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 $\alpha = 0.05$ (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 ($\alpha = 0.05$).

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)

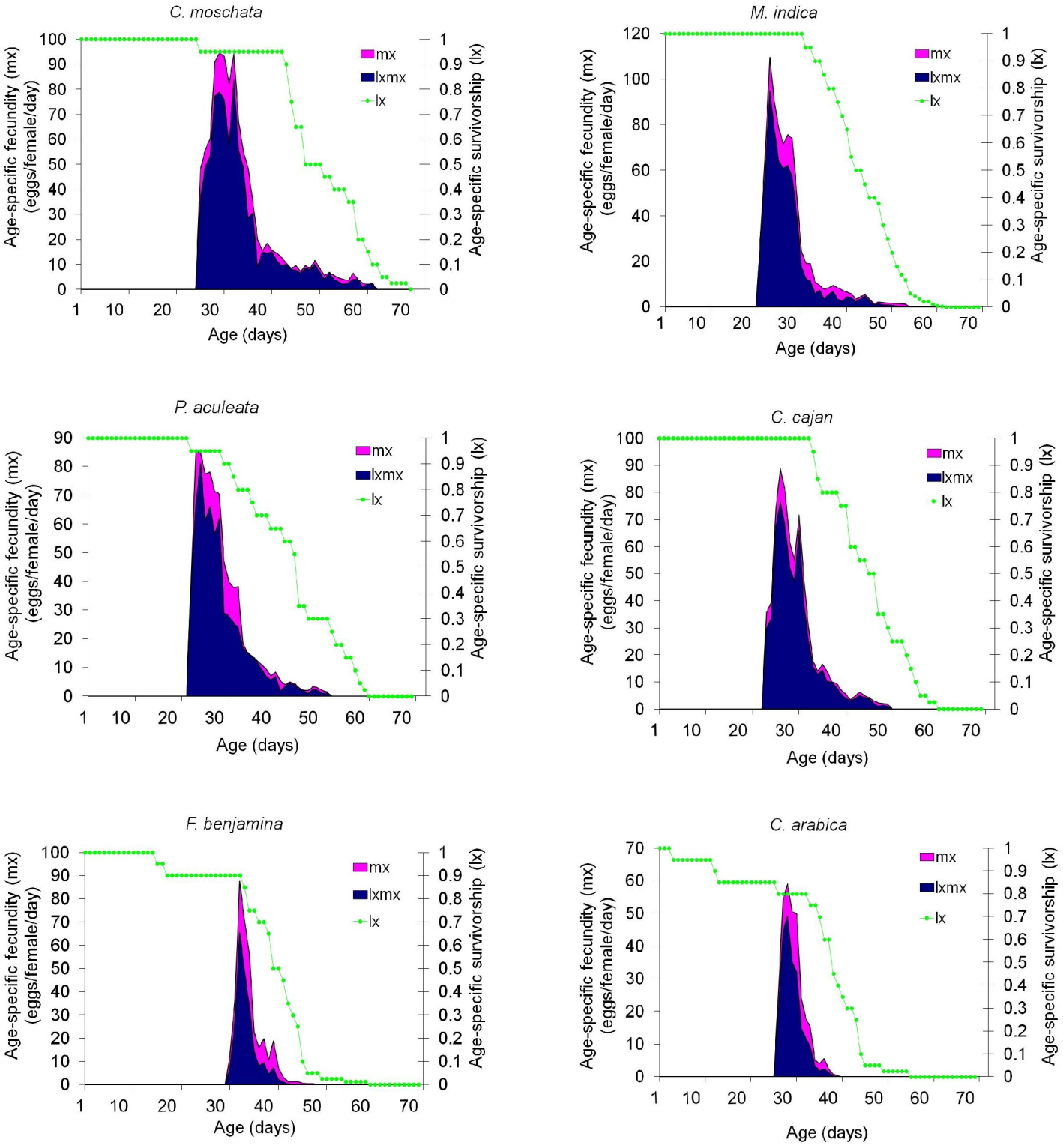

Figure 4. 1: Age-specific fecundity (m_x), age-stage specific maternity ($l_x m_x$), and age-specific survivorship (l_x) of *R. iceryoides* reared on six host plant species.

