate higher levels of climatic suitability (chosen arbitrarily), with red the highest strength for climatic suitability.

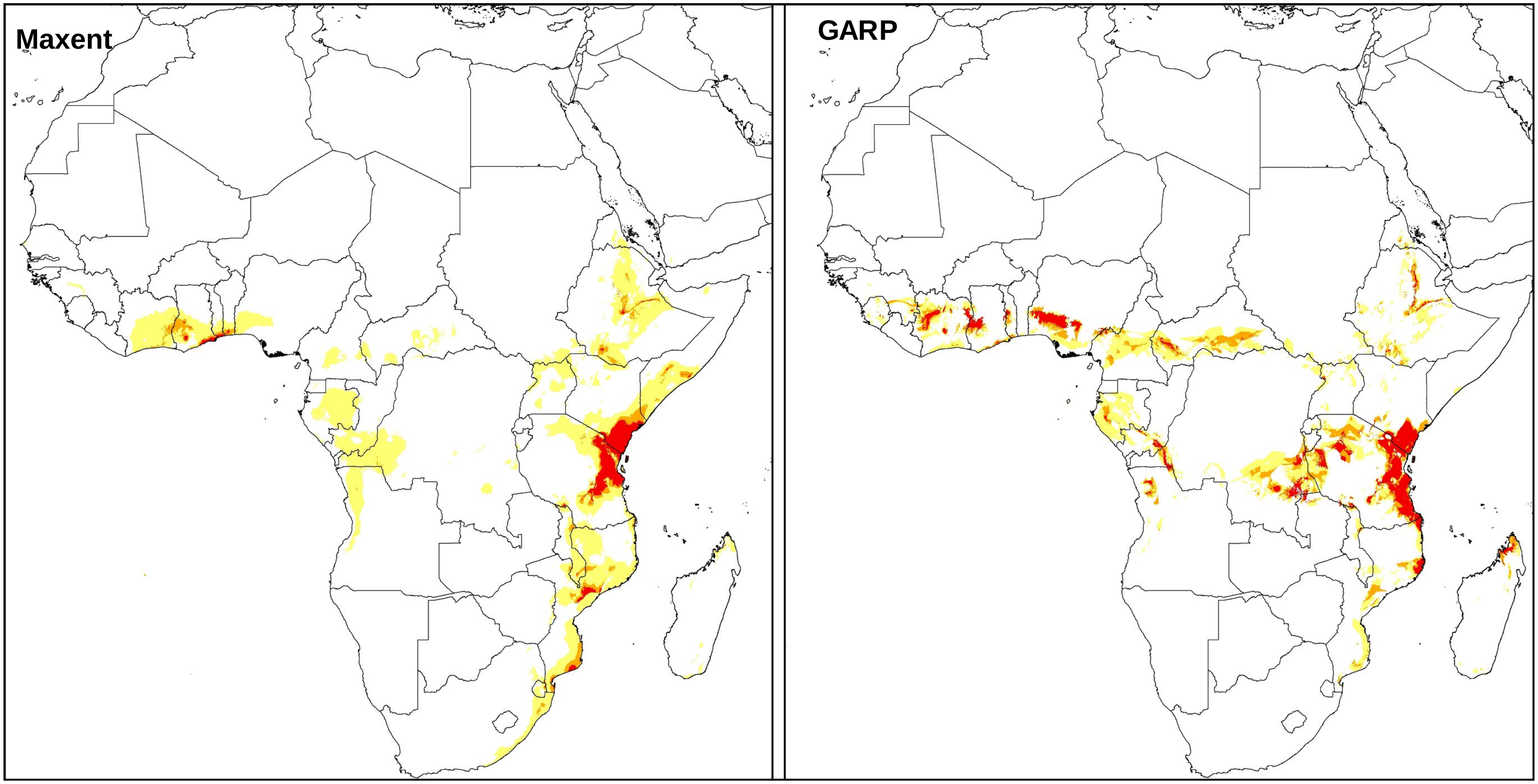


Figure 8. 5: Climatic suitability for *R. iceryoides* in its non-native range, Africa, (using India distribution records), using genetic algorithm for rule-set prediction (GARP) and maximum entropy method (Maxent). White, predicted no suitability, as indicated by the LTPT thresholding; shades of orange indicate higher levels of climatic suitability (chosen arbitrarily), with red the highest strength for climatic suitability.

