

**ASPECTS OF ECOLOGY OF WEAVER ANTS (*Oecophylla longinoda*
Latreille) (HYMENOPTERA: FORMICIDAE) IN TANZANIA**

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**A THESIS SUBMITTED IN FULFILMENT OF THE REQUIREMENTS FOR
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EXTENDED ABSTRACT

Studies were undertaken between 2012 and 2015 in Mtwara region, Tanzania to establish an understanding of the factors affecting population dynamics in *Oecophylla longinoda*. Experiments included assessment on; how viable offsprings are produced, when nuptial flights occur and environmental cues that trigger flights. *Oecophylla longinoda* queens were collected at four steps: before flight, immediately after leaving the nest, up to 12 h after leaving the nest and after settling naturally following the nuptial flights. Only eggs produced by queens (n=65) collected after settling naturally hatched into larvae. None of the 527 winged queens that were collected before flights and immediately after leaving the nests produced viable eggs. Observations on 56 flights recorded from 25 colonies revealed that, flights took place in the evenings. Days with flights were associated with higher relative humidity ($p=0.018$), less sunshine ($p=0.045$) and lower wind speed ($p=0.034$) when compared to days without flights. This knowledge offers a tool to improve forecasts of *O. longinoda* flights, facilitating collection of mated queens to stock ant nurseries that would supply ant colonies for IPM-programs. Furthermore, feeds that can be used as food supplements to the ants during food scarcity namely anchovy, earthworm, fish intestines and chicken intestines were tested on 20 colonies. *O. longinoda* preferred anchovy to the rest of the provided feeds. More ants' activity was observed on fresh-ground anchovy compared to the dry form. Large sized particles were easily removed than smaller particles. Analytical Hierarchy Process showed that, earthworm and fish intestines were the most available feeds. Thus, during reduced food availability, farmers in the study area can use such materials as feed supplement for *O. longinoda* colonies. Fresh or dry anchovy of large particle sizes can be used wherever available. On factors

affecting temporal abundance of weaver ants, the percentage of shoots with weaver ants was significantly ($p=0.0003$) associated with cashew growth phases. More weaver ants were recorded during cashew vegetative and reproductive phases than dormancy. Rainfall and temperature negatively affected number of nests, while relative humidity was negatively related to the number of shoots with weaver ants. Thus, weaver ant augmentations strategies should consider the phenology of cashew crop and weather parameters.

DECLARATION

I, WILSON A. NENE, do hereby declare to the Senate of Sokoine University of Agriculture that this thesis is my own original work done within the period of registration and that it has neither been submitted nor being concurrently submitted for a degree award in any other institution.

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The above declaration is confirmed

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Date

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LIST OF ABBREVIATIONS AND SYMBOLS

%	Percentage
AHP	Analytical Hierarchy Process
DANIDA	Danish International Development Agency
DFC	Danish Fellowship Centre
FAO	Food and Agriculture Organization
IITA	International Institute of Tropical Agriculture
IPM	Integrated Pest Management
M	Meters
masl	meters above sea level
m ²	Square meters
mm	Millimeter
NARI	Naliendele Agricultural Research Institute
RH	Relative Humidity
SUA	Sokoine University of Agriculture
UDSM	University of Dar Es Salaam
US\$	United State Dollar
USA	United States of America
χ^2	Chi Square

CHAPTER ONE

1.0 GENERAL INTRODUCTION

1.1 Classification and distribution of weaver ant

Weaver ants are eusocial predatory insects which belong to the ant genus *Oecophylla* of the family Formicidae. The ants belong to the order Hymenoptera of the subfamily Formicinae which contains two closely related living species; *Oecophylla longinoda* Latreille and *Oecophylla smaragdina* Fabricius (Cole and Jones, 1948; Lokkers, 1986; Crozier *et al.*, 2009). Some of the extinct species of *Oecophylla* are *O. ataviana*, *O. perdita*, *O. megarche*, *O. praeclora*, *O. superba*, *O. sicula*, *O. obesa*, *O. leakeyi*, *O. xiejiaheensis* and *O. grandimandibula* (Dlussky *et al.*, 2008).

The genus *Oecophylla* occurs in Asia, Australia and Africa (Cole and Jones, 1948; Way, 1954; Lokkers, 1986). *Oecophylla smaragdina*, the green tree ant has been found in Southeastern Asia and Northern Australia including many tropical western Pacific Islands (Cole and Jones, 1948; Lokkers, 1986). *Oecophylla longinoda* is found in Africa (Way, 1954; Van Mele and Cuc, 2007) with a wide band across equatorial Africa (Crozier *et al.*, 2009).

Distribution of *Oecophylla* may be limited by two physical parameters; mean annual rainfall and average minimum temperature. Low temperatures below 17 °C inhibit larval development. Both rainfall and temperature levels limit the distribution of the forest-wood land vegetation required by these arboreal ants (Lokkers, 1986). The *O. smaragdina* prefers environmental temperatures between 26 °C and 34 °C and relative humidity between 62 % and 92 % (Van Mele and Cuc, 2007). The predators

are found in area with evergreen tree and bushy vegetation (Way, 1954). For example, in East Africa, the ants have been reported to colonize plant hosts in areas such as along the coasts of Kenya and Tanganyika and on the Islands of Pemba, Zanzibar and Mafia (Way, 1954).