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	Reproductive period (days)	Lifetime fecundity (progeny #)	Mean development time (in days ± s.e.)			Statistics (♀ and ♂)		
			♂♀	♂	♀	*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

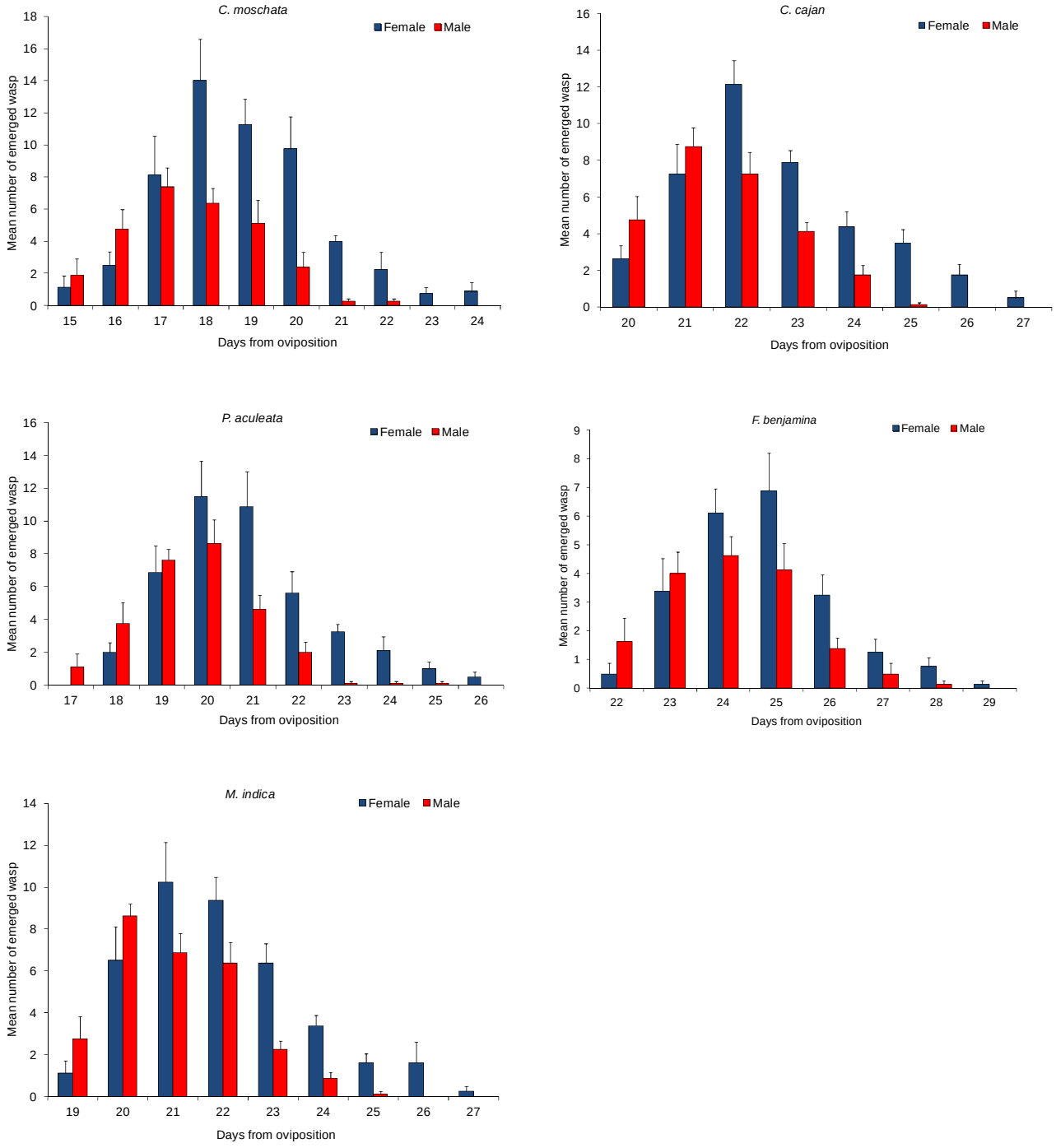

Figure 5. 1: Number of *A. pseudococci* males and females emerging on different days after 24 h exposure period to *R. iceryoides* cultured on five different host plants.