The GARP model predicted higher suitability in areas farther removed from the coast, particularly in Kenya, Tanzania, Côte d'Ivoire, Ghana, Togo, Benin, Nigeria, Cameroon, Somalia and Mozambique. Also, the latitudinal limits identified by GARP predictions were broader, especially north-wards, with high suitability being predicted for much of the Tanzania, Côte d'Ivoire, Ghana, Togo, Nigeria and Cameroon; these differences were less dramatic once lower thresholds were considered in Maxent. The climatic suitability of parts of East and West Africa, and their close proximity to Central Africa underlines the threat of invasion to this area by this emerging polyphagous pest. Additionally, a large part of Mozambique also appears to be vulnerable to invasion. The regions identified above are at risk of unintentional introductions of *R. iceryoides*, and when intentionally introduced might flourish. Madagascar's proximity to the primary source of *R. iceryoides*, the Indian Subcontinent, also puts it at high risk of colonization.

Climatic suitability for *R. iceryoides* globally using India and Africa distribution records revealed that GARP predicted somewhat broader potential distributional areas in South and Central, and the Caribbean that appeared climatically similar to the regions of the state of Tamil Nadu, India and Africa, not yet colonized. For the GARP model, seven fairly large areas are indicated as being highly suitable, which covers southern and eastern Brazil, Southern Mexico, Cuba, Venezuela, Honduras, Guatemala and Haiti. Small isolated regions along the slopes of the Andes mountain range in Bolivia, Guyana and Suriname as well as in Nicaragua are also indicated as being suitable (Figure 8.6). In contrast, Maxent indicated that much of Mexico, Guatemala, Honduras, Nicaragua, Cuba, Haiti, Domanica Republic, Venezuela, Guyana, Suriname, Brazil, Paraguay and Bolivia were climatically similar to Africa and India, although much of this was marginal similarity. Only Maxent indicated that Paraguay was climatically suitable or similar to Africa and India. The GARP model indicated a broader region of climatic similarity than the Maxent in all part of the world predicted by the two models. One of the regions of highest suitability in the Maxent model is located in eastern Brazil whereas the region of highest similarity in the GARP model was found in Cuba. Northern Territory of Australia to a lesser extent was also predicted as a potential climatically suitable zone by GARP.

Non-native populations of *R. iceryoides* in Africa were used as a means of testing model predictivity regarding suitable areas for the species globally. In both cases, model predictions were considerably better than expectations under random (null) models (binomial tests, both $P <$

10^{-14}), indicating that both approaches offer significant predictivity regarding the global potential distribution of the species. Inspecting ROC plots for the two model predictions based on independent testing data on a landscape distant from that where the models were trained, it is clear that the two models are slightly similar in performance.