1.2 Importance of *Oecophylla* spp.

The *Oecophylla* spp, in particular, *O. smaragdina* is the earliest biological control agent recognized in China dating from 304 A.D (Hölldobler and Wilson, 1990; Van Mele, 2008). The *O. smaragdina* and *O. longinoda* can control more than 50 different pests in multiple crops and forest trees (Way and Khoo, 1992; Peng *et al.*, 1995). Their ability to control pests can be similar or more than chemical pesticides (Peng and Christian, 2005a; Dwomoh *et al.*, 2009; Offenberg *et al.*, 2013).

The ants successfully reduce damages caused by four most important cashew insect pests; the tea mosquito bug, *Helopeltis* spp, the mango tip borer, *Penicillaria jocosatrix*, the fruit spotting bug, *Amblypelta* spp and the leaf roller, *Anigraea* spp. (Peng *et al.*, 1995; Peng *et al.*, 1997; Christian *et al.*, 2008; Van Mele, 2008). Farmers in Vietnam reported that, cashew nuts in plots with weaver ants were cleaner and shiner than those with no weaver ants, and suggested that, weaver ants were either better than or similar to insecticide control in terms of overall tree performance and the main insect pest damage (Van Mele, 2007; Christian *et al.*, 2008).

The ant, *Oecophylla* controls fruit flies (Van Mele *et al.*, 2007), seed weevil and leafhopper in mango (Peng and Christian, 2007). The ants also control bugs in coconut, cocoa, cashew and mango (Van Mele, 2008). The ants protect African

mahogany trees against shoot borers (Peng *et al.*, 2010; Peng *et al.*, 2011). In Tanzania, the presence of *O. longinoda* was reported to reduce damage caused by *Helopeltis anacardii* Miller and *Pseudotheraptus wayi* Brown, the most important insect pests of cashew (Stathers, 1995; Olotu, 2012). In Palm oil plantations, *O. smaragdina* control *Pteroma pendula* (Lepidoptera: Psychidae) (Pierre and Idris, 2013). The ants also protect eucalyptus, mahogany red cedar and other timber trees against damaging insect pests (Van Mele and Cuc, 2007).

Weaver ants reduced cost of production in citrus farms in Vietnam (Van Mele, 2008). Vietnamese farmers who took care of weaver ants spent on average half the amount of money on agrochemicals compared with those farmers who did not have *Oecophylla* in their orchards (Peng and Christian, 2005b; Van Mele, 2008).

Other uses of weaver ants as listed by Crozier *et al.* (2009) include a source of food and medicine in South-east Asia and valued food for song birds. Extracts from the ants are used by Indian traditional healers to treat a range of common diseases. Indigenous Australian women use weaver ants to produce a remedy for colds and flu.

1.3 Mating strategy of weaver ant

Reproduction has been cited as a determinant in population ecology (Gomez and Abril, 2012). As such mating behaviour including nuptial flight in ants have been stated as an essential process for reproduction (Dhami and Booth, 2008; Gomez and Abril, 2012).

There are two schools of thought on mating strategy of weaver ants. Vanderplank (1960) indicates that, winged females of *O. longinoda* are already inseminated when they emerge from their nests during rainy season. This implies no need for winged females and males to perform nuptial flights like other ant species, because mating occur within the nests before the flight. He found that, queens collected when emerging from their natal nests laid eggs that subsequently produced viable offsprings. However, the work by Peng *et al.* (2013) revealed that, winged females and males of *O. smaragdina* a closely related species of *O. longinoda* fly for mating (nuptial flight). After mating, the new queens find a suitable place for new colony establishment. Queens shed out wings and start to lay eggs (Van Mele and Cuc, 2007; Peng *et al.*, 2013). Mated new queens returning from nuptial flight, can be assisted to establish colonies by making artificial nests on trees (Peng *et al.*, 2013).

Timing of nuptial flight for reproduction is a key component of an organism's life history (Tschinkel, 1991; Kaspari *et al.*, 2001). It influences the timing of subsequent life stages and the forms of reproduction that are possible (Dunn *et al.*, 2007). The flight involves the aggregation of males (Van Veen and Sommeijer, 2000) and tends to be short in time in many ant species (Levin *et al.*, 2009).

Weather condition is one among vital determinants of the nuptial flight to occur (Gomez and Abril, 2012). The nuptial flight of social ant *Solenopsis invicta* generally occur after a day or two days of rainfall, at temperatures between 21-33 °C and RH of above 80% (Dhami and Booth, 2008). Under nuptial flights many ant species have an opportunity to mate and mixing up genetically where in turn their persistence and survival increases (Dhami and Booth, 2008). Given a lot of

unknown, mating strategies of *O. longinoda* needs to be resolved through systematic field and screen house observations.

1.4 Weaver ant life cycle and developmental physiology

The work by Bolton (1994) indicated that there are four stages in the life cycle of weaver ants; egg, larvae, pupae, and adult and it takes approximately 30 days to develop from an egg to an adult worker. There are also at least three larval instars (larval growth periods) before the immature ant can pupate. *O. longinoda* pupates without enclosing itself in silk, as it spends its silk on nest construction. Instead, it remains exposed as it undergoes a complete metamorphosis from its larval, grub-like form into an adult ant (Bolton, 1994).

The alate queens shed their wings in the first or second day after mating (Vanderplank, 1960). Time taken for brood development is influenced by temperatures (Vanderplank, 1960). The number of days taken for an ovum to hatch is 12 and half at 24 °C, six and half at 28 °C, five and half at 30 °C and 4 at 33.3 °C (Vanderplank, 1960).

