

## CHAPTER SIX

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

#### **ABSTRACT**

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

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

## 6.1 Introduction

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

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

In agricultural and natural ecosystems, ants' interaction with the assiduously attended Hemipteran insects such as mealybugs reveals benefits such as access to a constant defensible and renewable carbohydrate energy-rich food source (Carroll and Janzen, 1973). In return, the ants render protection against parasitoids, predators and even their competitors (Steyn, 1954; Barlett, 1961; Bradley, 1973; Adenuga, 1975; Pierce and Mead, 1981; Hölldobler and Wilson,

1990; Jiggins et al., 1993), as well as sanitation and sometimes transport services to sedentary Hemiptera (Buckley, 1987; Lach, 2003). By affording protection to the mealybugs from natural enemies, the presence of certain species of ant can be detrimental to the impacts of biological control (Bartlett, 1961; Buckley, 1987; Itioka and Inoue, 1996; Martinez-Ferrer et al., 2003). For example, Whitehead (1957) and Myburgh et al. (1986) reported that ant foraging on plant canopies reduces natural enemy activity and promotes mealybug infestation and therefore, biological control of the insect pest is compromised. In California, Daane et al. (2007) found that *L. humile* promoted populations of *Pseudococcus viburni* (Signoret) while lowering populations of its parasitoids *Pseudaphycus maritimus* (Erhorn) accompanied by a serious reduction in its parasitoid populations. Buckley and Gullan (1991) reported a very low parasitism rates (< 10%) of coccids in the presence of *Oecophylla* and *Solenopsis* species and > 15% in the presence of the more aggressive *Tapinoma* and *Iridomyrmex* species. Itioka and Inoue (1996) in a comparative field investigation found a 94% decrease of the mealybug *Pseudococcus citriculus* Green (Hemiptera: Pseudococcidae) populations in a Satsuma orange orchard by natural enemies in the absence of the attendant ant, *Lasius niger* Linnaeus (Hymenoptera: Formicidae).

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

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

## 6.2 Materials and Methods

### 6.2.1 Insect colonies

#### 6.2.1.1 Mealybug colonies

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

#### 6.2.1.2 *Oecophylla longinoda* Latr. colonies

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

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

#### 6.2.1.3 Parasitoid colonies

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

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

Butternut fruits infested with 100 third instar nymphs of *R. iceryoides* were used in this experiment. A total of 100 adult *O. longinoda* workers were transferred to the fruits and allowed to forage for 3 h on the mealybug-infested butternut. Thereafter, 10 three-day old fertilized female parasitoids were aspirated from the colony and gently introduced into the experimental

cages. An ant-free cage with mealybugs and parasitoids only was used as control. After 24 h exposure, the ants and parasitoids were removed. Cages with mealybugs were then allowed to stand for 10 days, after which mummified mealybugs were checked daily and recorded. Percent mummified nymph was computed based on the initial number of exposed host (100 nymphs) while the percent parasitoid emergence was computed based on the number of the mummified nymphs for each treatment. Sex ratio (percent female) was computed as percentage emerging females over the total emerging wasps. The experiment was maintained at  $27 \pm 1^\circ\text{C}$ , 50 - 80% RH with a 12:12 (L: D) photoperiod. The experiment was replicated 10 times.

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

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

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

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

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

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

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

In this study, host handling time is defined as the sum of a female's examination of the mealybug, probing and oviposition time during the observation period in both ant-attended and ant-excluded treatments. For *A. pseudococci*, when a female wasp encountered a host, they



exhibited a stereotypical host examination and ovipositional behaviour that has been well described (Hcidari and Jahan, 2000; Chong and Oetting, 2007). Following each host encounter, host handling was divided into four responses: (a) *host rejection* – occurs if the parasitoid moves away from the host without initiating probing (b) *host acceptance* – this is defined as a behavioural activity where foraging female wasp rotates her body to face away from the host, showing clearly that it had recognized the potential host; (c) *ovipositor probing and penetration* – occurs when the foraging female wasp flexed the tip of her abdomen such that the tip of the ovipositor touches the host body plus a fast and rhythmical insertion of ovipositor into the host.