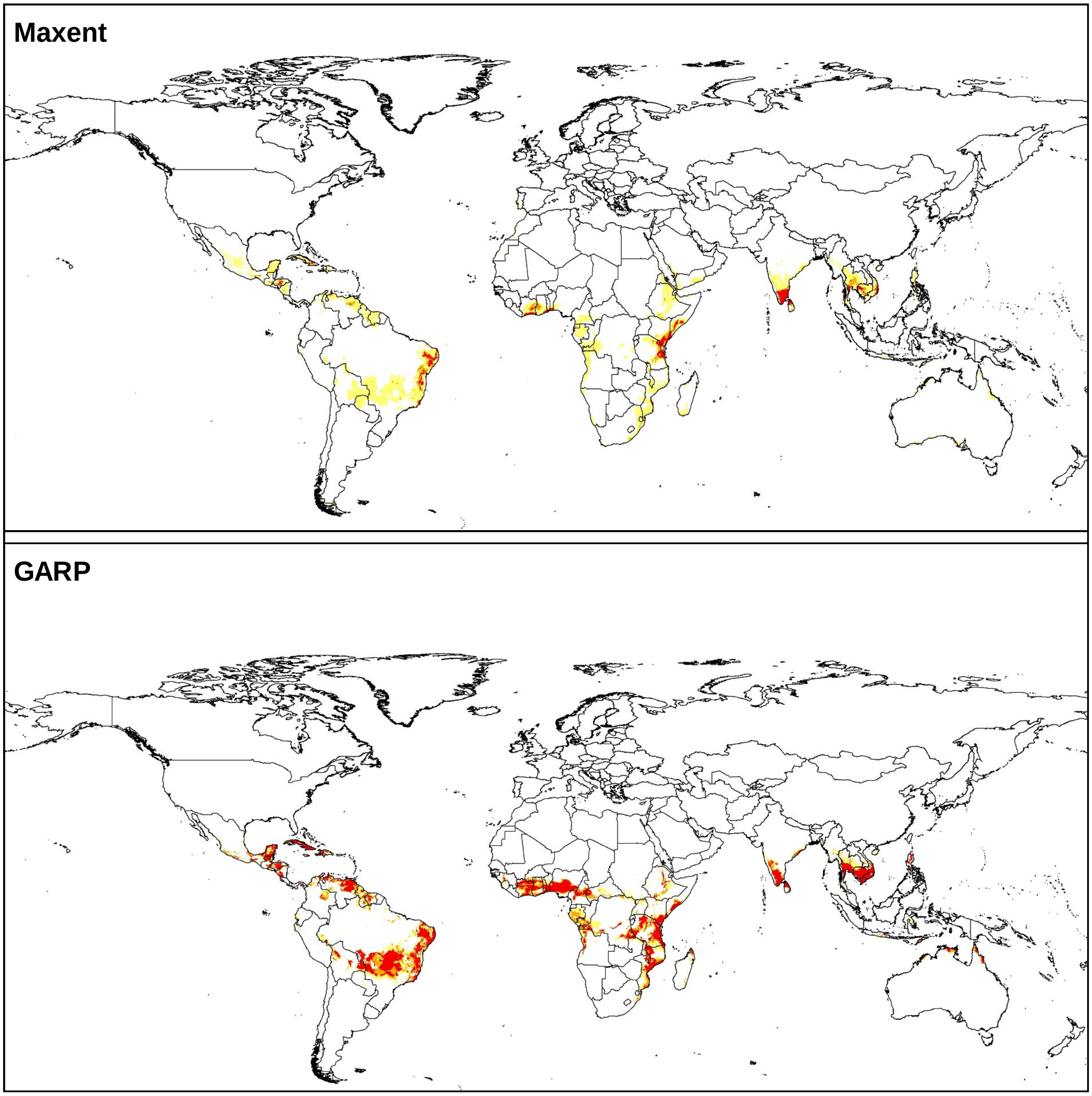


Figure 8. 6: Climatic suitability for *R. iceryoides* globally, (using India and Africa distribution records), using genetic algorithm for rule-set prediction (GARP) and maximum entropy method (Maxent). White, predicted no suitability, as indicated by the LTPT thresholding; shades of orange indicate higher levels of climatic suitability (chosen arbitrarily), with red the highest strength for climatic suitability.

8.3.7 Biological interpretation

Based on jackknife analyses for models including each variable alone, ‘temperature seasonality’ was the bioclimatic variable that contributed most to the ecological niche model with the highest predicted suitability for the likelihood of establishment of the parasitoids in Africa, followed by ‘annual temperature range’, ‘annual precipitation’, and ‘precipitation of the coldest quarter’ while the maximum temperature of the warmest month was the least important. This ecological niche model prediction does in fact appear quite similar to the distributions of the species and beyond. The areas indicated by the models contain conditions suitable for both pest and their natural enemies, because successful establishment may also be influenced by other factors, biotic and abiotic due to some geographical barriers. Maxent and GARP predictions are continuous, and within those areas suitable for the species, they further distinguish between those with a marginally (but sufficiently) strong prediction versus those with increasingly stronger predictions. This represents an important advantage for both models, and explains part of their consistently higher AUC values. Most strikingly, the models correctly indicate an expansive region of unsuitable environmental conditions for the pest and its associated natural enemies.

These parasitoid species occurs naturally in India and most of the predictions for the success of this species to become established in Kenya and Tanzania were correct. This was not surprising as there were many countries in Africa and Southern Asia that climatically matched the area-of-origin and suggests a high likelihood of establishment in these regions. Climatic modeling of potential distribution by Maxent and GARP models showed that the predicted optimal climate suitability of the parasitoids in Southern Asia and Africa (introduced home) are confined mainly to the coastal zones, where temperatures are warmer and humidity was high with the exception of desert regions where dry stress and/or hot stress might limit their establishment.

8.4 Discussion

Rastrococcus iceryoides is a primary mealybug pest of mango in India but the overall survey in the State of Tamil Nadu, India demonstrates that although *R. iceryoides* is widely distributed throughout the mango belt, they are certainly not of anything other than local importance. This is because of the existence of a complex of natural enemies capable of suppressing the mealybugs population (Tandon and Lal, 1978; Narasimham and Chako, 1988). In this study the parasitoid community was composed of eleven primary parasitoid species recovered from *R. iceryoides*, out of which seven are new records. Among these parasitoid species, *P. viridis* and *A. chryos* were clearly the most abundant and widely distributed primary parasitoids of *R. iceryoides* in the State of Tamil Nadu. Both parasitoids are highly host specific, cosmopolitan, solitary endoparasitoids of *R. iceryoides* (Narasimham and Chacko, 1988).

However, despite their important role in suppressing the populations of *R. iceryoides*, they have never been used in biological programmes (classical or inundative) against *R. iceryoides* anywhere in the world. Given the high parasitism rate of these two parasitoids across the different host plants in the field, they should be considered as candidates for biological control against *R. iceryoides* in regions other than India where they are scarce or absent in new-association strategy. *Praleurocerus viridis*, in particular has been attributed with remarkable regulation of population of *R. iceryoides* on guava resulting in complete disappearance of the pest in some part of the year in India (Mani and Krishnamoorthy, 1998b**)**. Pre-release quarantine evaluation of these parasitoids on *R. iceryoides* is now needed although it must be noted that for a parasitoid to successfully establish in a locality it must overcome a series of challenges including interspecific competition with native parasitoid species, host plant-parasitoid interaction in the invaded range among others.