1.5 Colony composition and division of labours

A colony is composed of one or more queens, males, large workers and small workers (Van Mele and Cuc, 2007). The distinctions of weaver ant forms in a colony are as follows (Van Mele and Cuc, 2007; IITA-CIRAD, 2008): queens; are big with large abdomen and produce eggs. Queens are winged at first, and lose wings after mating. Males are; smaller than the queens, have blackish coloured body with wings. Sole role of males is to mate with the queen and they die after mating. Major worker

ants are; the largest group in the colony responsible for building nests, defending them and collecting food for the whole colony. Minor worker ants; often remain inside the nest, administering to the needs of the hatch and other domestic tasks (Plate 1.1)

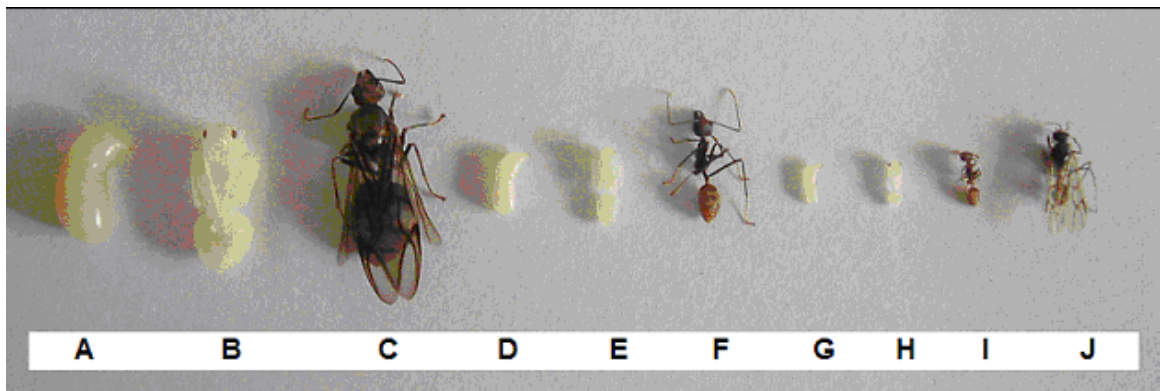


Plate 1. 1: Different life stages and forms of weaver ants. A – queen larva; B – queen pupa; C – winged queen; D – male or major worker larva; E – male or major worker pupa; F – major worker; G– minor worker larva; H – minor worker pupa; I – minor worker; J – male.

Source: (IITA-CIRAD, 2008).

1.5.1 Division of labour

The division of labour by weaver ants is according to the size of workers. Major workers forage, defend, maintain and expand the colony whereas minor workers tend to stay within the nests where they care for the brood. Major workers are also responsible for nest construction. They draw leaves together with the use of their mandibles and glue them together with larval silk (Way, 1954; Lokkers, 1990; Crozier *et al.*, 2009). Ants' pupae are not enclosed in silk because it is spent on nest construction (Plate 1.2).



Plate 1. 2: Weaver ant worker squeezing a larvae to produce silk for nest construction

Source: (Maffett, 2011)

1.6 Weaver ants' food requirements and their foraging behavior

Weaver ants require sugar and protein for their growth and development (Lockers, 1990; Van Mele and Cuc, 2007). However, weaver ants prefer protein to sugar (Van Mele and Cuc, 2007). They get protein food as prey on multitudes of insect pests (Way and Khoo, 1992, Peng *et al.*, 2008). Weaver ants also get their sugar from honeydew produced by sap-sucking insects or plant nectars (Van Mele and Cuc, 2007).

1.6.1 Effects of nutritional composition of food on ant

Nutritional composition of food influences the foraging decisions of ants (Kay, 2002). Ants sense presence of food using odour trails released from the sternal and rectal glands in the abdomen (Hölldobler and Wilson, 1978). Weaver ants have varied preferences of feed types (protein and sugar sources). Lim (2007) reported

greater counts of *O. smaragdina* in mealworm feeding bowl than in other feeds such as fish, honey and weaver ant formula. Ant species that collect exudates prefers food containing protein to carbohydrates (Kay, 2002). Ant species of *Aphaenogaster cockerelli* and *Pogonomyrmex occidentalis* rejected 2 % sucrose but accepted a mixture of 2 % sucrose + 2 % casein and the acceptance levels increased with percentage increase in casein concentration (Kay, 2002). Food imbalance and reduced feed availability, particularly protein and sugar have been reported to negatively affect the population of ants and other social insects (Dusstour and Sympson, 2012). During food scarcity, colonies can be supplemented with foods to maintain and sustain them (Lim, 2007). Therefore, it was imperative that feed type and forms to feed *O. longinoda* colonies be examined in this study.

1.7 Regulation of weaver ant abundances and management practices

1.7.1 Suitability of hosts and weather conditions

Suitability of hosts and weather conditions greatly determine the stability and population build-up of weaver ant colonies. Presence of enough prey, honey dew secretion on tender shoots by the respective host is considered to promote high and stable weaver ant's colonies (Van Mele and Cuc, 2007). New and flexible leaves can easily facilitate nests construction. Therefore, the presence of nectar and new flexible leaves at some stages of host plant growth phases, for instance cashew, can influence temporal abundances of weaver ants. Weather conditions such as temperature, rainfall and relative humidity can affect weaver ant abundances. Temperature below 21°C and above 34 °C negatively affects brood development (Lokker, 1990).

Conversely, harsh conditions such as heavy rainfall reduce weaver ant populations (Peng *et al.*, 2008).

