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Host-plant relationships and natural enemies of the invasive mealybug, *Rastrococcus iceryoides* Green in Kenya and Tanzania

C.M. Tanga^{1,2}, S. Ekesi¹, P. Govender² & Samira A. Mohamed¹

¹International Centre of Insect Physiology and Ecology (*icipe*), PO Box 30772-00100 GPO, Nairobi, Kenya; Tel. +254-20-8632000; Fax: +254-20-8632001/2;

²Department of Zoology and Entomology, University of Pretoria, South Africa

Running title: Bio-ecology of *Rastrococcus iceryoides*

***Correspondence to:** S. Ekesi, **Tel.:** + 254-20-8632150; **Fax:** +254-20-8632001/2; **Email:**

sekesi@icipe.org

Abstract

The invasive mango mealybug, *Rastrococcus iceryoides* Green (Hemiptera: Pseudococcidae) believed to be native to Southern Asia has rapidly invaded Kenya and Tanzania. A survey was carried out from February 2008–July 2009 to study its geographical distribution, host plant relationships and associated parasitoids in both countries. Our results infer that *R. iceryoides* is widely distributed across the coastal belts of both countries. *Rastrococcus iceryoides* was recorded from 29 cultivated and wild host plants from 16 families. Twenty-one of these host plants are new records. Among the cultivated host plants, *M. indica* (8153.6±19.2/20 twigs and 6054.3±29.2/80 leaves in Kibaha, and 2979.3±33.8/5 fruits in Kinondoni) and *Cajanus cajan* (L.) Millspaugh (1452.2±44.7/80 leaves and 4672.3±54.7/twig in Morogoro) recorded the highest levels of infestation. *Parkinsonia aculeata* (7892.3±25.1/20 twigs, 11.6±1.25/80 leaves and 42.2±5.1/5 fruits in Kinango), *Caesalpinia sepiaria* Roxb (266.3±6.3/80 leaves and 3116.1±17.5/20 twigs in Kinondoni) and *Deinbollia borbonica* Scheff., (215.7±10.3/80 leaves and 2253±22.9/20 twigs in Kibaha) were found to be the most heavily infested wild host plants. Six parasitoid species were recovered and are reported here for the first time to parasitize *R. iceryoides*. *Anagyrus pseudococci* Girault was the most dominant species accounting for 21% parasitism on *M. indica* and 20% parasitism on *P. aculeata* in Tanzania and Kenya, respectively. Despite this, the ability of the parasitoid to regulate the population of *R. iceryoides* was inadequate. Therefore, there is a 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.

Keywords: Invasive mango mealybug, distribution, infestation levels, parasitoids, biological control

Introduction

Mealybugs (Hemiptera: Pseudococcidae) are an 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 important exotic mealybug species native to Southern Asia that commonly colonize mango, *Mangifera indica* L. (Anacardiaceae). The former devastated mango production in West and Central Africa, but was brought under control through the introduction of the exotic parasitoid *Gyranusoidea tebygi* Noyes (Hymenoptera: Encyrtidae) from India (Noyes 1988; Bokonon-Ganta and Neuenschwander 1995). Owing to the devastating nature and the socioeconomic impact it had on the livelihood of farmers in West and Central Africa, *R. invadens* has been the subject of several studies and a considerable amount of information has been gathered and documented such as its host range, geographical distribution and natural enemies (Agouunké et al. 1988; Willink and Moore 1988; Williams 1989; Matokot et al. 1992; Bokonon-Ganta et al. 1995; Tobih et al. 2002). Most recently, *R. iceryoides* has been reported to cause localized failure of the mango crop (i.e., heavy quality losses and low production of mango fruit) in part of Malawi in 1992 (Luhanga and Gwinner 1993; CABI 2000). Although, several authors (Williams 1989; CABI 2000) reported the presence of *R. iceryoides* in Kenya and Tanzania, there is little documented information on the geographical distribution of the pest, no quantitative impact data on host plant species and yield losses are also largely unquantified in the invaded areas.

The nymphs and adults of mango mealybug species sucks sap from tender leaves, young shoots, inflorescences and fruits. As a result, the affected inflorescences are shrivelled and get dried. In case of severe attack, fruit settings are severally affected with majority of the mango fruit-lets shedding off pre-maturely. They also excrete sugary honeydew on which sooty mould develops on leaves, shoots and fruits, thus causing a drastic reduction in the photosynthetic activity in the plants, worsened by the premature drop of mature leaves. The presence of the resultant thick black layer of sooty mould on the fruits also severely affects their marketability, as export opportunities are often impaired due to quarantine regulations (CPC 2002). Infestation levels by mealybugs of the genus *Rastrococcus* on fruits has been reported to cause significant

reduction in the weight and size of fresh mango fruits, which is reflected in significant reductions in the ash content, crude fibre and reducing sugar levels of both ripe and unripe fruits (Tobih et al. 2002) and protein, fat and carbohydrate levels (Pitan et al. 2002). In village homesteads, heavy infestations of mango trees usually render them unsuitable for shade due to leaves dropping sticky honeydew around the canopy of the tree, which attracts clouds of flies causing nuisance to villagers and tourists; who are unable to fulfil their social functions under the trees (Vögele et al. 1991). In Kenya and Tanzania, damage can range from 30% to complete crop failure in orchards without any intervention measures (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) (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 new cultivars. This is supported by the findings of Karar et al. (2007) who demonstrated that mango mealybugs have a varietal preference to mango cultivars, and their population varies from variety to variety due to the degrees of susceptibility of each cultivar to the pest. Variations in the susceptibility of mango cultivars to other mealybug attacks or severity of their feeding symptoms has also been documented by Rosas-García et al. (2011), with important ramification for management programs.

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 is no comprehensive knowledge on the host plants of *R. iceryoides* apart from the damage observed on mango. To make an informed decision concerning orchard sanitation and mixed-cropping, growers must understand the host-plant range of *R. iceryoides*.

Natural enemies play an important role in regulating mealybug populations and globally there are several success stories of biological control of different species of mealybugs 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 mealybug populations, no information exists in the 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 characterized and quantified. Information on the distribution, host range, abundance and associated natural enemies of *R. iceryoides* should provide basic information for developing reliable and cost-effective management methods for the pest. As part of an ongoing project on integrated pest management (IPM) of major mango pests, the objectives of this study were to: (i) establish the geographic distribution of *R. iceryoides* in Kenya and Tanzania, (ii) establish its host-plant relationships, and (iii) document the parasitoids associated with *R. iceryoides* in these countries.

Materials and Methods

Sampling sites

Field surveys were conducted in 22 localities across the Coast province of Kenya and 12 localities in five regions of Tanzania (Fig. S1, Tab S1). The sampling sites in both countries were chosen based on horticultural production (especially mango). The province and regions are regarded as the major mango production areas (Greisbach 2003; Nyambo and Verschoor 2005). In both countries, sampling was carried out in cultivated fields, backyard gardens, woodlands, roadsides, forested areas and protected reserves. The GPS (global positioning system) readings were recorded for the surveyed sites (Tab S1).