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

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



## 6.2.6 Statistical analysis

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

## 6.3 Results

### 6.3.1 The mean ( $\pm$ SE) number of ants and parasitoids on the mealybug-infested butternut squash

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

During the 1 min observation periods, the number of *A. pseudococci* on the mealybug-infested butternut in the presence of *O. longinoda* increased from 0 to 7 and averaged  $2.83 \pm 0.13$  wasps. While in the ant-free cage, the number of *A. pseudococci* on the infested butternut ranged from 1 to 9 and averaged  $5.39 \pm 0.16$  wasps during 1 min period of observation. The mean

number of *A. pseudococci* foraging on the infested butternut differed significantly over the 2 h period in the presence and absence of *O. longinoda* ( $t = 12.07$ ;  $df = 23$ ;  $P < 0.0001$ ). The number of foraging parasitoids on the mealybug-infested butternuts increased with time in the presence or absence of *O. longinoda*. Figure 6.2 illustrates the trends in the number of parasitoids on the mealybug-infested butternuts in the presence or absence of ants.

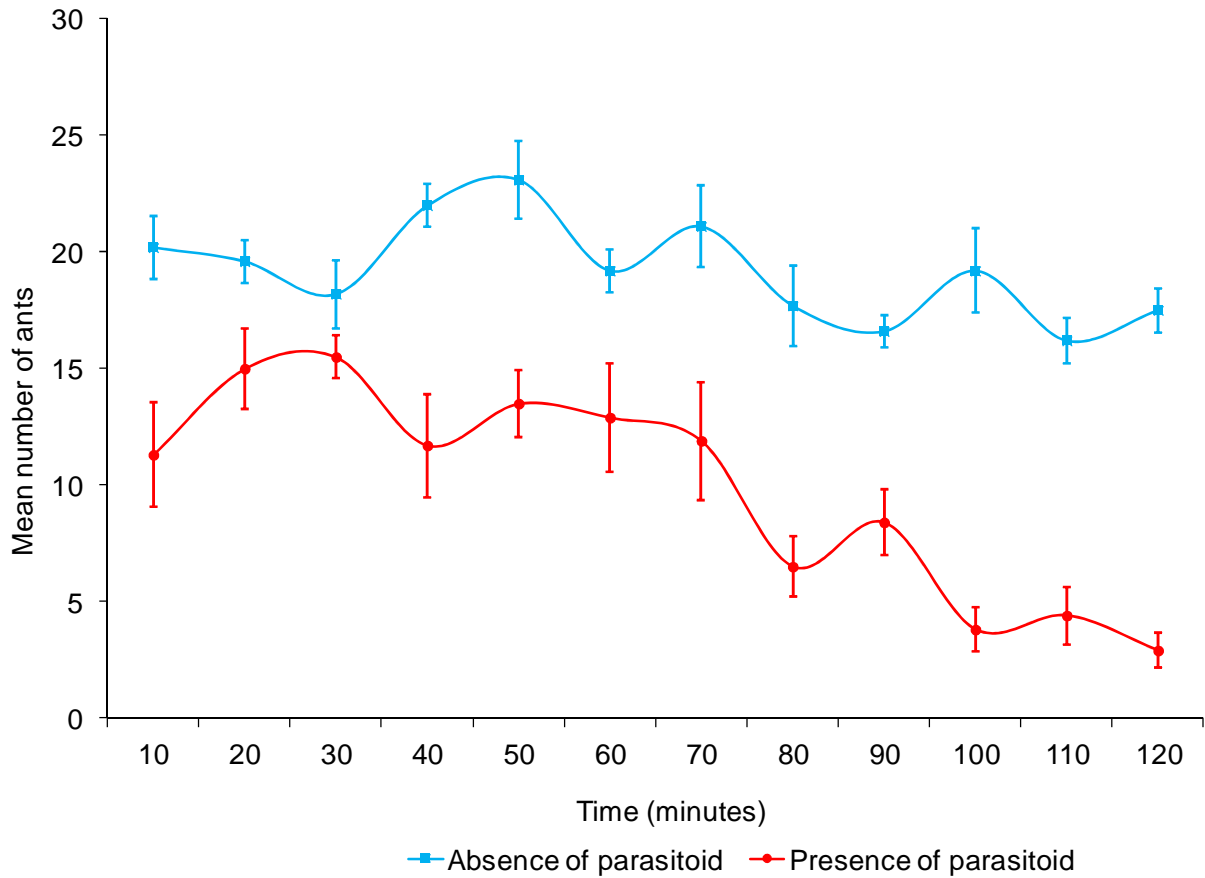


Figure 6. 1: The mean ( $\pm$  SE) number of *O. longinoda* observed on *R. iceryoides*-infested butternut during 1 min intervals over a 2 h observation period in the presence and absence of *A. pseudococci*.