The findings from the field survey reveal that these two parasitoids coexist together and also complement each other in all the localities and the host plants surveyed. Among all the host plant species sampled, the highest percent parasitism by *P. viridis* and *A. chryos* was frequently found on mango plants and occasionally on *C. pentandra, P. guajava* and *T. grandis*. Our study provides information that predicts the distribution of parasitism across host plants, which is crucial for rational conservation and augmentation of the parasitoid species. The remaining parasitoid species played a minor role and are observed to have a patchy distribution occuring in very low numbers throughout the survey. The number of new records generated from this study emphasizes the lack of attention to this pest in India because of its minor pest status. Despite the wide geographic distribution of *R. iceryoides,* this paper represents the first comprehensive study of the field-population analysis of the pest, host plant preferences and the possible role of its associated parasitoids.

The two niche modeling algorithms used in this study present a similar overall picture, although Maxent is somewhat more conservative. Comparing with the updated Koppen-Geiger climate classification (Kottek et al., 2006), most suitable areas identified by our models fall within the Equatorial climate categories (minimum temperatures (≥ 18°C)). Our result suggests that *R. iceryoides* prefers hot and humid environments with low annual precipitation, although it does not have to be continuous. This equatorial climate type should have a distinct dry period with driest-month precipitation of 0 mm and wettest-month precipitation of < 300 mm rainfall (C. M. Tanga, unpublished data). Population dynamics study for *R. iceryoides* conducted in the coast region of Tanzania for 77 weeks from June 2008 to June 2010 showed that the Coastal region of Tanzania situated in the transition zone between bimodal and unimodal rainfall belts with a distinct dry season is suitable for *R. iceryoides* survival all the year-round, although populations increase dramatically during the dry season between December to February (Tanga, unpublished data).

Mealybugs are generally known to be excellent invaders (William, 1986; Williams and Granara de Willink, 1992; Sagarra and Peterkin, 1999; Kumashiro et al., 2001; Larrain, 2002; Neuenschwander, 2003; Williams, 2004; Abbas et al., 2005; Zaka et al., 2006; Akintola and Ande, 2008; Muniappan et al., 2008; Charleston and Murray, 2010; Winotai et al., 2010; Muniappan et al., 2011) but for majority of the species, no analysis of their global invasive potential has yet been undertaken. Species distributions represent the combined effects of abiotic requirements of species, biotic interactions with other species, and limitations on dispersal (Soberon and Peterson, 2005) and ecological niche modeling provides a means of evaluating ecological factors that participate in delineating a species' geographic distribution. These analyses represent an attempt to address this question making use of computational tools to model the ecological niche of *R. iceryoides* and predict its worldwide potential distribution. The tests in three regions (Native range, Southeast Asia, Central America, South America, West Africa and East Africa) suggest that these areas are indeed climatically suitable for *R. iceryoides* populations' establishment and useful in predicting biological agent introduction. This situation contrasts with the ‘‘pest status’’ of *R. iceryoides* populations in the Central and South America (especially Mexico, Cuba, Guatemala, Nicaragua, Guyana, Suriname, Honduras, Haiti, Bolivia and Brazil) as well as in Africa (especially Cameroon, Nigeria, Benin, Togo, Ghana, Côte d'Ivoire, Central African Republic, Mozambique, Gabon, Congo, Angola, Somalia and Ethiopia), where our models show high agreement predicting potential presence. These areas predicted as potentially suitable are novel relative to known *R. iceryoides* occurrences but implies that the species is likely to expand its range further in these regions if the six barriers that a species has to overcome to become invasive can be breached by the mealybug (Richardson and van Wilgen, 2004). Given the apparently high dispersal capabilities of this species (C.M. Tanga, unpublished data), particularly in light of huge pockets of population outbreaks now developing in Tanzania and Kenya that may act as new nuclei for expansion, these areas should be considered susceptible to invasion by *R. iceryoides*. Madagascar's proximity to the primary source of *R. iceryoides*, the Indian Subcontinent, also puts it at high risk of colonization. Finally, these distributional possibilities may be in the process of shifting, given current global shifts in climates, which would in general act to broaden the species' distributional potential at the equatorial limits of its pre-change distribution.