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.

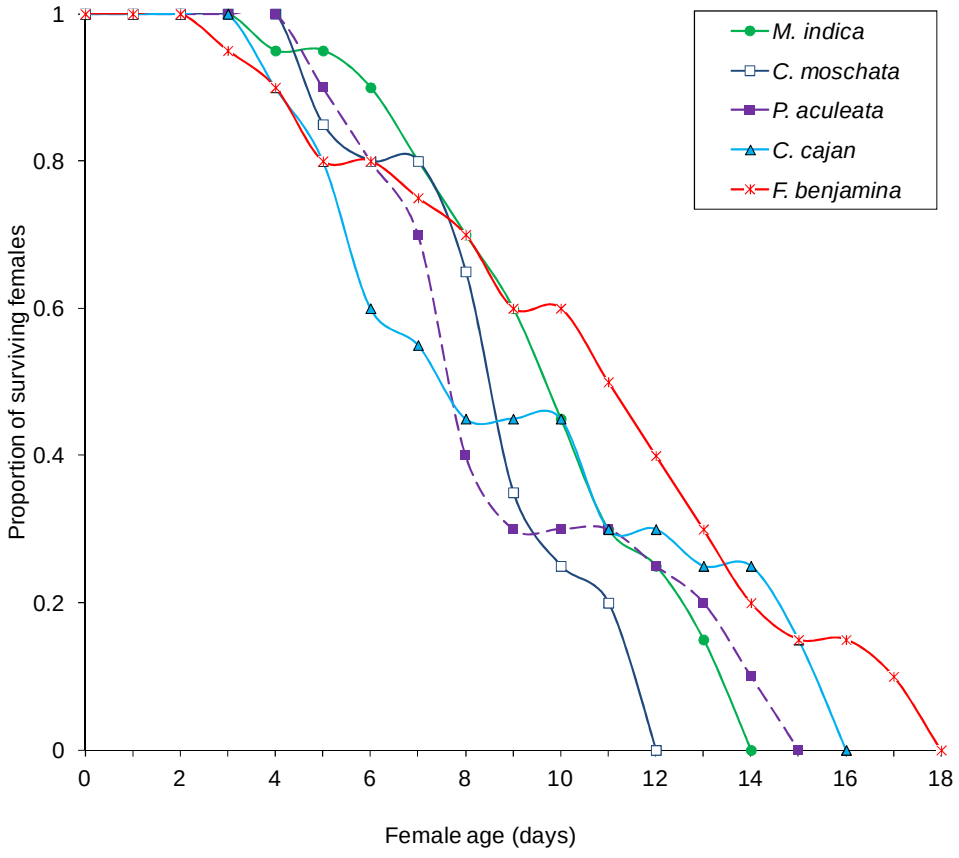

Figure 5. 2: Survival of ovipositing females of *A. pseudococci* reared on different mealybug-infested host plants at 22.3 ± 1.07 ˚C, 40 - 80% RH, with 12L: 12D photoperiod.

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. pseudoccoci* (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.