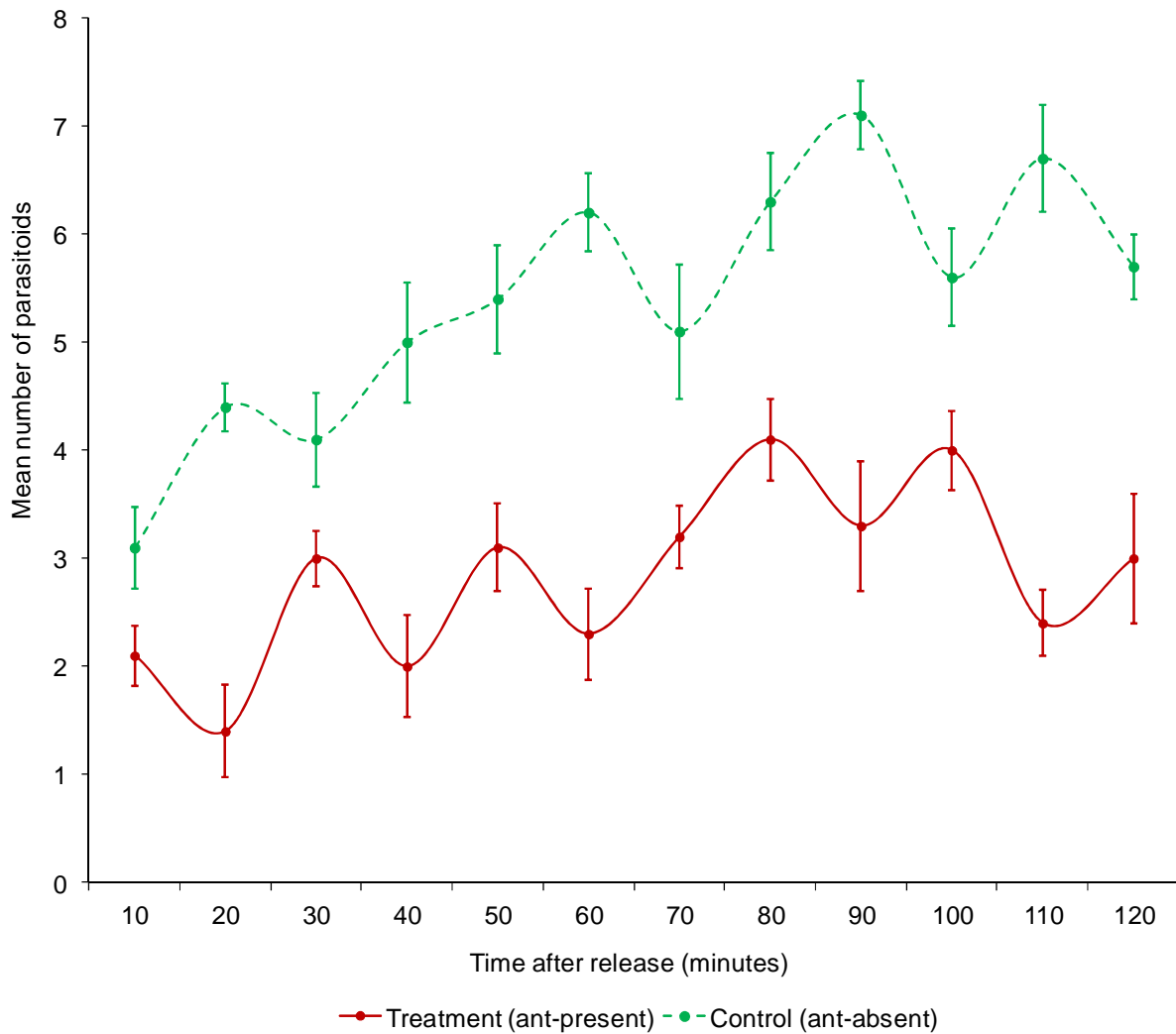


Figure 6. 2: The mean ( $\pm$  SE) number of *A. pseudococci* observed on *R. iceryoides*-infested butternut during 1 min intervals over a 2 h observation period in the presence and absence of *O. longinoda*.

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

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

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

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

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

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

Means with different letters are significantly different at  $P \leq 0.05$ .