1.7.2 Presence of Weaver ant antagonists such as *Pheidole* and *Anoplolepis* spp

Pheidole spp is among the antagonists of the weaver ant (Van Mele, 2008). In Tanzania, *Pheidole megacephala* is the species most known to antagonize *O. longinoda* (Zerhusen and Rashid, 1992; Seguni *et al.*, 2011). *Pheidole* spp tend to nest around the base of tree trunks. They forage on the ground and stream up the trunks and can be found on tree canopies (NARI, 1995). *Pheidole megacephala* are aggressive towards other ant species and their highly efficient predatory abilities favour the success of the invasive (Dejean *et al.*, 2007). O'Connor (1950) reported that *O. longinoda* that passes through *Pheidole* spp territory at the base of the trunk would often be destroyed.

Crazy ants, *Anoplolepis* spp gets its name because of its erratic and rapid movements (appears to be “acting like crazy”). Crazy ants are very much adaptive, having the ability to survive in both dry and moist conditions. The ants nest a distance away from its foraging area. NARI (1995) reported that in cashew, *Anoplolepis castodiens* are observed swarming up trunks and competing with *O. longinoda* workers. Other ants that have reported to reduce weaver ant populations are *Crematogaster* sp and Black ant, *Dolichoderus thoracicus* (Peng *et al.*, 2008).

1.7.3 Application of synthetic insecticides against insect pests

Proper use of insecticides for the control of insect pest leads to the production of more crops (Dix *et al.*, 1995). However, may result to insect pest resistance to

pesticides, unintentional pesticide damage to non-target organisms, and contamination of the environment (Dix *et al.*, 1995). Application of toxic chemicals should be avoided in order to maintain the existence of weaver ants in an orchard (IITA-CIRAD, 2008). Christian *et al.* (2008) reported that, the use of insecticides could result into increased costs and reduction of pollinators.

Application of broad-spectrum pesticides had swept aside many of the natural enemies of pests and trigger secondary pest outbreaks (Van Mele, 2008). Spraying of some toxic chemicals that kill beneficial insects such as weaver ants could lead to outbreaks of insect pests such as aphids, scales and mealybugs (Van Mele and Cuc, 2007). Organophosphates and synthetic pyrethroids should be completely avoided due to their negative effects on weaver ant and the health status of sprayer operators (Van Mele and Cuc, 2007). The work by Christian *et al.* (2008) in Vietnam, found no weaver ant in 15 cashew orchards which were sprayed with insecticides three times or more per year.

1.7.4 Weaver ant management practices

Several research reports indicate that, utilization of weaver ant as both predators and sources of food needs a high population density and stable weaver ant colony. For instance, Stathers (1995) revealed that, cashew trees colonized (abundantly >500 foraging *O. longinoda*) by *O. longinoda* were less damaged by *H. anacardii* and *P. wayi* than those with few *O. longinoda* (1-20). According to Way (1953), *O. longinoda* provided adequate palm protection when two or more nests were found in the palm crown. Sporleder and Rapp (1998) reported that population of *P. wayi* sunk to zero after a long and stable occupation of the palm by *O. longinoda*. A crop can be

adequately protected against insect pests if more than 50 % of tree's main branches hold weaver ant trails (Peng *et al.*, 2008). Therefore, weaver ant colonies must be managed to keep their populations higher for adequate protection of crops against insect pests.

Different weaver ant management practices have been recommended. *Pheidole* spp have been controlled through the use of Amdro bait (Seguni, 1993), spraying with insecticides like Dieldrin or killing the weeds with Paraquat (Van Mele, 2008) and ecological management through weeding regimes (Seguni *et al.*, 2011). Farmers in Vietnam use pesticides that are less harmful to the weaver ants (Van Mele and Cuc, 2007). Colony expansion is facilitated by using strings and poles to connect trees (Van Mele and Cuc, 2000; 2007). Furthermore, suitable artificial nesting sites are provided to the weaver ants under unfavourable nesting conditions (Offenberg, 2014).

1.8 Justification for the study

Weaver ants are useful natural enemies in fruit crop production particularly cashew, citrus and mangoes (Van Mele and Cuc, 2007). In Tanzania, the insect naturally colonises the crops offering continued protection thereby minimizing the need for application of costly insecticides most of which are environmentally unfriendly (Stathers, 1995).

Weaver ant populations in several fruit crops have been observed to decline at some periods of the year. Whole colonies can even shift to non agricultural fields (Van Mele and Cuc, 2007) resulting in inadequate crop protection against insect pests. The

causes of such decline particularly on cashew and citrus fruit in Tanzania have never been clearly determined. Factors affecting the reproduction, feed availability and habitat, largely contributed to population decline in several insects (Pedigo and Rice, 2009). Knowledge on reproductive strategy by *O. longinoda* was scarce. No clear information on whether mating occurs within the nests before flight or after flights. Associations between time from nuptial flights to egg laying and shedding wings with eggs hatching had never been determined. Time of flight for *O. longinoda*, colony behavior such as aggregations before flights and nest conditions after flights were not well documented. Aggregations and flights durations, quantifications of winged males and females (sexual) that aggregate on nest surface or perform nuptial flights had never been determined. Effects of weather parameters such as rainfall, temperature, relative humidity and wind speed on nuptial flight by *O. longinoda* was also unclear. As the need for weaver ant-based bio-control increased in lieu of excessive use of insecticides and advocacy to organically produced produce, understanding of their reproductive strategies became paramount.