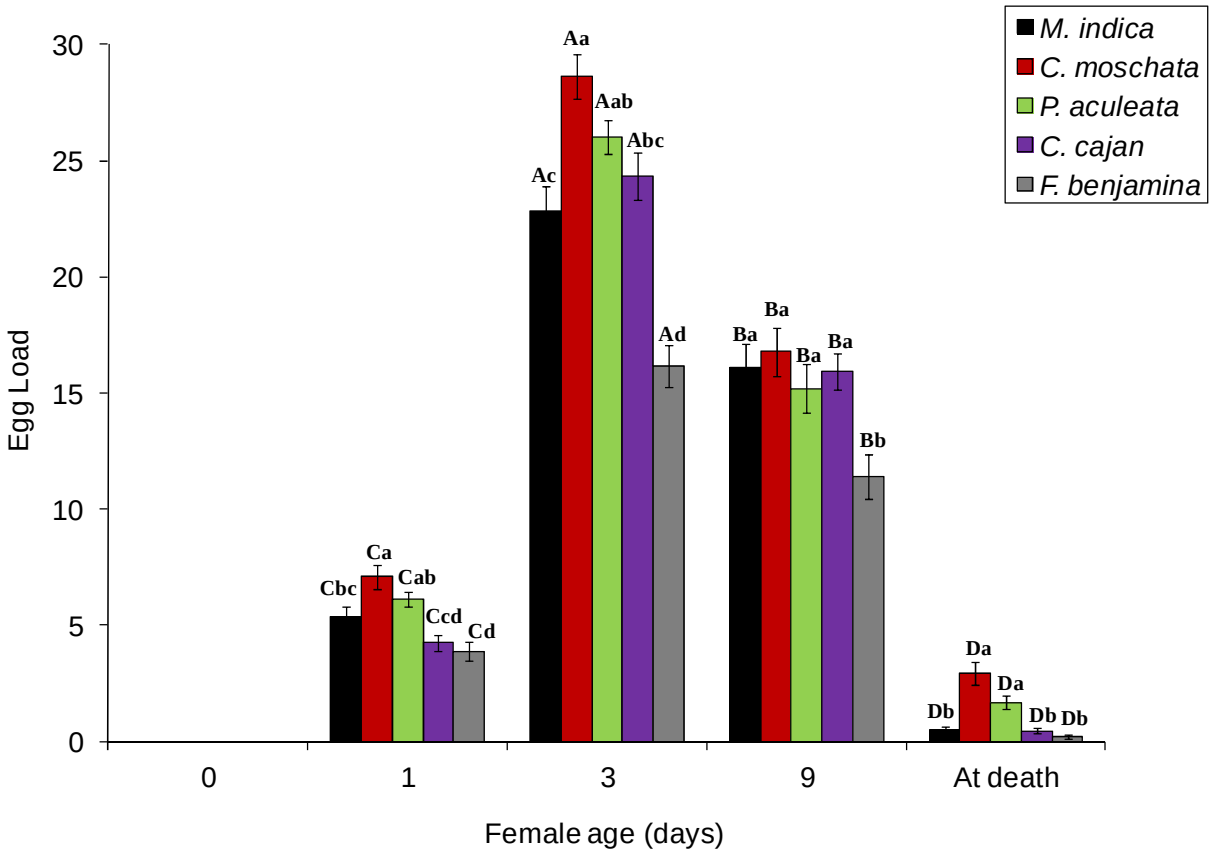

Figure 5. 3: Egg maturation and resorption by *A. pseudococci* females provided honey in the absence of hosts. Bars show mean egg loads ± SEM. Sample sizes for days 0, 1, 3, 9 and at death (> 30 day) are for *C. moschata*: 35, 25, 30, 19 and 30; *M. indica*: 23, 25, 25, 15 and 24; *P. aculeata*: 18,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