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 zones of Kenya and Tanzania. 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 each transect, five plants of same host plant species were selected using random bearing at fixed distances before commencing the sampling. For each host plant the sample units consisted of 80 leaves, 20 twigs (~10 cm length) and 5 fruits selected at random within a surface area of 1 m² 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 plant. During the survey, care was taken to make sure that no plant was sampled twice within the same location. Sampling along the transect leading away from the locations was discontinued after several stops without *R. iceryoides* infestation (Bokonon-Ganta and Neuenschwander 1995).

The sample units of each plant sampled above were placed individually in transparent plastic bags and then transported to the laboratory in cool boxes. In the laboratory, all female mealybugs (i.e., third instar nymphs, adults and ovipositing females) per sampled plant part was counted and recorded with the aid of a head lens (Donegan OptiVISOR LX Binocular Magnifier-Lensplate #10, Magnification 3X at 10" focal length) or stereomicroscope - [Leica MZ 125 Microscope (Leica Microsystems Switzerland Limited)], fitted with Toshiba 3CCD camera using the Auto Montage software (Syncroscopy, Synoptics group, Cambridge, UK) at magnification of X25. We scored the severity of mealybugs infestation in each locality and host plant from the infested foliage, twigs and fruits following the scale developed by Tobih et al. (2002) for *R. invadens* with slight modification (see Tab S2). For each locality and sampling unit (i.e. 80 leaves, 20 twigs and 5 fruits), infestation by *R. iceryoides* was expressed as the cumulative number of mealybugs of all developmental stages in a sampling unit.

From the field-collected mealybugs, three to five adult mealybug samples were randomly selected and slide-mounted at the *icipe* Biosystematics Support Unit (BSU), using the

methodology of Watson and Kubiriba (2005), for further confirmation of their identity. All mealybug samples were identified by Dr. Gillian Watson, Plant Pest Diagnostics Branch, California Department of Food and Agriculture, Sacramento, U.S.A and Dr. S. Suresh, Department of Agricultural Entomology, Tamil Nadu Agricultural University (TNAU). Voucher specimens for collected mealybug samples were deposited at the BSU. 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*. Only plant species positive for mealybug infestation are presented. Plant nomenclature used conforms to the International Plant Names Index database (IPNI 2004) and the Missouri Botanical Garden database W³ TROPICOS (MBOT 2006).

Parasitoid recovery from field-collected mealybug samples

After the tally of mealybugs on infested plant parts, live and mummified specimens were transferred into plastic paper bags with well-ventilated minute 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 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 the insects were maintained in the laboratory at $70 \pm 5\%$ RH, 12:12 (L: D) h photoperiod and 26–28 °C ambient temperatures 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 of

Annamalai University, India and later confirmed by Dr G. L. Prinsloo of Agricultural Research Council (ARC), Pretoria, South Africa.

Statistical Analysis

Data for field surveys are presented according to plant species, family, location, infestation levels, severity of attack, species of emerged parasitoids and their percentage parasitism on the different host plants in different localities. Infestation by *R. iceryoides* was expressed as the total number of mealybugs of all developmental stages per number of plant parts sampled for each locality. Parasitism of the solitary parasitoids was expressed as percentage of the number of emerged parasitoid species to the total number of hosts in the samples for each host plant and locality. The data on mealybug abundance and parasitism rates were compared across plant parts by subjecting the data to *t* test or one-way ANOVA using Proc T Test or Proc GLM after being $\log(x + 1)$ transformed. Comparisons were also made on mealybug abundance on different host plants as well as between different localities. Data on percentage parasitism obtained during the study were arcsine transformation to comply with homogeneity of variance and normality assumptions before subjecting them to *t* test or one-way ANOVA as described above. All computations were performed using R 2.13.1 software (R Development Core Team, 2013)

Results

Distribution and infestation levels by R. iceryoides

In Kenya, *R. iceryoides* was recorded from 12 of the 22 sites sampled but with varying degrees of infestation (Table 1). For the wild host plants, the heaviest infestation levels were recorded on the twigs of *P. aculeata* in Kinango. The infestation levels on the twigs of *P. aculeata* was significantly higher compared to the leaves and fruits ($F = 12.25$; $d.f. = 2, 51$; $P < 0.0001$). On cultivated host plant, the heaviest infestation on twigs was recorded on *M. indica* in Matuga followed by Mombasa and Malindi (Table 1). The mealybug infestation on the leaves ($F = 228.22$; $d.f. = 8, 451$; $P < 0.0001$) and twigs ($F = 319.47$; $d.f. = 8, 223$; $P < 0.0001$) of *M. indica*

Table 1 Distribution, host plants and infestation levels by *Rastrococcus iceryoides* in Kenya and Tanzania, February 2008-July 2009

Country/ Locality	Plant species	Plant family	No. of <i>R. iceryoides</i>			Severity of attack			Statistics		
			Leaves	Twigs	Fruits	S	M	L	T or F	d.f.	P
Kenya											
Mombasa	<i>Mangifera indica</i> L.	Anacardiaceae	422.2±21.5	971.5±8.1	-		+		2.85	12	0.0146
	** <i>Ficus benghalensis</i> L.	Moraceae	190.5±12.7	358.9±15.3				+	1.56	14	0.1423
Malindi	<i>Manilkara zapota</i> L.	Sapotaceae	7.0±1.22	69.2±2.6	-			+	2.97	8	0.0178
	<i>Mangifera indica</i> L.	Anacardiaceae	374.1±9.67	881.2±21.4	-		+		5.11	25	<0.0001
Matuga	<i>Mangifera indica</i> L.	Anacardiaceae	516.9±20.4	3654.6±38.7	-	+			6.94	21	<0.0001
	<i>Citrus aurantifolia</i> Swingle	Rutaceae	3.1±1.11	27.2±3.27	-			+	2.70	4	0.0539
	<i>Psidium guajava</i> L.	Myrtaceae	66.8±4.20	271.1±6.45	-			+	0.03	17	0.9729
	<i>Parkinsonia aculeata</i> L.	Fabaceae	17.3±2.15	3467.6± 17.3	-	+			6.96	23	<0.0001
Kinango	<i>Parkinsonia aculeata</i> L.	Fabaceae	11.6±1.25	7892.3±25.1	42.2±5.1	+			12.25	2,51	<0.0001
Kilifi	<i>Mangifera indica</i> L.	Anacardiaceae	215.9±5.51	568.5±9.4	-		+		4.25	14	0.0008
Voi	<i>Mangifera indica</i> L.	Anacardiaceae	161.2±11.3	723.2±8.9	-		+		3.01	29	0.0021
Ikanga	<i>Mangifera indica</i> L.	Anacardiaceae	9.0±2.13	23.4±3.2	-			+	2.11	17	0.0441
Mwatate	<i>Mangifera indica</i> L.	Anacardiaceae	34.1±3.22	101.1±4.4	-			+	0.21	21	0.6043
Kigala	<i>Parkinsonia aculeata</i> L.	Fabaceae	13.6±2.19	3101.2±16.4	-		+		7.03	32	<0.0001
Ndome	<i>Mangifera indica</i> L.	Anacardiaceae	26.8±3.22	115.4±6.75	-			+	2.76	11	0.0201
Kamleza	<i>Mangifera indica</i> L.	Anacardiaceae	17.4±1.27	72.2±3.3	-			+	2.18	10	0.0167
Taveta	<i>Mangifera indica</i> L.	Anacardiaceae	43.3±4.26	215.7±7.7	-			+	2.44	14	0.0232
Tanzania											
Bagamoyo	<i>Mangifera indica</i> L.	Anacardiaceae	455.8±9.47	674.1±6.9	-		+		0.51	17	0.6169
Tanga	<i>Mangifera indica</i> L.	Anacardiaceae	3603.4±14.6	5154.8±22.76	-	+			3.55	39	0.0010
	<i>Cajanus cajan</i> L.	Fabaceae	98.9±6.52	1578.3±20.16	-		+		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; - = fruits were either not infested and omitted from analysis or not available during sampling; ^aSeverity of attack: S = Severe; M = Moderate; L = Low; + = degree of attack.