This may guide the strategies for collection and artificial rearing of weaver ant colonies for ultimate packaging and supply to the needy farmers in the organic farming sector. Supplemental feeding, particularly during food scarcity is one of management practices that maintain and boost weaver ants population. However, weaver ants have varied preferences of feed types (Lim, 2007). Greater counts of *O. smaragdina* were observed in mealworm feeding bowl than other feeds such as fish, honey and weaver ant formula (Lim, 2007). Supplementing for *O. longinoda* is limited by inadequate knowledge on type of feeds that can be accessed by farmers to

feed the ant. Knowledge on food preference could guide decisions on supplementary feeding for better establishment and expansion of the ant colonies.

Suitability of hosts that offers the right habitat to the weaver ant greatly determines the stability and population build up of weaver ant colonies (Van Mele and Cuc, 2007). Weather parameters such as rainfall, temperature and relative humidity affect weaver ant abundances (Van Mele and Cuc, 2007). Information on associations between cashew phenology and weather parameters with weaver ant abundances is scant.

The current study intended to explore the key factors affecting weaver ant population. The knowledge generated from this study can provide valuable information on population build up and colony stability of *O. longinoda* and its use in integrated pest management (IPM).

1.9 Objectives

1.9.1 Overall Objective

The overall objective of the study was to establish an understanding of the key factors affecting population dynamics of African Weaver ant, *Oecophylla longinoda*.

1.9.2 Specific objectives

The specific objectives were:

- (i) To explore mating strategies of Weaver ant, *O. longinoda*
- (ii) To determine feed preferences by Weaver ant, *O. longinoda*

(iii) To identify factors affecting temporal distribution of Weaver ant, *O.*

longinoda in cashew orchard

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CHAPTER TWO

2.0 Mating Behavior of the African Weaver Ant, *Oecophylla longinoda* Latreille (Hymenoptera: Formicidae)

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Abstract

Mating in most species of ants occur during nuptial flights. In the African weaver ant, *Oecophylla longinoda* Latreille, mating was previously hypothesized to take place within the nest before the nuptial flight but no research data has ever been presented to support this. Understanding the mating strategy of *O. longinoda* is important for its successful application in biological control programs. Here we report on the findings from studies conducted in Tanzania to determine whether mating occur prior to dispersal flight. Winged *O. longinoda* queens collected at four approaches; before taking flight, immediately after leaving the nest, up to 12h after leaving the nest and after settling naturally following the nuptial flights were examined. Mating in captivity with varied number of males and queens was also assessed. Results showed that no eggs hatched from any of the 527 winged queens that were collected prior to their dispersal flights and no mating attempts in captivity lead to viable offspring. Only eggs produced by queens collected after settling naturally (N=65) hatched into larvae. High percentages (88.73) of eggs that hatched were laid by queens that shed wings and laid their eggs within 3 days after nuptial flights. Findings from the current study suggest that mating of *O. longinoda* queens take place during a nuptial flight and does not take place within the nest, as previously suggested. Time from nuptial flights to shedding of wings and egg laying translates to hatchability of the eggs.

Key words; African weaver ant, mating behavior, nuptial flight, *Oecophylla longinoda*

2.1 Introduction

The African Weaver Ant (AWA), *Oecophylla longinoda* Latreille (Hymenoptera: Formicidae) is a predatory species which controls a wide range of insect pests in multiple crops (Way and Khoo, 1992; Van Mele, 2008). The specie is increasingly being used in biological control programs in tropical plantations (Van Mele, 2008). A stable and high population level is usually required for effective control of insect pests by *O. longinoda* (Sporleder and Rapp, 1998). Introduction of *O. longinoda* into crop fields is done after mapping of colonies in natural habitat, subsequent harvesting and transplanting. However, the transplantation of colonies into new plantations is hampered by the difficulties in finding the single maternal queen in colonies comprising up to several hundreds of nests (Ouagoussounon *et al.*, 2013; Rwegasira *et al.*, 2014).

Oecophylla spp. queens disperse from their natal colonies at the onset and/or during the rainy season (Vanderplank, 1960; Offenbergl and Wiwatwitaya, 2010; Peng *et al.*, 2013). Copulation of unrelated sexual forms during nuptial flights (Depa, 2006), provides an opportunity for genetic mixing and plays an important role in the life cycle of many ant species (Dhami and Booth, 2008). There are mainly two mating systems which have been reported in ants namely; male aggregation and female calling. Male aggregation (MA) is a system that winged males and females meet during nuptial flights. Females enter a swarm, which is dominated by males and subsequently mate with one or more of these males and return from the flight as fertile queens (Kaspari *et al.*, 2001). Female calling (FC) is a system that winged females position themselves near their natal nests and emit pheromones to attract winged males with whom they mate, before they disperse or re-enter their natal colony as fertile queens (Kaspari *et al.*, 2001). In rare cases, mating occur within the nest, for instance in *Formica aquilonia* (Yarrow) (Fortelius, 2005) and *Linepithema humile* (Myr) (Aron, 2001). In independent founding species, including

Oecophylla spp., fertilized queen's search for nesting places and shed their wings after the mating (Hölldobler and Wilson, 1978; Dhami and Booth, 2008). Queens then rear their first brood using food reserves stored in the fat body and their wing musculature (Keller and Passera, 1989).

Mating strategy of *O. longinoda* has never been established. Understanding the mating strategy of *O. longinoda* is an important prerequisite for their successful use in integrated pest management (IPM), particularly in collection of mated queens to stock ant nurseries (Ouagoussounon et al., 2013; Peng *et al.*, 2013). Way (1954) collected alate and dealated queens of *O. longinoda* in the field, during the ants' mating seasons in Tanzania which laid eggs that subsequently hatched into viable offspring. However, it was not known how and when mating occurred. Vanderplank (1960) hypothesized that mating by *O. longinoda* takes place within the nest, before dispersal of the queens. In a latter case, males liberated from their natal nests, disperse and enter into the nests of other colonies, where mating with alate queens takes place. Reported facts about sexuals of the closely related Asian green tree ant, *Oecophylla smaragdina*, unveiled that mating occurs during nuptial flights (Peng *et al.*, 2013). Similar case would be true to *O. longinoda* but evidence based on data was required to disprove Vanderplank's hypothesis.