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

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

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

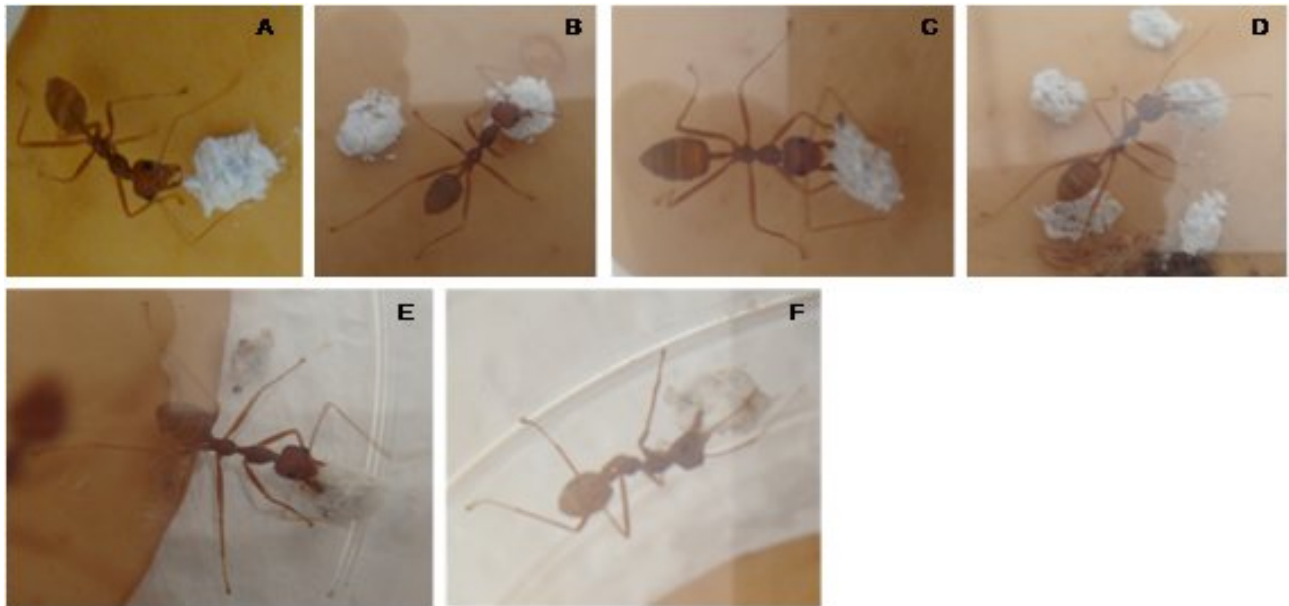


Figure 6. 3: Behavioural interactions between *O. longinoda* and mummified *R. iceryoides* in foraging cages.