Table 1_Continues Distribution, host plants and infestation levels by *Rastrococcus iceryoides* in Kenya and Tanzania, February 2008-July 2009

Country/ Locality	Plant species	Plant family	No. of <i>R. iceryoides</i>			Severity of attack			Statistics		
			Leaves	Twigs	Fruits	S	M	L	T or F	d.f.	P
Tanzania											
Tanga	<i>Psidium guajava</i> L.	Myrtaceae	54.3±2.1	213.2±4.7	218.8±8.72			+	1.51	2,13	0.2567
	<i>Citrus aurantifolia</i> Swingle	Rutaceae	8.1±1.45	38.4±2.6	5.6±1.67			+	16.43	2,7	0.0023
Kibaha	** <i>Sorindeia madagascariensis</i> Thouars	Anacardiaceae	4.3±0.67	39.7±3.3	-			+	5.56	5	0.0026
	** <i>Annona stenophylla</i> Engl. & Diels.	Annonaceae	15.7±3.5	66.1±4.6	-			+	0.99	6	0.3589
	** <i>Phyllanthus engleri</i> Pax	Euphorbiaceae	112.5±5.52	837.9±11.8	-		+		3.15	18	0.0055
	** <i>Artocarpus heterophyllus</i> Lam.	Moraceae	77.6±4.15	321.3±8.4	-			+	1.90	20	0.0721
	** <i>Annona squamosa</i> L.	Annonaceae	13.2±1.7	278.6±5.5	-			+	3.97	13	0.0016
	<i>Psidium guajava</i> L.	Myrtaceae	6.6±1.92	123.2±2.4	435.2±12.7		+		3.33	2,18	0.0587
	<i>Musca paradisiaca</i> L.	Muscaeeae	8.1±3.14	0.0±0.00	-			+	1.86	2	0.2036
	** <i>Annona senegalensis</i> Pers.	Annonaceae	2.8±0.92	11.3±0.94	-			+	0.76	2	0.5264
	** <i>Ficus vallis-choudae</i> Delile	Moraceae	0.0±0.00	25.6±3.2	-			+	1.79	3	0.1713
	** <i>Dialium holtzii</i> Harms	Caesalpiniaceae	127.5±3.7	566.2±17.9	-		+		1.51	11	0.1604
	<i>Cajanus cajan</i> (L) Millsp.	Fabaceae	388.2±11.5	3359.2±32.7	-	+			4.19	39	0.0002
	** <i>Annona muricata</i> L.	Annonaceae	234.4±9.7	1334.4±18.9	-		+		2.94	9	0.0165
	** <i>Dalbergia melanoxydon</i> Guill & Perr	Papilionaceae	0.0±0.00	66.2±4.7	-			+	1.75	3	0.1778
	** <i>Flueggea virosa</i> Voigt	Euphorbiaceae	0.0±0.00	23.7±2.8	-			+	2.49	4	0.0675
	** <i>Clerodendrum johnstonii</i> Oliv.	Verbenaceae	1.2±0.21	4.4±1.2	-			+	0.50	2	0.6667
	** <i>Lecaniodiscus fraxinifolius</i> Baker	Sapindaceae	44.3±3.47	231.5±6.4	-			+	1.60	10	0.1403
	<i>Mangifera indica</i> L.	Anacardiaceae	6054.3±29.2	8153.6±19.2	-	+			2.25	68	0.0277
	** <i>Solanum indicum</i> L.	Solanaceae	63.9±6.8	314±9.6	-			+	0.86	9	0.4124
	** <i>Deinbollia borbonica</i> Scheff.	Sapindaceae	215.7±10.3	2253±22.9	-			+	2.73	36	0.0099
Mkuranga	<i>Mangifera indica</i> L.	Anacardiaceae	1223.4±14.7	3417.4±36.8	-	+			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; - = fruits were either not infested and omitted from analysis or not available during sampling; ^aSeverity of attack: S = Severe; M = Moderate; L = Low; + = degree of attack.