Given the apparent rapid spread of *R. iceryoides* in Tanzania and Kenya, and its impact on local horticulture, the risk of this species being introduced, establishing and invading other regions of the world should be considered. The two models used in this study clearly indicate regions of the world that are climatically suitable for *R. iceryoides*, but this does not implies that these regions will unavoidably become invaded by the species. This is because for a species to invade in a new region, it must overcome a series of challenges (Richardson and van Wilgen, 2004; De Meyer et al., 2008). Our analyses are only able to assess one of them, which is the likelihood of the species surviving in the new region. Therefore, as this study has not explored the entire invasion challenges reported by Richardson and van Wilgen (2004) that non-native species face, these maps should not be interpreted as maps of invasion risk or likelihood of establishment. However, it is important to note that regions presenting suitable climatic conditions for the species, as indicated by the models, are more likely to be invaded than regions that have a low suitability. Most of the regions highlighted as highly suitable by the models include areas already invaded by the species, giving some confidence in the models. Several regions in the native-range of the pest, India: Andaman and Nicobar Islands, Assam, Bihar, Delhi, Gujarat, Jammu and Kashmir, Kerala, Madhya Pradesh, Uttar Pradesh and West Bengal, has been reported by Williams (1989) to harbour populations of *R. iceryoides* but were not identified to be climatically similar using the two climate matching approach. This could probably be attributed to the fact that a climate matching approach that only samples part of the distributional range of the pest has been reported to give an incomplete description of the species fundamental niche, which results in a spatial prediction that described only part of its potential range (Wharton and Kriticos, 2004; Soberon and Peterson, 2005). However, these models clearly identified Karnataka where the two parasitoids have been reported to coexist as climatically similar to Tamil Nadu (Noyes and Hayat, 1994).

In order to avoid prediction errors due to the presence of its natural enemies, wherever possible, when modeling the potential climatic suitability, use is made of distribution information from the species' exotic range, as well as its native range (Kriticos and Randall, 2001). Given that *R. iceryoides* has a much broader ecological niche in its native range, a more thorough inventory for the species in its native region, or at least detailed inspection and re-evaluation of *R. iceryoides* records from the region, might present additional information that could improve the models. Currently, however, such information is not available.

Predicting the probability of successful establishment and invasion of alien species at a broad scale, by climatic matching is a priority for the risk assessment. These models identified several areas in Africa having maximum suitability for the invasive mealybug, *R. iceryoides*, entailing a maximum risk of successful invasion. *Rastrococcus iceryoides* has already been introduced into three of these areas and in some of them invasion is ongoing. The observation stresses the importance of implementation of an early detection and eradication plan of this alien invasive species within the areas affected and showing high suitability for the pest. However, the low suitability of some areas of Africa does not mean that *R. iceryoides* can be introduced without any risk of invasion. The approach presented here can be used to focus on preventive monitoring in areas that are more at risk. Indeed, being a correlative method, these approaches does not consider directly the effects of biotic interactions that are known to be fundamentally important for the establishment and spread of introduced species or their natural enemies.

It is very useful for estimating climatically suitable areas for biocontrol introductions, for estimating the effect of climate on invasions by the exotic mealybug, *R. iceryoides* and for conservation decisions. Maxent and GARP accurately predicted when there was going to be successful establishment of the parasitoids probably because these species were overridingly influenced by climatic conditions. The accuracy of the predictions also depended on the preciseness of the physiological parameters derived for the species. This emphasizes the importance of determining the exact original distribution of the species to accurately derive the parameters and iterate the models until the highest values are obtained. Both models operate on a wider spatial and temporal climatic scale with less emphasis on microclimatic factors such as amount of solar radiation, degree of cloudiness or frequency of wind, all of which are important to a small ectotherm (Unwin and Corbet, 1991). Although climate may appear similar between two sites, other factors such as distance from the sea and elevation can also influence local fluctuations in temperatures and rainfall during the year. This could explain the disparity in the models in equally predicting the potential establishment of the pest and parasitoids in different regions of Africa and the world at large. The models for the invasive pest did correctly predict the likelihood of establishment of the parasitoid species in new geographical areas of Africa and suggested that overall climate might be the sole determinant of establishment, although other factors can play a major role.

Because the Maxent and GARP models were fitted primarily to the currently known native distribution, the model probably represents the realized niche of *R. iceryoides* and, therefore, it is likely to be partially conservative because of the presence of biotic constraints namly competitors, predators and natural enemies in the native range (Davis et al., 1998). As noted by Brown et al. (1996), where biotic factors are constraining ranges, they are most likely to manifest themselves in those regions of the potential range that are relatively warm and wet, and where environmental resources such as heat and moisture are not limiting. Hence, as the warm and wet range limits for *R. iceryoides* are not encountered in Kenya and Tanzania, the model can be considered to be reasonably reliable, and potential climatic range expansion due to biotic release is unlikely. Therefore, for critical decision making, it is prudent to consider the sensitivity of the affected system to be the probable full range of expected climatic suitability.