The current study aimed at examining the mating strategies of *O. longinoda* by testing the fertility of queens at different dispersal stages (pre-dispersing, dispersing, post-dispersing and natural settling).

2.2 Materials and Methods

2.2.1 The study area

Field Experiments were conducted from 2012 to 2014 at Naliendele Agricultural Research Institute (NARI) in Mtwara region, southern Tanzania (40°09' 57.05" E, 10°

21° 22.49' S). The region has a unimodal rainfall lasting from November/December to April/May and the mean annual rainfall ranges from 810 to 1090 mm. The mean maximum and minimum temperatures are 27 °C and 23 °C respectively.

2.2.2 Collection of queens at different stages of dispersal

Winged queens were caught during different stages of their dispersal from their natal nests. Four different stages of nuptial flights were considered; i) pre-dispersal, ii) dispersal, iii) post-dispersal and iv) after naturally settling. In the first stage, alate queens were caught before dispersal, either from surface of the nests or from vegetation in the immediate neighborhood of nests. In the second stage, queens were collected immediately after they took off from their nests. The queens were trapped with mosquito nets that covered cashew trees in the field as well as plastic sheets that covered potted mango and cashew seedlings. In the third stage, queens that attempted to disperse from four colonies established on cashew seedlings were collected from the roof of the screen house, twelve hours after each dispersal flight. In the fourth stage, queens were collected by using artificial nests, after settling naturally as described by Peng *et al.* (2013). In this technique, a total of 1000 artificial nests were constructed on 10 citrus trees.

2.2.3 Testing for fertility of the queens collected at different stages of dispersal

Each collected queen was kept in a match box (53x36x17mm) placed on tables in a screen house and protected from other ants as described by Way (1954). Queens were provided with distilled water every day. A 20% sucrose solution was also provided after the first workers emerged. Rearing continued for 60 days after the queens were collected to allow complete growth development period. Number of queens that shed wings, numbers of queens that laid eggs, and queens whose eggs hatched and developed into larvae, pupa and imago were recorded. The mortality of queens after 60 days of development was calculated and compared between the tested groups.

2.2.4 Testing for fertility of queens in captivity

Queens and males were collected from nest surfaces and nearby vegetation just before mating. A queen was paired with 0, 1, 2, 3, 4, or 5 males in a matchbox during the first mating season (December 2012 to April 2013). The same combinations of males were paired with two queens in a matchbox, except in the second season (December 2013 to April 2014). Each combination was composed of two different kinship relations; i.e with queens and males originating from the same or different colonies and replicated three times. Queens and males were maintained for 60 days as in the queen fertility tests at varied stages of dispersal described above. The number of queens that shed their wings, numbers of queens that laid eggs, and queens whose eggs hatched and developed into larvae, pupa and workers were recorded.

2.2.5 Developmental times of initial stages of weaver ants

Queens were collected using artificial nests placed on citrus trees at Moma ($40^{\circ}18' 25.1''$ E, $10^{\circ} 39' 71.7''$ S, 34 m asl) and Sogea ($40^{\circ}14' 71.4''$ E, $10^{\circ} 37' 79.1''$ S, 142 m asl) sites in Mtwara from January to April 2015. Founding queens were reared in matchboxes placed in cages in the shade house. Number of queens that shed wings ($n=103$) as well as number of queens that laid eggs ($n=103$) within 3, 6 and above six days from nuptial flights were recorded. Also recording was done on number of queens with hatched eggs from 10th to 14th and beyond 14th days after nuptial flights ($n=69$). The temperature during rearing period ranged between 28 and 29 °C. Queens were provided with distilled water *ad libitum*.

2.2.6 Data analysis

Collected data were analysed using JMP 10.0 software (SAS, 1995). Chi Square was used to test dependency of wing shedding, egg laying, egg hatching and queens' survival,

on different stage of queen dispersal. The dependency of wing shedding, egg laying and egg hatching on days from nuptial flights were also analysed by Chi Square test.

2.3 Results

2.3.1 Fertility status and survival of collected queens

All collected queens produced eggs, regardless of stage of collection. Egg production was not dependent on stage of collection in both the first ($X^2=7.418$, $p=0.06$) and second ($X^2=5.88$, $p=0.12$) season. Only queens that settled naturally after dispersal flights produced viable eggs (Fig. 2.1). The proportion of queens that produced eggs was higher than the proportion that shed their wings (Fig. 2.1). The proportion of queens that shed their wings varied between 60.6 and 100%. Shedding of wings was significantly dependent on stage of queen collection in both first ($X^2=24.83$; $p<0.0001$) and second season ($X^2= 28.60$; $p<0.0001$). The highest proportion of queens that shed wings were those collected after settling naturally.