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			Leaves	Twigs	Fruits	S	M	L	T or F	d.f.	P
Tanzania											
Kinondoni	<i>Mangifera indica</i> L.	Anacardiaceae	3865.2±34.6	6868.4±74.5	2979.3±33.8	+			4.70	2,73	0.0120
	<i>Citrus aurantifolia</i> Swingle	Rutaceae	34.7±3.9	122.2±7.4	-			+	0.53	7	0.6150
	<i>Citrus sinensis</i> L.	Rutaceae	118.3±8.4	313.5±5.8	-			+	1.81	12	0.0952
	** <i>Artocarpus heterophyllus</i> Lam.	Moraceae	129.8±12.4	326.2±13.9	-			+	2.23	20	0.0372
	** <i>Morus alba</i> L.	Moraceae	1.1±0.02	5.4±0.67	-			+	1.0	2	0.4226
	<i>Parkinsonia aculeata</i> L.	Fabaceae	24.2±2.5	5567.3±38.4	-	+			4.82	47	<0.0001
	** <i>Osyris lanceolata</i> Hochst. & Steud.	Santalaceae	2.6±0.12	2356.1±88.9	-	+			3.25	13	0.0063
	** <i>Harrisonia abyssinica</i> Oliv.	Simaroubaceae	573.3±29.6	358.2±8.5	-			+	4.70	7	0.0022
	** <i>Indigofera spicata</i> Forsk	Papilionaceae	34.4±4.2	221.4±4.2	-			+	1.57	9	0.1518
	** <i>Caesalpinia sepiaria</i> Roxb.	Fabaceae	266.3±6.3	3116.1±17.5	-	+			3.97	35	0.0003
Vomero	<i>Mangifera indica</i> L.	Anacardiaceae	335.2±9.8	142.2±5.8	-			+	1.14	20	0.2695
Turiani	<i>Mangifera indica</i> L.	Anacardiaceae	211.6±5.1	967.5±14.6	-			+	3.56	26	0.0015
	** <i>Annona muricata</i> Linn	Annonaceae	5.2±1.9	49.2±2.9	-			+	3.47	5	0.0179
	<i>Citrus aurantifolia</i> Swingle	Rutaceae	3.1±1.7	21.3±0.77	-			+	2.47	3	0.0903
Mikese	<i>Mangifera indica</i> L.	Anacardiaceae	814.8±13.5	3578.2±49.9	-	+			4.88	41	<0.0001
Kilosa	<i>Mangifera indica</i> L.	Anacardiaceae	87.4±4.6	237.1±8.1	-			+	0.64	15	0.5326
	<i>Psidium guajava</i> L.	Myrtaceae	9.2±2.1	40.4±4.3	-			+	1.44	6	0.2002
Ilonga	<i>Mangifera indica</i> L.	Anacardiaceae	62.6±1.7	421.6±12.7	-			+	4.92	19	<0.0001
Kyela	<i>Mangifera indica</i> L.	Anacardiaceae	263.8±4.8	1700.1±16.9	-			+	5.96	23	<0.0001
	<i>Cajanus cajan</i> L.	Fabaceae	87.3±3.5	457.2±22.8	-			+	2.70	14	0.0171
Morogoro	<i>Mangifera indica</i> L.	Anacardiaceae	2563.8±68.9	8325.6±77.4	-	+	+		2.89	67	0.0051
	<i>Cajanus cajan</i> (L) Millsp.	Fabaceae	1452.2±44.7	4672.3±54.7	-	+			2.0	35	0.0530
	<i>Citrus aurantifolia</i> Swingle	Rutaceae	2.6±0.28	28.7±2.7	-			+	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; - = fruits were either not infested and omitted from analysis or not available during sampling; ^aSeverity of attack: S = Severe; M = Moderate; L = Low; + = degree of attack.

were significantly different when compared between the different localities. There was also a significant difference in the infestation levels recorded on the twigs ($F = 134.12$; $d.f. = 1, 97$; $P < 0.0001$) of *P. aculeata* in Matuga, Kinango and Kigala. However, for *P. aculeata* leaves, infestations did not vary significantly with locations.

In Tanzania, all the 12 localities sampled in the five regions were found to be infested with *R. iceryoides* although with different levels of infestation (Tables 1). The highest levels of infestations were recorded on *M. indica* in Kinondoni, followed by Morogoro, Kibaha, Tanga and Mkuranga (Table 1). The infestation levels on the leaves ($F = 567.72$; $d.f. = 10, 617$; $P < 0.0001$) and twigs ($F = 478.89$; $d.f. = 10, 458$; $P < 0.0001$) of *M. indica* were significantly different when compared among the different localities. There were also significant differences in the infestation levels recorded on the twigs ($F = 348.17$; $d.f. = 2, 234$; $P < 0.0001$) and the leaves ($F = 339.82$; $d.f. = 2, 435$; $P < 0.0001$) of *C. cajan* when compared among the different localities.

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* infestations included both cultivated and wild plants (Table 1).

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 1). On cultivated host plants, mealybug infestations on *M. indicar* ranged from 215.9 ± 5.51 mealybugs/80 leaves to 568.5 ± 9.4 mealybugs/20 twigs in Kilifi while in Matuga, mealybug infestations ranged from 516.9 ± 20.4 mealybugs/80 leaves to 3654.6 ± 38.7 mealybugs/20 twigs (Table 1). The most heavily infested wild host plant was *P. aculeata* with infestation ranging from 11.6 ± 1.25 – 17.3 ± 2.15 mealybugs/80 leaves and 3467.6 ± 17.3 – 7892.3 ± 25.1 mealybug/20 twigs (Table 1). On heavily

infested host plants such as *M. indica* and *P. aculeata*, infestations were significantly higher on the twigs compared to 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 1).

In Tanzania, *R. iceryoides* attack was noted on 27 host plants. Host plants with heavy infestation levels included *M. indica*, *P. aculeata*, *Osyris lanceolata* Hochst & Steud [Santalaceae], *Caesalpinia sepiaria* Roxb. [Fabaceae], *Artocarpus heterophyllus* Lam., *Cajanus cajan* (L.) Millsp. [Fabaceae], *Annona muricata* L. [Annonaceae] and *Deinbollia borbonica* Scheff. [Anacardiaceae]. Among the cultivated host plants, infestation levels were severe on *M. indica* (211.6±5.1–6054.3±29.2 mealybugs/80 leaves, 142.2±5.8–8325.6±77.4 mealybugs/20 twigs, 2979.3±33.8 mealybugs/5 fruits), *C. cajan* (87.3±3.5–1452.2±44.7 mealybugs/80 leaves and 457.2±22.8–4672.3±54.7 mealybugs/20 twigs) and *P. guajava* (6.6±1.92–54.3±2.1 mealybugs/80 leaves, 40.4±4.3–213.2±4.7 mealybugs/20 twigs and 218.8±8.72–435.2±12.7 mealybugs/5 fruits) compared to the other cultivated host plants sampled among the different localities (Table 1). On heavily infested *M. indica* plants in Kinondoni ($F = 4.70$; $d.f. = 2, 73$; $P = 0.0120$), Morogoro ($t = -2.89$; $d.f. = 67$; $P = 0.0051$), Mkuranga ($t = -2.39$; $d.f. = 32$; $P = 0.0231$), Kibaha ($t = -2.25$; $d.f. = 68$; $P = 0.0277$) and Tanga ($t = -3.55$; $d.f. = 39$; $P = 0.0010$), mealybug infestation on the twigs were always significantly higher compared to the leaves and the fruits (Table 1). Infestation levels recorded on the twigs of *C. Cajan* was also found to be significantly higher compared to the infestation on the leaves in Tanga ($t = -3.86$; $d.f. = 19$; $P = 0.0011$), Kibaha ($t = -4.19$; $d.f. = 39$; $P = 0.0002$), Kyela ($t = -2.70$; $d.f. = 14$; $P = 0.0171$) and Morogoro ($t = -2.0$; $d.f. = 35$; $P = 0.0530$).