CHAPTER NINE

General discussion, Conclusion and Recommendations

9.1 General discusion and conclusion

Mealybugs (Hemiptera: Pseudococcidae) are important group of phytophagous insects that cause significant damage on a variety of horticultural crops worldwide. In Africa, *R. invadens* and *R. iceryoides* are regarded as two important exotic mealybug species native to Southern Asia that commonly colonize mango, *M. indica*. The former devastated mango production in West and Central Africa, but was brought under biological control through the introduction of the exotic parasitoid *Gyranusoidea tebygi* Noyes (Hymenoptera: Encyrtidae) from India. Owing to the devastating nature and the socioeconomic impact that *R. invadens* had on the livelihood of farmer in West and Central Africa, it has been the subject of many studies and a considerable amount of information was gathered and documented with regards the pest host range, geographical distribution and it natural enemies. *Rastrococcus iceryoides* on the other hand is so far restricted to East Africa (mainly Tanzania and coastal Kenya) and northern Malawi where it has remained as a major pest of *M. indica*.

Due to the novelty status of *R. iceryoides* compared to *R. invadens* there had been little known about the biology and ecology of this pest and its associated natural enemies that would inform development and implementation of any management strategies against this pest in the invaded areas in Africa. Consequently, this study was initiated to study the bio-ecology of *R. iceryoides*, assess the role of indigenous natural enemies in suppressing the pest; and explore for effective co-evolved natural enemies in its aboriginal home for introduction and release in target African countries. The effect of climatic factors on the seasonal and annual dynamics of the pest and its natural enemies in Tanzania was also investigated. Finally, laboratory studies were carried out to unravel the effect of host plants on the development and reproduction of *R. iceryoides* and *A. pseudococci* as well as the interference of *Oecophylla longinoda* in the biological control of *R. iceryoides* by *A. pseudococci*.

Chapter 3 describes the distribution, host-plant relationship and natural enemies survey of *R. iceryoides* in Kenya and Tanzania. The results showed that *R. iceryoides* is widely distributed across the coastal belt with heavy infestation levels extending up to 145 km inland in Kenya and 851 km in Tanzania. In Kenya, the high level of *R. iceryoides* infestations in Matuga is particularly disturbing because this locality represents one of the key mango production areas in the country while in Tanzania, the high level of attack on mango in Kinondoni and Mkuranga demands urgent management attention given the ongoing expansion of the horticulture industry and particularly mango in the region. *Rastrococcus iceryoides* was recorded from 29 plants species including cultivated and wild host plants from 16 families, 21 of which are new records for Kenya and Tanzania. The wild host plants recorded during the survey harbouring this invasive pest occur throughout the year and evidently ensured that sufficient reproductive bases existed for *R. iceryoides* as a persistent source of spread of the mealybug during the off-season when cultivated hosts were not adequately suitable for attack. The wide range of plant families attacked by *R. iceryoides* strongly suggests that this pest is an emerging polyphagous species capable of surviving in a wide range of host and can jeopardize the lucreative export of these crops from this region. There was evidence of altitudinal limits of distribution of *R. iceryoides* in both countries with indications that it is better adapted to low and mid altitudes, which exactly match its distributional range in its native home of India. Although the precise date of introduction of *R. iceryoides* to both Kenya and Tanzania is unknown, it is highly probable that current distribution and spread of the mango mealybug populations is assisted by fruits and plant materials transported across the region in commercial and private vehicles as is the case with the introduction of *R. invadens* into West and Central Africa. Although, six primary parasitoid species were recovered from *R. iceryoides* with some representing new associations only *A. pseudococci* showed promising performance (~20% parasitism). This study provides information that predicts the distribution of parasitism across host plants, which is very crucial for rational conservation and augmentation of the parasitoid. Therefore, conservation of this parasitoid (through habitat management), and its augmentation with periodical releases of laboratory reared wasps, will enhance the effectiveness of this parasitoid in suppressing the population of *R. iceryoides* in areas with low infestations.

In laboratory host preference studies carried out in chapter 4, results showed that although the six host plant species tested supported the development of *R. iceryoides*, they differed significantly in their suitability. *Mangifera indica*, *C. moschata*, *P. aculeata* and *C. cajan* were the most preferred host plants in view of improving laboratory mass rearing of this pest. The suitability of host plant species described in this study should help in the making of informed decisions regarding the invasion risk associated with the insect in countries where *R. iceryoides* has not invaded. Base on these findings, it is likely that more important host plants will be shown to support development of *R. iceryoides* as further studies are carried out. It is mostly likely that this information will become increasingly important as *R. iceryoides* continues to colonize new geographical areas. In addition to helping predict the population growth potentials, the life table findings from the different host plants in this study have practical implications to more efficient and effective production of the mealybugs for parasitoid mass rearing and releases.