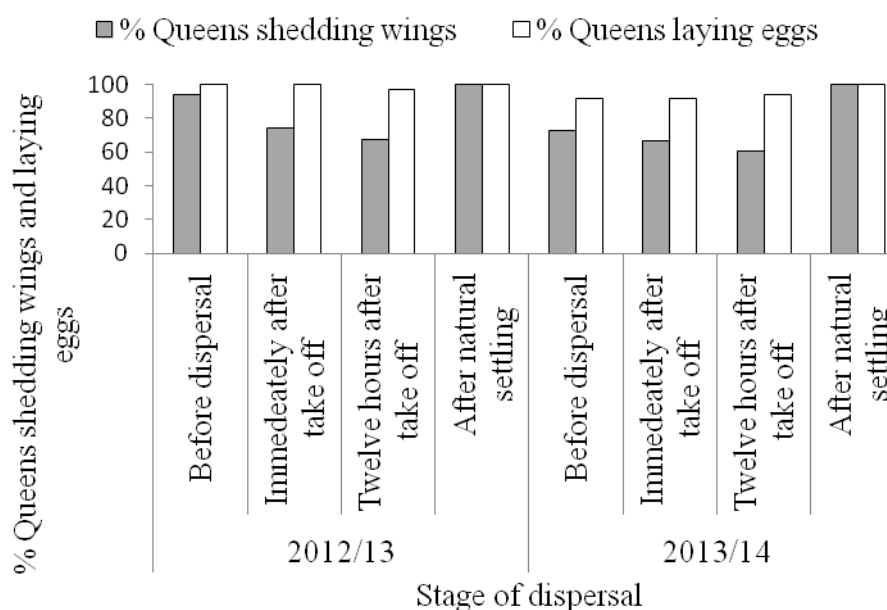


Figure 2. 1: Reproductive success of queens collected at different dispersal stages

Results on survival of queens collected at different stages of dispersal are presented in Fig. 2.2. In all cases, survival was above 88 %, except for queens that settled naturally after dispersal (73.7% to 94.1). Survival of queens was significantly dependent on stage of collection in the first season ($X^2=8.2$, $p=0.043$) but not in the second season ($X^2=0.80$, $p=0.85$). Queens collected pre-dispersal to 12 hours after dispersal had higher survival rates while those collected after settling naturally had lowest survival rate.

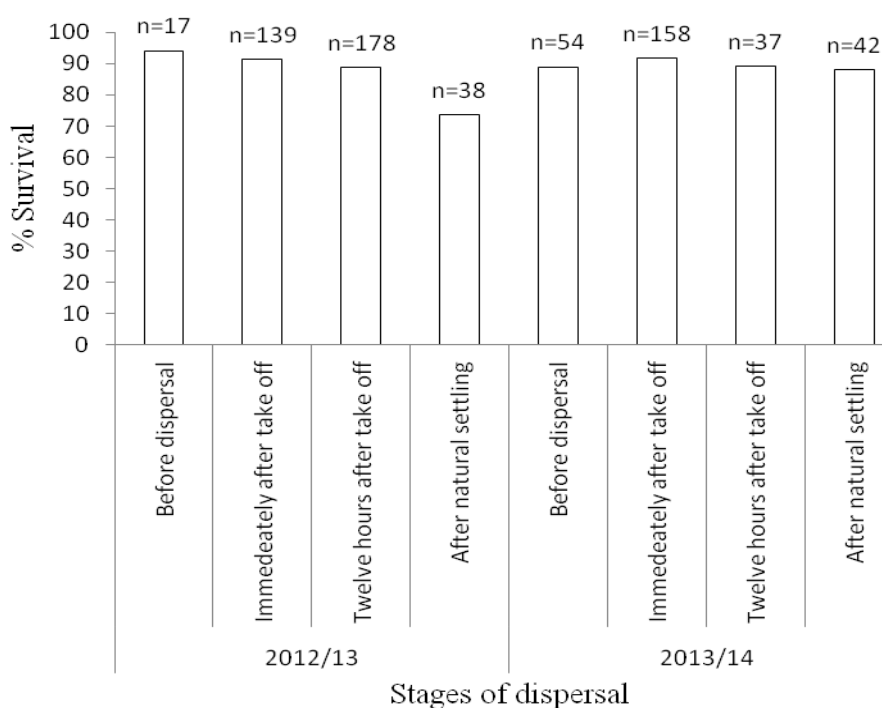


Figure 2. 2: Survival rates of queens caught at different stages of dispersal from their natal nests in 60 days of rearing

2.3.2 Mating in captivity

None of the males that were introduced to alate queens in match boxes survived, regardless of the combination. Males died within approximately two days after they were placed in the match boxes of alate queens. Dead males were removed from the rearing chamber (match boxes). Many of the test queens (N=83) kept in captivity shed off their wings and initiated egg laying but none of them (N=90) produced viable eggs.

2.3.3 Developmental times of eggs and wing shedding

All the collected queens (experiment conducted from January to April 2015) laid eggs and shed their wings. However, only eggs from 71 queens were hatched out of 103, making eggs hatching successful by 68.93%. Wing shedding was significantly dependent on time from nuptial flights (Table 2.1). More queens shed their wings within 3 days after nuptial flights. Similarly, egg laying was significantly dependent on time from nuptial flights (Table 2.1). More queens (68.93%) laid eggs within 3 days after flights. A high percentage of eggs that hatched (88.73%) were laid by queens that shed their wings and laid eggs within 3 days after nuptial flights ($\chi^2=21$, $p<0.0001$, $n=71$). Only 11.27% of eggs that hatched were laid by queens between 4th and 6 days after nuptial flights. Egg hatching was also dependent on days from nuptial flights (Table 2.2). A significantly high number of queens had their eggs hatched from day 11 to 13 after nuptial flights. No hatching was recorded in eggs laid 6 days after nuptial flights.