Other host plants with low to moderate infestation in Tanzania included *Artocarpus heterophyllus* Lam. [Moraceae], *Harrisonia abyssinica* Oliv. [Simaroubaceae], *Indigofera spicata* Forsk [Papilionaceae], *Annona squamosa* L. [Annonaceae], *Dialium holtzii* Harms [Caesalpiniaceae], *Lecaniodiscus fraxinifolius* Baker [Sapindaceae], *C. aurantifolia*, *C. sinensis* L. and *Solanum indicum* L. [Solanaceae]. On these plants, infestation ranged from 34.4±4.2–

129.8±12.4 mealybugs/80 leaves and 221.4±4.2–321.3±8.4 mealybugs/20 twigs, across the various localities sampled (Table 1). *Rastrococcus iceryoides* was also recorded from *Morus alba* L. [Moraceae], *Sorindeia madagascariensis* Thou. [Anacardiaceae], *Annona stenophylla* Engl. & Diels. [Annonaceae], *Musca paradisiaca* L. [Musaceae], *Annona senegalensis* Pers. [Annonaceae], *Ficus vallis-choudae* Delile [Moraceae], *Dalbergia melanoxylon* Guill & Perr [Papilionaceae], *Flueggea virosa* Voigt [Euphorbiaceae], and *Clerodendrum johnstonii* Oliv. [Verbenaceae] but infestation on these host plants did not exceed 66.1±4.6 mealybugs/20 twigs.

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

Parasitoids complex associated with R. iceryoides

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). Among these parasitoids, *A. pseudococci* accounted 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 2). The percentage parasitism by *A. pseudococci* (72.73± 4.56%) and *L. dactylopii* (18.18±1.97%) on the twigs was found to be significantly higher compared to parasitism on the leaves in Kinango (Table 2). The highest percentage parasitism of *R. iceryoides* by *Anagyrus pseudococci* on *M. indica* was recorded in Mombasa, Matuga and Malindi. However, there was no significant difference on the percent parasitism by *A. pseudococci* on the twigs and leaves in Mombasa and Malindi except in Matuga ($t = 8.56$; $d.f. = 35$; $P = 0.0060$). The highest percent parasitism by *L. tecta* was recorded

Table 2 Parasitoid complex associated with the different host plants and their relative contribution in the biological control of *Rastrococcus iceryoides* in Kenya and Tanzania, February 2008-July 2009

Country	Regions/province	Parasitoid species	Plant species	Percentage parasitism (%)			T or F	d.f.	P
				Leaves	Twigs	Fruits			
Kenya	Mombassa	<i>Anagyrus pseudococci</i> Girault	<i>Mangifera indica</i> L.	12.32±2.35	8.75±3.25	-	0.17	14	0.6890
		<i>Anagyrus pseudococci</i> Girault	<i>Ficus benghalensis</i> L.	4.21±1.22	4.75±1.18	-	0.01	14	0.9297
	Matuga	<i>Anagyrus pseudococci</i> Girault	<i>Mangifera indica</i> L.	5.43±1.66	19.65±2.77	-	8.56	35	0.0060
		<i>Anagyrus pseudococci</i> Girault	<i>Psidium guajava</i> L.	3.03±0.22	6.64±1.67	-	1.03	16	0.3244
		<i>Anagyrus pseudococci</i> Girault	<i>Parkinsonia aculeata</i> L.	17.65±3.54	14.05±3.44	-	0.00	23	0.9748
	Kilifi	<i>Anagyrus pseudococci</i> Girault	<i>Mangifera indica</i> L.	3.72±1.11	12.68±2.86	-	11.84	21	0.0025
	Malindi	<i>Anagyrus pseudococci</i> Girault	<i>Mangifera indica</i> L.	8.29±2.71	10.78±2.97	-	1.33	35	0.2568
	Kinango	<i>Anagyrus pseudococci</i> Girault	<i>Parkinsonia aculeata</i> L.	72.73± 4.56	20.12±5.32	-	3.16	48	< 0.0001
		<i>Leptomastrix dactylopii</i> Howard	<i>Parkinsonia aculeata</i> L.	18.18±1.97	0.09±10 ⁻³	-	2.66	48	< 0.0001
<i>Leptomastidea tecta</i> Prinsloo		<i>Parkinsonia aculeata</i> L.	9.09±2.53	0.13±0.04	-	7.31	48	0.0094	
Tanzania	Kinondoni	<i>Anagyrus pseudococci</i> Girault	<i>Mangifera indica</i> L.	8.38± 3.22	3.13±0.87	5.1±1.21	4.47	2, 73	0.0147
		<i>Anagyrus aegyptiacus</i> Moursi	<i>Parkinsonia aculeata</i> L.	0.21± 0.01	0.31±0.01	0.10±0.01	0.40	2, 73	0.6737
		<i>Leptomastrix dactylopii</i> Howard	<i>Parkinsonia aculeata</i> L.	0.05±10 ⁻⁴	0.19±0.03	-	3.20	67	0.0783
		<i>Agarwalencyrtus citri</i> Agarwal	<i>Parkinsonia aculeata</i> L.	0.08± 10 ⁻³	-	-	-	-	-

** = indicate host plants native to Africa; - = indicates infested plant parts that were not available at the time of sampling.

Table 2 Continues Parasitoid complex associated with the different host plants and their relative contribution in the biological control of *Rastrococcus iceryoides* in Kenya and Tanzania, February 2008-July 2009

Country	Regions /province	Parasitoid species	Plant species	Percentage parasitism (%)			<i>T</i> or <i>F</i>	<i>d.f.</i>	<i>P</i>
				Leaves	Twigs	Fruits			
		<i>Anagyrus pseudococci</i> Girault	<i>Artocarpus heterophyllus</i> Lam.	5.43±1.01	2.76±0.34	-	0.37	20	0.5515
		<i>Anagyrus pseudococci</i> Girault	<i>Parkinsonia aculeata</i> L.	20.83±3.08	5.64±1.44	-	1.07	47	0.0065
		<i>Anagyrus pseudococci</i> Girault	** <i>Indigofera spicata</i> Forsk	5.88±0.69	2.71±0.12	-	0.03	11	0.8609
		<i>Anagyrus pseudococci</i> Girault	<i>Caesalpinia sepiaria</i> Roxb.	4.14±0.58	1.64±0.01	-	0.99	35	0.3270
	Mkuranga	<i>Anagyrus pseudococci</i> Girault	<i>Mangifera indica</i> L.	10.96±2.22	9.45±3.23	-	0.30	47	0.5862
	Kibaha	<i>Anagyrus pseudococci</i> Girault	** <i>Phyllanthus engleri</i> Pax.	7.14±1.11	8.84±2.17	-	0.07	18	0.7941
		<i>Anagyrus pseudococci</i> Girault	<i>Artocarpus heterophyllus</i> Lam.	2.60±0.23	4.98±1.03	-	3.52	20	0.0755
		<i>Anagyrus pseudococci</i> Girault	** <i>Annona squamosa</i> L.	7.69±2.42	12.95±3.82	-	12.46	13	0.0037
		<i>Anagyrus pseudococci</i> Girault	<i>Psidium guajava</i> L.	0.0±0.00	3.25±0.41	2.53±0.13	0.61	1, 17	0.4439
		<i>Anagyrus pseudococci</i> Girault	** <i>Dialium holtzii</i> Harms	5.51±1.02	3.36±0.25	-	4.12	11	0.0673
		<i>Anagyrus pseudococci</i> Girault	<i>Cajanus cajan</i> (L) Millsp.	8.51±1.27	2.44±0.07	-	3.65	39	0.0635
		<i>Anagyrus pseudococci</i> Girault	** <i>Lecaniodiscus fraxinifolius</i> Baker	4.55±0.46	2.60±0.02	-	0.49	10	0.4996
		<i>Anagyrus pseudococci</i> Girault	<i>Mangifera indica</i> L.	11.22±2.53	18.43±3.93	-	7.12	68	0.0095
		<i>Anagyrus aegyptiacus</i> Moursi	<i>Mangifera indica</i> L.	0.38±0.01	0.07±10 ⁻⁴	-	0.03	68	0.8600
		<i>Leptomastrix dactylopii</i> Howard	<i>Mangifera indica</i> L.	0.18±10 ⁻²	0.27±10 ⁻²	-	2.71	68	0.1046
		<i>Agarwalencyrtus citri</i> Agarwal	<i>Mangifera indica</i> L.	0.03±10 ⁻⁴	0.06±10 ⁻⁴	-	2.61	68	0.1108
		<i>Aenasius longiscapus</i> Compere	<i>Mangifera indica</i> L.	0.03±10 ⁻⁵	0.18±10 ⁻³	-	3.11	68	0.0824