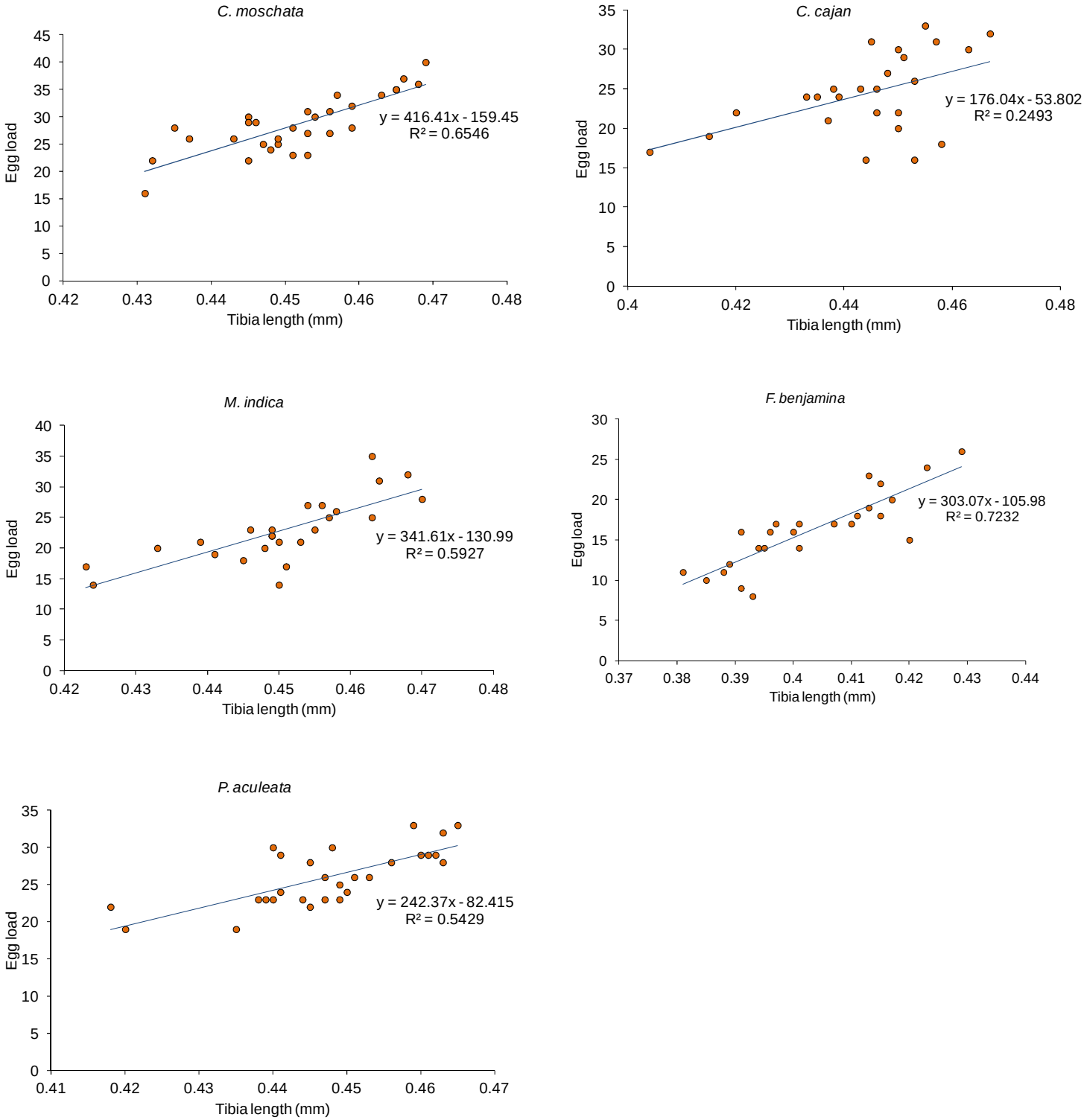

Figure 5. 4: Influence of the size of *A. pseudococci* females (tibia length) reared from *R. iceryoides* maintained on different host plants on their egg loads. GLM based on Poisson distribution for 3 day-old females.

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.