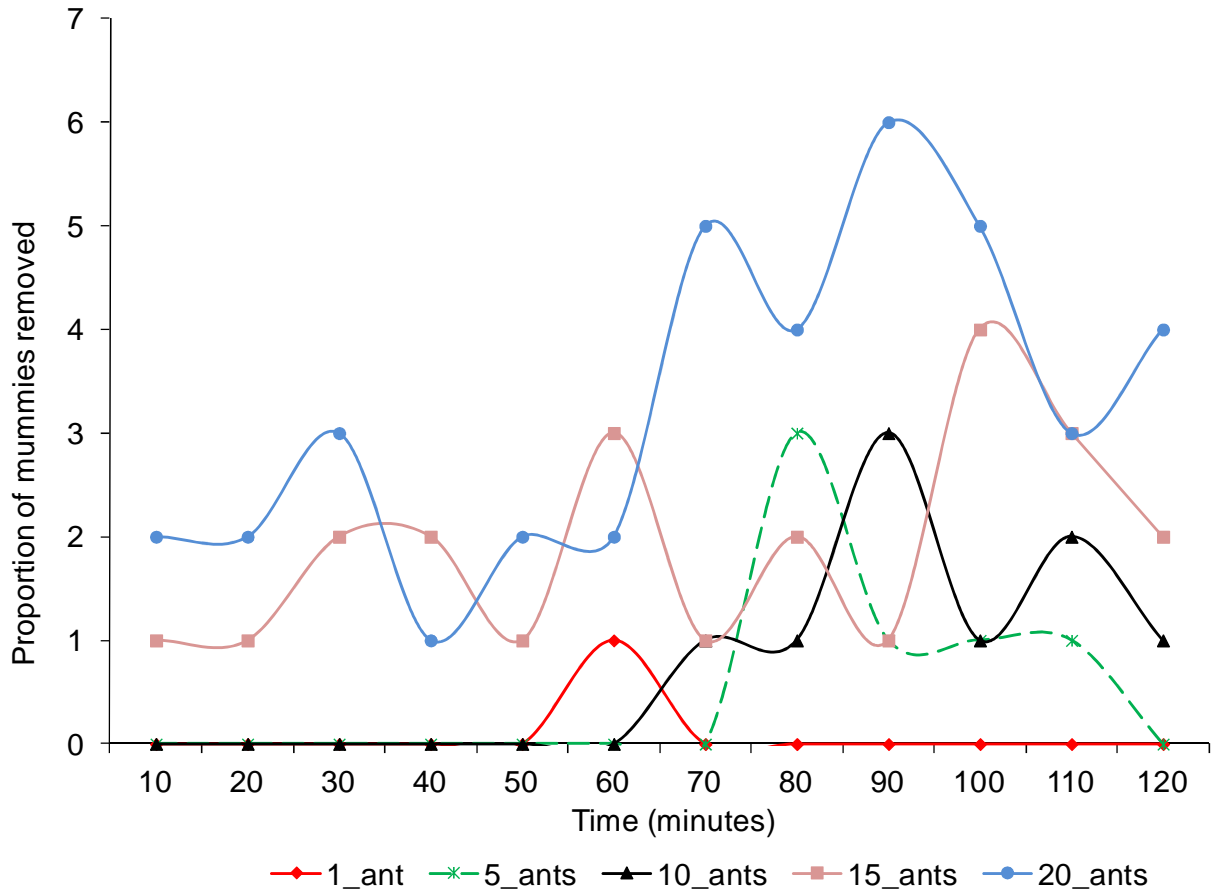


Figure 6. 4: The mean percentage ( $\pm$  S.E) of mummified mealybugs removed by *O. longinoda* at 10 min intervals on butternut during a 2 h observation period.

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

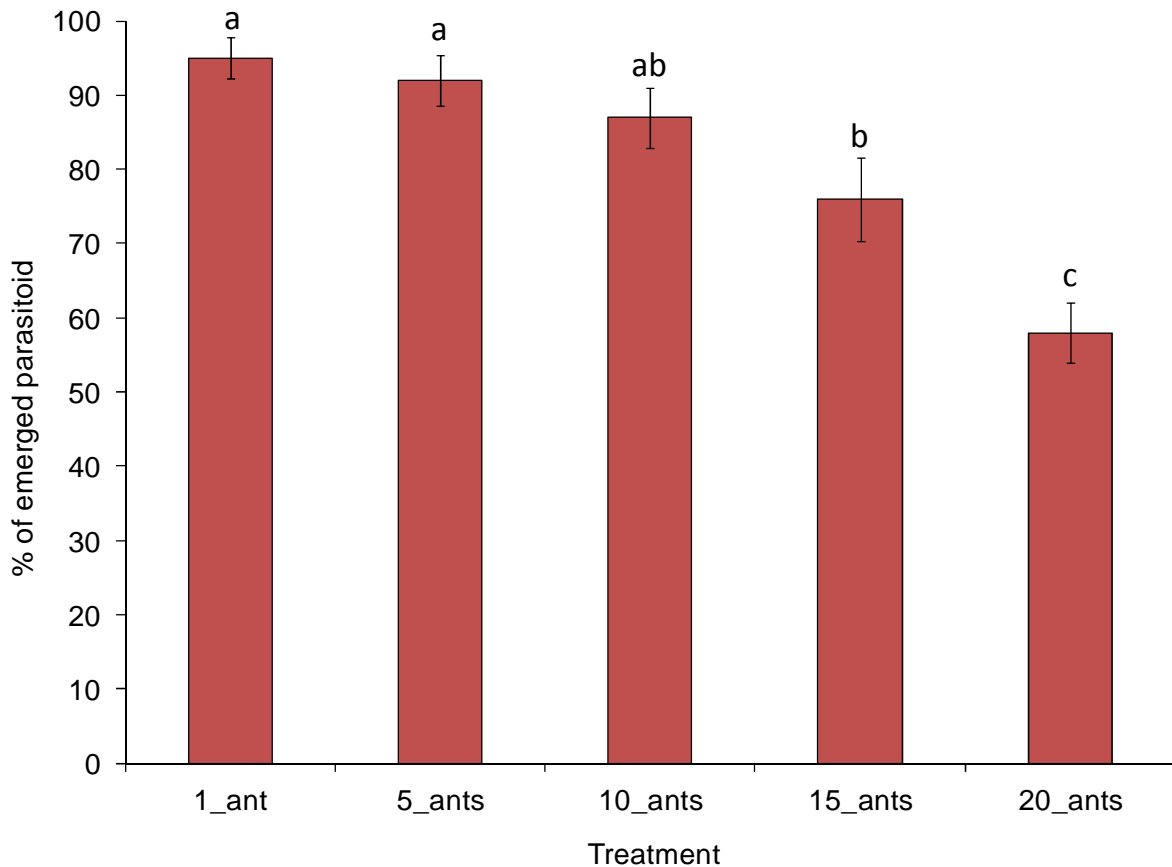


Figure 6. 5: Mean percentage ( $\pm$  S.E) adult parasitoid eclosion when mummified mealybugs were allowed access to different groups of *O. longinoda* workers during a 2 h period.