** = indicate host plants native to Africa; - = indicates infested plant parts that were not available at the time of sampling.

Table 2 Continues Parasitoid complex associated with the different host plants and their relative contribution in the biological control of *Rastrococcus iceryoides* in Kenya and Tanzania, February 2008-July 2009

Country	Regions/province	Parasitoid species	Plant species	Percentage parasitism (%)			<i>T</i> or <i>F</i>	<i>d.f.</i>	<i>P</i>
				Leaves	Twigs	Fruits			
		<i>Anagyrus pseudococci</i> Girault	** <i>Solanum indicum</i> L.	3.17±0.78	6.69±1.64	-	6.23	9	0.0341
		<i>Anagyrus pseudococci</i> Girault	** <i>Deinbollia borbonica</i> scheft	17.21±3.46	13.14±2.75	-	9.74	36	0.0035
	Bagamoyo	<i>Anagyrus pseudococci</i> Girault	<i>Mangifera indica</i> L.	9.67±2.27	16.62±4.21	-	0.82	17	0.3774
	Morogoro	<i>Anagyrus pseudococci</i> Girault	<i>Mangifera indica</i> L.	2.61±0.03	5.48±0.97	-	2.85	67	0.0960
		<i>Anagyrus pseudococci</i> Girault	<i>Cajanus cajan</i> (L) Millsp.	3.86±0.51	2.10±0.04	-	0.66	35	0.4208
	Mikese	<i>Anagyrus pseudococci</i> Girault	<i>Mangifera indica</i> L.	9.58±2.47	5.93±1.53	-	0.94	41	0.3390
	Turiani	<i>Anagyrus pseudococci</i> Girault	<i>Mangifera indica</i> L.	2.37±0.39	5.48±0.72	-	3.81	26	0.0618
	Vomero	<i>Anagyrus pseudococci</i> Girault	<i>Mangifera indica</i> L.	6.57±1.72	9.15±2.97	-	4.81	20	0.0402
	Kilosa	<i>Anagyrus pseudococci</i> Girault	<i>Mangifera indica</i> L.	3.45±0.11	7.17±1.83	-	0.02	15	0.9039
	Ilonga	<i>Anagyrus pseudococci</i> Girault	<i>Mangifera indica</i> L.	8.06±1.92	9.98±2.61	-	4.22	19	0.0541
	Tanga	<i>Anagyrus pseudococci</i> Girault	<i>Mangifera indica</i> L.	5.91±1.41	19.94±3.22	-	3.12	28	< 0.0001
		<i>Anagyrus pseudococci</i> Girault	<i>Cajanus cajan</i> (L) Millsp.	3.06±0.25	8.43±1.04	-	2.95	19	0.1019
	Kyela	<i>Anagyrus pseudococci</i> Girault	<i>Mangifera indica</i> L.	2.66±0.07	4.65±1.19	-	10.86	23	0.0032
		<i>Anagyrus pseudococci</i> Girault	<i>Cajanus cajan</i> L.	8.05±1.74	7.00±1.74	-	0.00	14	0.9906

** = indicate host plants native to Africa; - = indicates infested plant parts that were not available at the time of sampling.

on *P. aculeata* with significantly higher rates on the leaves compared to the twigs ($t = 7.31$; $d.f. = 48$; $P < 0.0001$). The percent parasitism by *A. aegyptiacus* and *A. citri* on the different host plants was found to be less 1% (Table 2). The percentage parasitism of *R. iceryoides* by *A. pseudococci* recorded on the leaves ($F = 169.36$; $d.f. = 2, 114$; $P = 0.0321$) and twigs ($F = 78.94$; $d.f. = 2, 58$; $P = 0.0121$) of *M. indica* were significantly different when compared across the different localities. There were also significant differences in the percent parasitism by *A. pseudococci* on the leaves ($F = 46.73$; $d.f. = 1, 34$; $P < 0.0001$) and twigs ($F = 34.92$; $d.f. = 1, 23$; $P < 0.0001$) of *P. aculeata* when compared across the different localities.

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 2). The highest percent parasitism by *A. pseudococci* was recorded on a wild host plant, *P. aculeata* with $20.83 \pm 3.08\%$ on the leaves and $5.64 \pm 1.44\%$ on the twigs ($t = 1.07$; $d.f. = 47$; $P = 0.0065$). Beside *P. aculeata*, *A. pseudococci* also had a high percent parasitism on another wild host plant called *D. borbonica* with significantly different rates on the twig and the leaves ($t = 9.74$; $d.f. = 36$; $P = 0.0035$). High percent parasitism on *M. indica* was recorded in Mkuranga, Kibaha, Bagamoyo and Tanga. However, there was no significant difference in the percent parasitism recorded on the leaves and twigs of *M. indica* in Bagamoyo and Mkuranga, except in Kibaha and Tanga (Table 2). The percent parasitism of *R. iceryoides* by the other parasitoid species across the different host plants in the different localities in Tanzania was less than 1%. On *M. indica*, the percentage parasitism of *R. iceryoides* by *A. pseudococci* recorded on the leaves ($F = 969.56$; $d.f. = 10, 424$; $P = 0.0348$) and twigs ($F = 784.59$; $d.f. = 10, 358$; $P = 0.0425$) were found to vary significantly between localities.