Chaper 5 describes the effect of five host plant species on host acceptability for oviposition (as measured by % parasitized nymphs) and suitability (as measured by day to mummification, percent mummified host, percent parasitoid adult eclosion, sex ratio and pre-maginal developmental time) for immature development of *A. pseudococci***.** Effect host plant on fitness trait (parasitoid size, egg load and longevity) and life table parameters was also assessed. Although *A. pseudococci* accepted mealybugs regardless of the host plant species on which they were reared and exposed, the level of acceptability varied significantly, with the highest and lowest percent parasitized nymphs on butternut and weeping fig respectively. Host suitability was also strongly affected by the host plant and largely mirrored host acceptability for all the parameters evaluated. To judge from the R_o and r_m values, mass rearing would be suitable on the four most optimal host plants (*M. indica*, *C. moschata*, *P. aculeata* and *C. cajan*). These observations provide important information for future management of the mealybug. On non-crop host plants such as *P. aculeata*, targeted management methods including parasitoid conservation and augmentation on this host should result in the build-up of the parasitoid populations ahead of the mango fruiting season before heavy infestations on the mango fruits start. Overall, the information provided in this investigation should be essential in understanding the dynamics of the parasitoid on different host plants and form vital part of an integrated management plan that allows for targeted suppression of the mealybug in East Africa.

Chapter 6 revealed that *O. longinoda* showed aggressive behaviour toward *A. pseudococci,* which greatly affected the foraging activities and significantly reduced the oviposition success of *A. pseudococci*. Worker ants were also observed to remove mummified mealybugs as food source, which resulted in significantly reduced percentage of adult parasitoid eclosion. Although, *A. pseudococci* manifested some behavioural responses to escape ant aggression, they were significantly attacked and killed by worker ants. Therefore, mass release programmes of *A. pseudococci* to control low mealybug infestation in ant infested mango orchards via inundative releases should be done with considerable caution as this may not be effective depending on the ant species present. These results are important to growers who should be aware of the species of ants foraging in their orchards.

The studies on the seasonal and annual abundance of *R. iceryoides* carried out in chapter 7, indicates that the activities of *R. iceryoides* were governed by temperature, relative humidity and rainfall. The results indicated that the population dynamics of *R. iceryoides* followed an annual cycle which is synchronized with the mango fruiting season, with a peak incidence occurring during the dry season (December-February) on all plant parts at both sites. Mealybug incidence was low throughout the rainy season (March to October) and this prolonged period of low infestation, thus provide a wider window of opportunity for management. Both predators and parasitoids demonstrated the highest level of activities between December and February for each year. The incidence of the predators and parasitoids were observed to increase correspondingly to that of *R. iceryoides* infestation, which clearly illustrates the enhance ability of the parasitoids and predator populations to track the host's population. However, they were not adequate to affect the pest population probably, because of the rapid developmental time, high survival rates, enormous reproductive capacity, and lack of early detection and control of the mealybug population.

The survey carried out in chapter 8 revealed that *R. iceryoides* is a primary mealybug pest of mango in the State of Tamil Nadu, India. Although, *R. iceryoides* is widely distributed throughout the mango belt, they are certainly not of anything other than local importance. This is because of the existence of a complex of natural enemies capable of suppressing the mealybugs population. This survey reported a total of eleven primary parasitoid species, out of which seven are new records. Among these parasitoid species, *P. viridis* and *A. chryos* were clearly the most abundant and widely distributed species. Both parasitoids are highly host specific, cosmopolitan, solitary endoparasitoids of *R. iceryoides*. However, despite their important role in suppressing the populations of *R. iceryoides*, they have never been used in biological programs (classical or inundative) against *R. iceryoides* anywhere in the world. Given the high parasitism rate of these two parasitoids across the different host plant species in the field, they should be considered as candidates for biological control against *R. iceryoides* in regions other than India where they are scarce or absent in new-association strategy. Priority areas identified by the two ecological niche models, GARP and Maxent for further search for natural enemies in Southern Asia were Sri

Lanka, Bangkok, Cambodia, Thailand, Philippines, and to a lesser extend Northern Territory of Australia. In India, additional priority areas determined for further exploration of natural enemies of *R. iceryoides* included three additional states: Karnataka, Orissa and Andhra Pradesh. The potential worldwide distributional range presented by the two models is indicative of the likely magnitudes of the expected changes in the potential range of *R. iceryoides* in response to climate suitability, highlighting areas at high risk. The extensive areas of potential mango production reported in Central and South America identified by the models has not yet invaded by this invasive pest. This suggests that proper policies should be formulated and implemented in the different areas to limit the expansion of *R. iceryoides* and for early detection of the pest. In Africa, the potential distributional range described by the models are of interest to improve the communication with the general public, policymakers and other stakeholders, who wish to understand the potential spatial extent of the invasion so that the impacts can be gauged and appropriate ameliorative measures, can be considered prior to the invasion reaching valuable mango production countries.