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

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



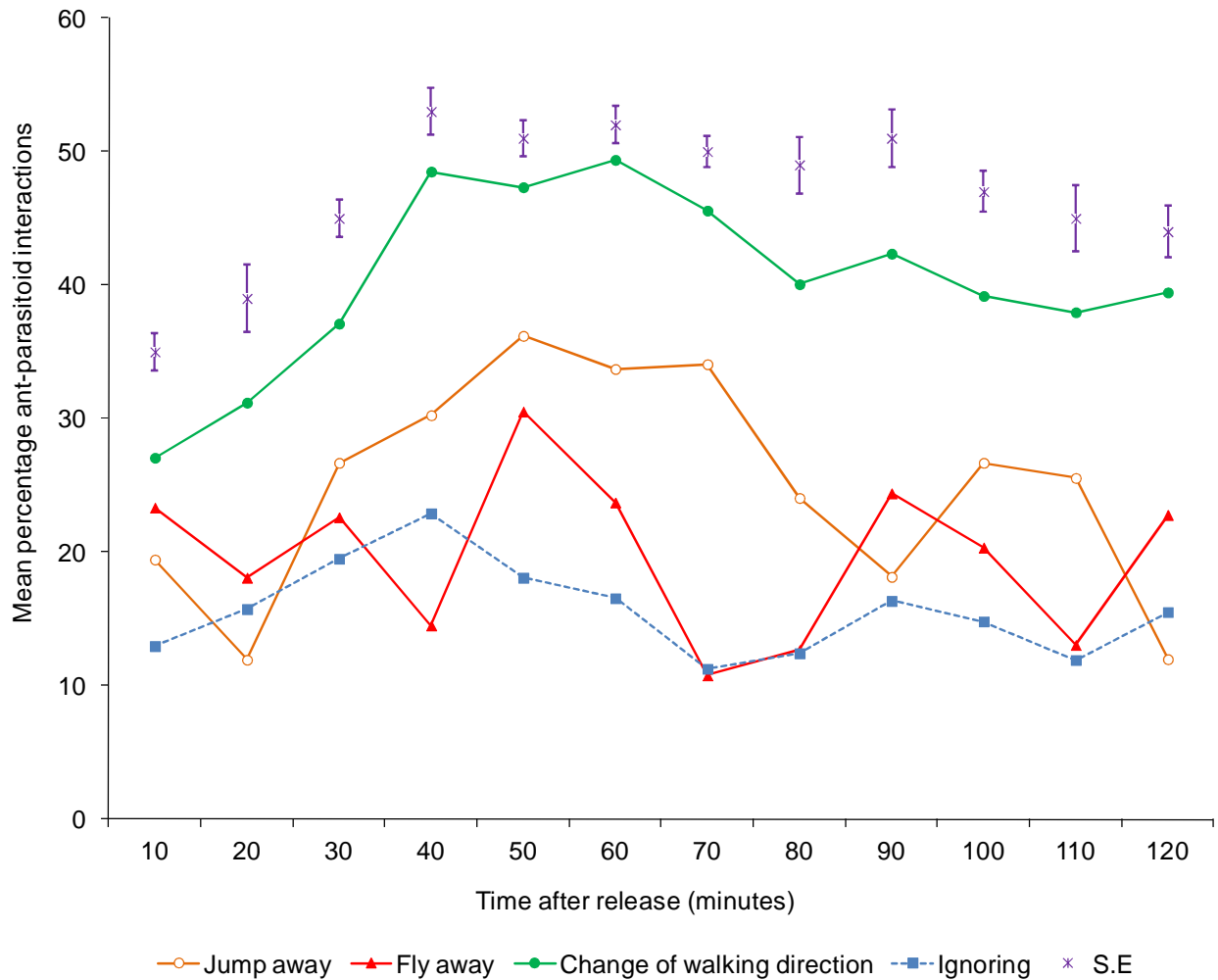


Figure 6. 6: The mean ( $\pm$  SE) percentage behavioural responses by *A. pseudococci* to evade encounters and attacks by *O. longinoda* during 5 min intervals on *R. iceryoides*-infested butternut during a 2 h observation period.