Discussion

Our 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 on both *M. indica* 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 *M. indica* in Mombasa, Kilifi and Malindi were also observed in Kenya. It is uncertain whether the infestation in these localities 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, the high level of infestation observed on *M. indica* in several areas demands urgent management attention given the ongoing expansion of the horticulture industry and particularly *M. indica* in the region (Nyambo and Verschoor 2005; Madulu and Chalamila 2007). We were able to record the pest 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. Despite the wide availability of preferred host plants in Madabogo, Dembwa, Wundanyi and Kungu (located at 943 to 1480 masl), *R. iceryoides* was absent at these sampling sites suggesting these elevations could be outside the altitudinal range of the pest. Our data strongly suggest that *R. iceryoides* may be better adapted to low and mid altitudes than to higher altitudes, which exactly match its distribution range in its native home of India (Rawat and Jakhmola 1970; Narasimham and Chacko 1988; Williams 1989; Narasimham and Chacko 1991; C. M. Tanga, unpublished data). Although the precise date of introduction of *R. iceryoides* to Kenya and Tanzania is unknown (Williams 1989), it is highly probable that current distribution and spread of the mango mealybug populations was 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 (Agouké et al. 1988).

In this study, significant differences were recorded in the intensity of mealybug infestation between host plant species and between plant parts (leaves, twigs and fruits). These

differences strongly suggest that all main parts of the plant should be considered when establishing host susceptibility. We found that host plant susceptibility changed considerably according to localities, climatic, growth and geographical conditions and due to the same factors also the infestation on the different plant parts. The most highly susceptible host plants found in this study were *M. indica* and *P. aculeata* with extremely high levels of infestation recorded on the twigs followed by the leaves and then the fruits. Heavy and prolonged infestations were observed to cause the twigs to wilt and die, reduced flowering, kill branches or entire plants when left uncontrolled. Severe attack on the leaves and fruits were worsened by the premature drop of mature leaves and fruit-lets shedding off pre-maturely, respectively. This implies that different plant part may have the same mean infestation level, but the yield loss will be more pronounced in the plant part with the greater aggregation of damage. Therefore, studies on yield loss quantitative impact data assessment should be a function of both level of infestations and spatial pattern of plant injury.

Rastrococcus iceryoides was recorded from 29 plant 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, include Anacardiaceae, Fabaceae, Sapindaceae and Santalaceae. Plants from the families 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 an earlier study carried out by CABI (1995) following the first detection of the pest in Tanzania, only six host plants, namely mango (*M. indica*), cacao (*Theobroma cacao* L.), *Albizia lebbek* L. (Indian siris), cotton (*Gossypium* spp.) and rain-tree (*Samanea saman* (Jacq.) Merr.) were reported to be attacked by this pest. The additional host plant records from our survey clearly suggest that *R. iceryoides* is expanding its host range in the continent, possibly due to lack of its coevolved natural enemies, and other co-generic competitors. Thus, *R. iceryoides* represents a serious emerging polyphagous mealybug pest in Tanzania and Kenya and the continent at large. Several *Rastrococcus* species have been reported from the different host plant families listed in our study. For example following the invasion of *R. invadens* in West Africa, Agouunké 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 attacks several host plant species (Williams 1989; Ben-Dov 1994; Williams 2004), it is envisaged that this list is likely to expand.

Among the members of the Anacardiaceae sampled in our study, mango scored the highest infestation with *R. iceryoides*. Agouké et al. (1988) also reported similar results in West Africa for the closely related species *R. invadens*. The authors found that mango suffered heavy infestation by *R. invadens*, along with other crops such as 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 were the most preferred host plants of *R. invadens* in Nigeria.

The heavy infestation of plants in the family Fabaceae (e.g. *P. aculeata* and *C. cajan*) by *R. iceryoides* is in line with the host range reported for this pest in its native home, and the finding of Ben-Dov (1994), who reported that these host plants (*P. aculeata*, *C. cajan*) are preferred and associated with this pest in Asia. The heavy infestation of *P. aculeata* is perhaps surprising given that the plant is not native to Asia; it is an invasive tree indigenous to tropical America (Cochard and Jackes 2005). Nevertheless, plants that are generally drought stressed easily favour high populations of mealybug (Gutierrez et al. 1993; Lunderstadt 1998; Calatayud et al. 2002; Shrewsbury et al. 2004) and *P. aculeata* is known to thrive in drought-prone environments with limited amount of water (Floridata 2001). Fully-grown *P. aculeata* can flower throughout the year (WNS 2011) and can harbour 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 its high potential as breeding ground for *R. iceryoides*.

The cat's claw, *C. sepiaria* is recorded here for the first time as a preferred host harbouring 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 Southern Asia. It is an Indo-Malayan species, indigenous to India (the putative aboriginal home of *R. iceryoides*), Burma, Sri Lanka, eastern China and Southeast Asia down to the Malay Peninsula (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 positively affected by increased level of plant nitrogen content. Hogendrop et al. (2006) demonstrated that higher nitrogen concentrations, in the form of supplemental fertilizers, led to an increase 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 an important reservoir host plant for *R. iceryoides*. High infestation levels were especially recorded in Kibaha, Tanzania (2253 mealybugs/10 cm twig). *Deinbollia borbonica* is a perennial tree and a crucial off-season host plant for *R. iceryoides* particularly when mango, the primary cultivated host plant, is off-season. Several plant species from the Sapindaceae family (e.g., *Nephelium lappaceum* L., *Harpullia* sp., *Guioa pleuropteris* Blume, *Heterodendrum* sp., and *Nephelium lappaceum* L.) 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 (Williams 1989; Ben-Dov 1994; Williams 2004).

Osyris lanceolata from the family Santalaceae was heavily attacked by *R. iceryoides*. To the best of our knowledge, there are no records of mealybug attack from this plant species and our 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 stems at 10 cm above the ground level. In Kenya, a root decoction of *O. lanceolata* is used to treat diarrhoea, while in Tanzania, a decoction of the bark and heartwood, is used to treat sexually transmitted diseases (including the killer Hepatitis B) and anaemia (Orwa et al. 2009). Thus *R. iceryoides*

invasion in Africa and its heavy infestation of this plant threatens the existence of a vital medicinal source for communities that have limited access to modern medical services. The roots and wood are scented and used to make cosmetics and perfume; and has a lucrative market in Germany, India, Indonesia and South Africa (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* and *A. muricata* is a new record for the insect. Studies elsewhere have shown that other species of *Rastrococcus* such as *R. invadens* and *R. spinosus* are pestiferous on this family (Boussienguet and Mouloungou 1993; Ben-Dov 1994; 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 pesticidal, antimalarial, cell growth inhibitory, antiparasitic, antimicrobial and cytotoxic activities (Colman-Saizarbitoria et al. 1995; Oberlies et al. 1997; Fujimoto et al. 1998; 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 were associated with noticeable deformation and distortion of the growing tip, 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 moderately infested by *R. iceryoides*. This is a common plant species and is 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 the 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 drunk to treat bilharzia,

sexually transmitted diseases (STDs), menstrual problems and abdominal and chest pain (Christopher et al. 2002).