9.2 Recommendations for application and future study

1) Host range of invasive phytophagous insects is a dynamic phenomenon particularly as a result of climate change. It is very likely that the host plant list presented here may not be conclusive data for *R. iceryoides* and may change over time. Further survey activity is therefore strongly recommended.
2) Areas without or with low pest prevalence identified in this study might benefit from a simple community-based quarantine system such as the restriction of uncontrolled movements of plant materials. So material from altitudes outside this range may offer potential for *R. iceryoides*-free source of planting material, especially if the production in these areas deploys an integrated approach to further minimize infestation risk. These findings are also likely to be of great significance in other regions of Africa, in case this pest is accidentally introduced into new regions where their natural enemies are absent as they may become particularly injurious.
3) Although several indigenous parasitoids have been found attacking the pest in Kenya and Tanzania, they are unable to keep the pest populations below economically damaging levels. Therefore, as chemical and mechanical measures have proved inefficient,

biological control remains the most efficient and cost-effective option, and would require additional species of efficient coevolved natural enemies from the aboriginal home of *R. iceryoides*, India where it is rarely a pest and under excellent natural control to solve this problem in Africa.

4) Host plant species played a significant role in influencing the life history parameters of *R. iceryoides* and the parasitoid. These differences may be attributed to nutritive factors; allelochemical compounds and physical differences in stem structures, although none of these factors involved in the variation of plant suitability was investigated.
5) This study therefore, concludes that *R. iceryoides* can adapt to the transferred host plant gradually, based on an initial adverse effect on development and reproduction during a few generations after shifting host plants. It is most likely that this information will become increasingly important as *R. iceryoides* continues to colonize new geographical areas in the African continent.
6) The findings from the tritrophic interactions between host plants, *R. iceryoides* and *A. pseudococci* demonstrate that the performance of *A. pseudococci* varied significantly on the various host plants. Therefore, for any successful biological control of the mealybugs by *A. pseudococci*, the host plant species should be taken into consideration as an important factor in the success of the biocontrol agent.
7) Disturbance by *O. longinoda* greatly affected the foraging activities and significantly reduced the oviposition success of *A. pseudococci*. Collectively, these data suggest *O. longinoda* could have a detrimental effect on populations of *A. pseudococci* in mango orchards. Therefore, ant populations should be suppressed or controlled prior to release of parasitoids to suppress populations of ant-tended Hemiptera in mango orchards.
8) The studies on the seasonal and annual abundance of *R. iceryoides* in relation to weather factors demonstrated that reproductive activities of *R. iceryoides* is continuous and its occurrence throughout the year has serious implications in their management. However, given that at lower temperatures during the southwest monsoon (June – October), populations of *R. iceryoides* declined sharply it thus provides a wider window of opportunity for management. Therefore, control programs can take advantage of the fact that the development of *R. iceryoides* is dependent on the prevailing weather conditions.

9) The two parasitoid species (i.e., *P. viridis* and *A. chryos*) identified during the foreign exploratory survey in the native home of the pest in India, were found to be very effective against the pest but have never be introduced in Africa for biological control of *R. iceryoides*. As growers await approval for possible importation of these important biocontrol agents, there is an urgent need to continue doing research on the development of *A. pseudococci* for possible suppression programs in low infestation areas in both countries.
10) Because *R. iceryoides* apparently occurs in low abundance in its native range of India, it indicates that the pest is under biological control, making the insect an excellent candidate for classical biological control in Kenya and Tanzania. However, further laboratory bioassays are necessary to test the efficacy of the dominant parasitoid species to fully incorporate them into IPM programs as prime candidate for releases.
11) The seasonal/annual population studies provide a baseline data for development of geospatial models for predicting areas where *R. iceryoides* can potentially establish. There is therefore need for the development of an early warning system especially for regions where the pest has not invaded.
12) Awareness campaigns and education of the growers about the importance of mealybugs in general and availability of management practices will be crucial for dealing with all the mealybug pests. Farmer field days and field demonstration of activities of available IPM packages should form an integral part of the mealybug pest management programs.
13) Presently management effort by mango growers in Kenya and Tanzania concentrates on the cultural methods, mainly cutting and burning of heavily infested plant materials because of reluctance of the continuous use of pesticides. As a matter of fact this practice must be revised because it is actually detrimental to predators' larvae and the parasitoid developing inside host body that died out of burning and if the infested plants are buried in the soil the parasitized mealybugs also get crushed.

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