The encounters between ant and parasitoid appeared to be random rather than oriented search by the ant workers. Ants frequently showed aggressive behaviour towards *A. pseudococci* and when *A. pseudococci* was seized, it was sometimes released and picked up again immediately by the same or other ant workers (Figure 6.7). This repeated capturing and recapturing of the same parasitoids resulted in serious injury and sometimes death of the parasitoid. The percentage mortality of adult parasitoids was observed to decrease with time

ranging from  $1.0 \pm 0.67$  to  $3.5 \pm 0.68\%$  (Figure 6.8). The mean percentage mortality of female wasps over the 2 h observation period was  $24.32 \pm 3.32\%$ . However, during non-aggressive interactions with *O. longinoda*, the proportion of flights was considerably high. Response by ant workers was either touching the female wasps with their antennae or their legs or they passed the parasitoid within a closed distance without exhibiting any obvious responses (Figure 9).



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

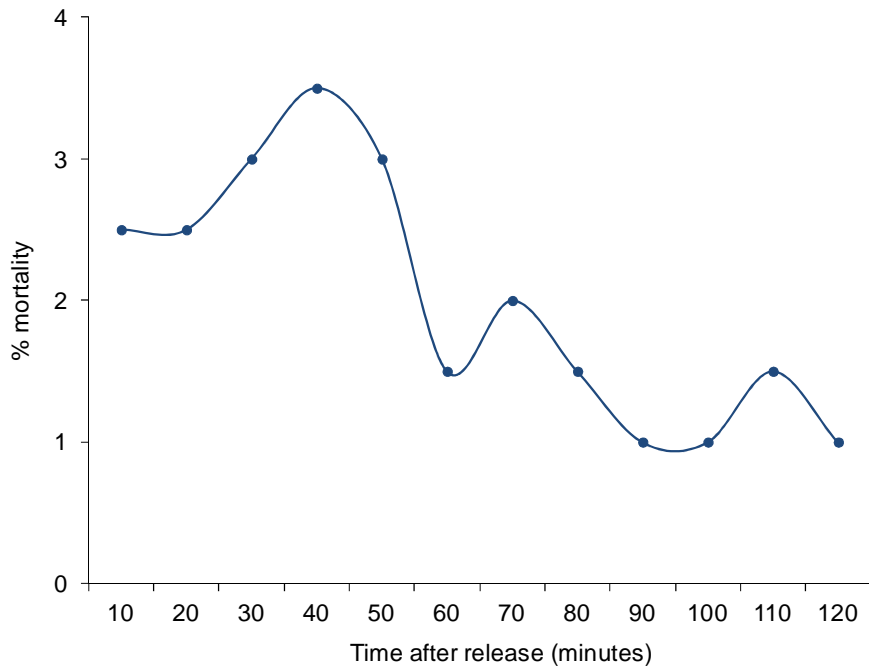


Figure 6. 8: The mean ( $\pm$  SE) percentage mortality of *A. pseudococci* observed in cages with *R. iceryoides*-infested butternut during a 2 h observation period in the presence of *O. longinoda*.



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

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

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

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

Some mealybugs were rejected after several failed attempts by the parasitoid to insert the ovipositor, due to either host defence (Figure 6.10) or repeated failure to insert the ovipositor in

an appropriate area of the host's body (Figure 6.11). The mean number of successful host defence was significantly ( $t = -4.3$ ;  $df = 28$ ;  $P = 0.0002$ ) higher in ant-excluded treatment ( $7.07 \pm 0.77$ ) compared to ant-attended treatment ( $2.93 \pm 0.57$ ).