Two plant species in the family Rutaceae (*Citrus sinensis* and *C. aurantifolia*) and one plant in the family Myrtaceae (*P. guajava*) warrant further discussion. These crops are known to host a variety of mealybug species worldwide including several species of *Rastrococcus* (Ben-Dov 1994; Williams 2004) (*Psidium 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 *C. sinensis* and *C. aurantifolia*. Although infestation was generally low in our 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; Boussienguet and Mouloungou 1993; Ben-Dov 1994), in addition to *C. sinensis* and *C. aurantifolia* (Ivbijaro et al. 1992).

In our study, although up to six parasitoid species were recovered from *R. iceryoides* (some representing new associations) only *A. pseudococci* showed promising performance (~21% parasitism). In its native home range (Southern Asia), Tandon and Lal (1978), Narasimham and Chako, (1988), and Tandon and Srivastava (1980) reported that *R. iceryoides* was attacked by several parasitoid species with parasitism rates exceeding 40%. This high level of parasitism maintained the pest at low levels resulting in this pest being of little or no economic significance in the region. The former authors listed *A. pseudococci* as one of the parasitoid guilds attacking *R. iceryoides*. Although our findings support that of Tandon and Lal (1978), the current observation requires further confirmation given that Noyes and Hayat (1994) reported the findings by Tandon and Lal (1978) as a misidentification.

Globally, *A. pseudococci* has been reported from 12 countries (Noyes and Hayat 1994) excluding the countries of our survey, which implies that the results presented herein add Kenya and Tanzania to the list of countries where the parasitoid exists. In Texas (USA), 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. pseudococci* on *R. iceryoides* was from mealybugs infesting mango and *P. aculeata*. Our study provides information that predicts the distribution of parasitism across a variety of host plants, which is 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 should enhance the effectiveness of this parasitoid in suppressing the *R. iceryoides* population. Also, parasitoid conservation and augmentation (particularly inoculative releases) could potentially be used to target the pest population on the preferred ornamental host plant, *P. aculeata* that is often abundant in and around *M. indica* orchards in the different localities sampled. Such practices should result in the build-up of the parasitoid populations ahead of the mango fruiting season before heavy infestations on the mango plants start.

Parasitism by the other parasitoid species encountered during the survey did not exceed 1% (Table 2). The reason for the general low level of parasitism by the parasitoid species is not well understood. However, one factor that could have contributed to the overall low parasitism by the endogenous parasitoids could have been the strong immune reaction of the invasive pest (though not investigated in this study) against the endogenous parasitoids due to lack of shared evolutionary history between the pest and the parasitoids. Other factors could include host plants, parasitoid population and climate conditions. Indeed, all these factors have been found to be crucial for successful parasitism by most encyrtid parasitoids on mealybugs (Cross and Moore 1992; McDougall and Mills 1997; Blumberg 1997; Islam and Copland 1997; Sagarra and Vincent 1999; Karamaouna and Copland 2000; Daane et al. 2004 a, b; 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 co-evolved natural enemies

capable of suppressing *R. iceryoides* populations to 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.

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Supporting information

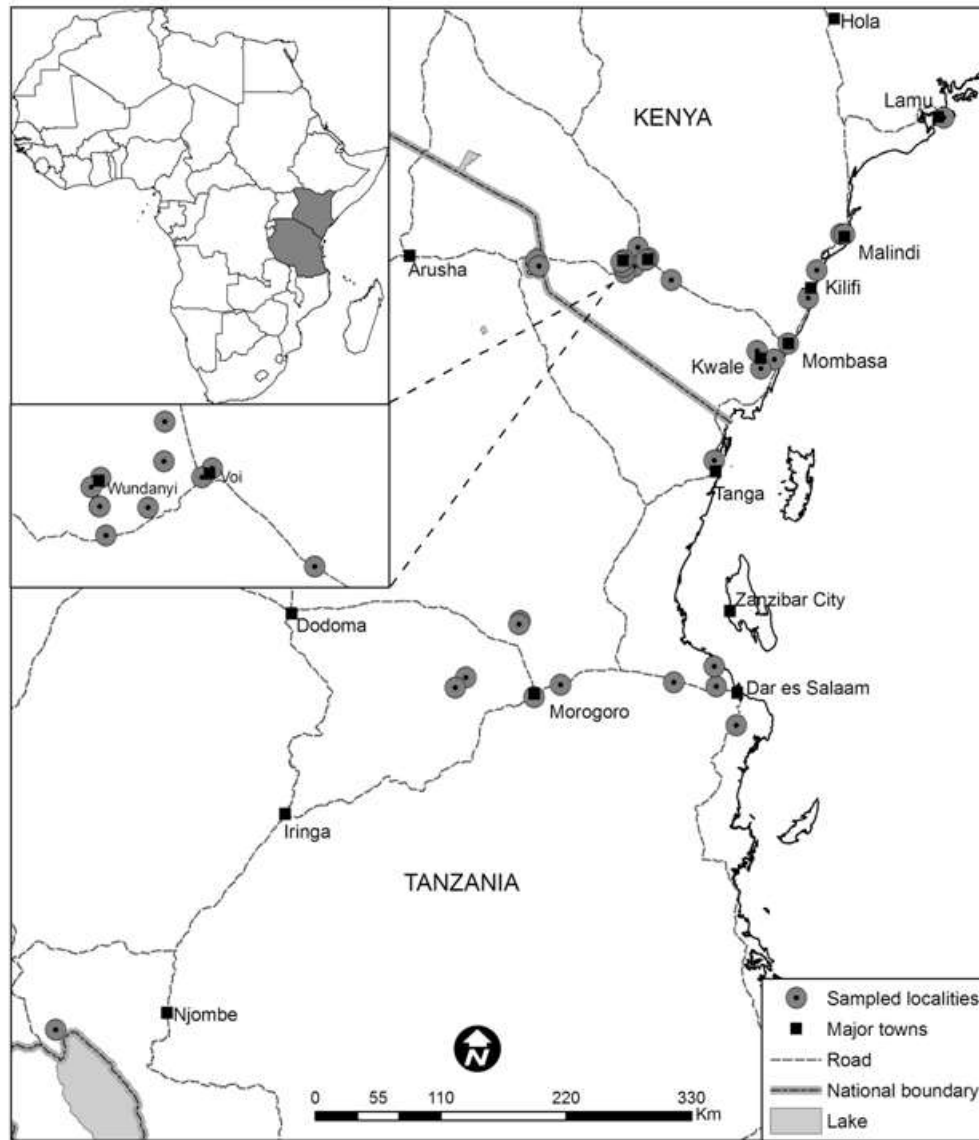


Fig. S1 Map showing locations where sampling of the mealybug *R. iceryoides* was conducted in Kenya and Tanzania, February 2008-July 2009.

Table S1 Sampling sites for *Rastrococcus iceryoides* and its associated natural enemies with geo-referenced positions and altitude

Country/locality	Longitude	Latitude	Elevation (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

Table S2 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