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

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

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

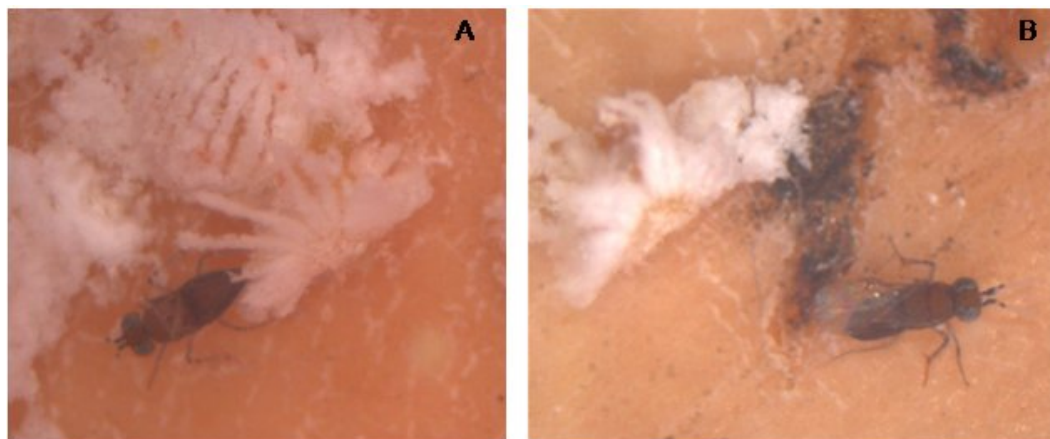


Figure 6. 10: (A) Third instar nymph of *R. iceryoides* exhibiting a more vigorous defense against an ovipositing female of *A. pseudococci*; (B) *A. pseudococci* terminates the oviposition process as it fails to subdue the host.

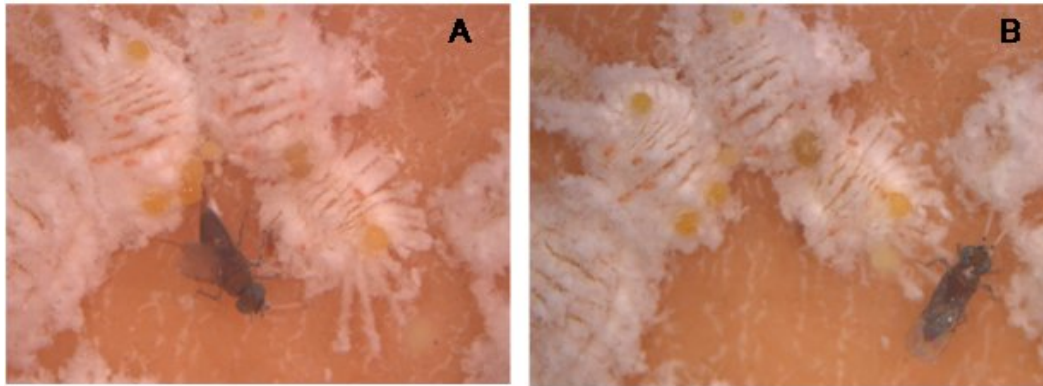


Figure 6. 11: (A) Repeated failure of *A. pseudococci* to insert the ovipositor in an appropriate area of the host's body as the host retreats; (B) Oviposition is terminated prematurely and *A. pseudococci* continued searching elsewhere.

#### 6.4 Discussion

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

However, given the invasive nature and wide distribution of *O. longinoda*, possibly perpetrated by suitable eco-climatic conditions from the coastal shorelines to 1220 meters above

sea level (Way, 1954), its present a serious threat to biological control of Hemipteran pests like *R. iceryoides*, as they are capable of affecting parasitoids in orchards over large areas. In most orchards, they construct numerous temporary nests on tree canopies, presumably providing better protection to mealybugs (Way, 1954), thereby posing a serious threat to mealybug parasitoids.

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

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



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

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

During our study we found that *A. pseudococci* has developed strategies to forage in ant-attended patches and to escape ant aggression based on behavioural responses, which were often combined with morphological adaptations to improve their efficacy. For example, the agile and quickly foraging behaviour of *A. pseudococci* is a typical representative of the avoidance type. The female wasp showed a striking high rate of avoidance behaviour, which prevented direct ant contacts and kept up a reasonable distance to the next ant worker. *Anagyrus pseudococci* females were observed to leave the mealybug-infested butternut immediately when coming in contact with *O. longinoda*. This sensitiveness toward any physical ant contact and following quick flight

response, which is supported by an excellent jumping ability, ensured a low mortality risks in the presence of the very aggressive *O. longinoda*. Similarly, this corroborates with studies reported by several authors on the behavioural strategies of other encyrtid species interacting with ants (Novak, 1994; Völkl, 1995). The flight strategy of *A. pseudococci* on the other hand resulted in short residence times in the arena and significantly low numbers of host contacts in ant-attended treatments. Our investigation of *O. longinoda*-parasitoid interactions in the laboratory gives a better insight into the overall effectiveness of the escape strategies of *A. pseudococci* that might be experienced in the field.

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

Therefore, mass release programs of *A. pseudococci* to control low mealybug infestation in ant infested mango orchards via inundative releases should be done with a lot of caution as

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

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