Table 2. 1: Time taken for initiation of wing shedding and egg laying in founding *O. longinoda* queens

Duration (days)	1-3 days	4-6 days	>6 days	Statistic	Number of queen
% Shedding wings	78.64 (81)	8.74 (9)	12.62 (13)	$X^2=52.12$, df, 2, $p<0.0001$	103
% Laying eggs	68.93 (71)	13.59 (14)	17.48 (18)	$X^2=67.79$, df, 2, $p<0.0001$	103

Table 2. 2: Time taken to hatching of eggs into larvae

Eggs hatch day	Number of queens hatched their eggs	% queens hatched their eggs	Statistic
Day 10	2	2.9	$X^2=60.07$, df, 5, $p<0.0001$
Day 11	11	15.94	
Day 12	16	23.18	
Day 13	32	46.38	
Day 14	4	5.8	
Above 14	4	5.8	
Total	69	100	

2.4 Discussion

In this study only the queens that dispersed and subsequently settled naturally were able to produce viable offspring. These queens had an opportunity to fly to heights beyond 10 meters (the size of a shade house). This observation suggests that mating took place after their dispersal from the nest and that a certain flying height was needed before mating occurred. This correlates well with the Australian species, *O. smaragdina* that mate at some greater heights in the air and not inside their nests (M.G. Nielsen et al., unpublished data). Mating at great heights is not a unique character to *Oecophylla* species (Peng *et al.*, 2013). Other ant species are known to fly high before mating. For instance, *Solenopsis invicta* has been reported to reach at height of 60-100 m during their mating flights (Dhamb and Booth, 2008).

The current study has shed light on the myth that surrounded the collected alate *Oecophylla* queens that produced viable offspring (Way, 1954; Lokkers, 1990) without clear understanding of the dispersal stage at which the queens were collected. It also brings to an end the speculation (Vanderplank, 1960) that mating by *O. longinoda* on Zanzibar took place inside their nests before dispersal. Vanderplank's alate queens were reportedly collected from the nest surface and that the queens subsequently produced viable offsprings with imago workers. Queens were collected after nuptial flights, and were most probably founding queens. Centrally to that, the difference between Vanderplank's and the present study might be hard to explain unless behavioral variation among populations exists. If the behavior observed by Vanderplank (1960) holds true, it would be a rare strategy only used by few species and not even by the sister species, *O. smaragdina* (Peeters and Molet, 2010).

In the present study, many of the infertile queens shed their wings and laid eggs. Thus, wing shedding and egg-laying cannot be used as indicators of fertility or egg viability

among *O. longinoda* queens. It is surprising that queens shed their wings while still unmated as they would need them in subsequent attempts to locate potential mating partners. Further studies would be needed to unveil triggers for wing shedding in queens which occurred regardless of mating or fertilization of the queen's eggs.

Queens that were allowed to settle naturally had lower survival rates exhaustion due to higher energy expenditure. Also these queens had been on a mating flight and subsequently fed their developing larvae with food derived from their body reserves. They used more energy than queens that did not participate in mating flights. This higher energy consumption led to a slightly lower survival (King'ori, 2012).

All the founding queens laid eggs and shed their wings but only eggs from 71 queens (68.93%) were hatched. Thus, mating for viable offsprings may sometimes be unsuccessful. We hypothesized that hatching of the laid eggs is affected by the environmental conditions that prevailed in rearing units particularly the temperature. Further research might be needed to confirm this hypothesis. Time from nuptial flights to egg laying and shedding wing was associated with eggs hatching. Early laid eggs hatched successfully unlike late laid ones. No eggs hatched from any queen that started laying eggs beyond 6th day after the flight. High percentage (88.73%) of queens with hatched eggs laid eggs within three days following nuptial flights. This suggests that, eggs hatching can be expected by more than 88 % within three days rearing.

Variation in time taken from first day of nuptial flight to egg hatching was also noted. More egg hatches were observed in the 11th, 12 and 13th days from first day of nuptial flight, with the highest on the 13th day. These results contrast with Vanderplank (1960) who reported that, time from nuptial flights to egg hatching is the same for all new

queens reared at the same temperature. He reported that eggs from 24 queens reared at 28 °C took 6 and half days to hatch whereas at 33 °C took only 4 days.

We therefore conclude that mating is most likely to take place after queens disperse. This is further supported by the fact that attempts to mate queens in artificial nests was unsuccessful, and that several hundred attempts to mate Australian and Thai *O. smaragdina* sexuals in artificial enclosures have been, equally, unsuccessful (Morgens G. Nielsen, Personal communication).

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CHAPTER THREE

3.0 Nuptial flights behaviour of the African weaver ant, *Oecophylla longinoda* Latreille (Hymenoptera: Formicidae) and weather factors triggering flights

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Abstract

Weaver ants (*Oecophylla* spp.) are intensively studied in basic and applied contexts. Yet, little is known about their mating behavior. Knowledge on their reproductive strategy is a prerequisite to the basic understanding of their life history and may provide valuable information facilitating their use in integrated pest management (IPM) and protein production (entomophagy). Here, we report on the behavior displayed by *O. longinoda* in relation with their nuptial flights in Tanzania and test for environmental cues that may trigger the flights. We found that sexuals aggregate on nest surfaces prior to flights based on observations of 56 flights recorded over two years. We also found that flights took place during the raining season, and all flights took place in evenings just before sunset. Further to these, days with flights were associated with higher relative humidity and less sun shine compared to days without flights. Also, flights mainly took place around full moons. However, this correlation was based on a total of only five full moon phases and

should, therefore, be interpreted with caution. The results also showed that flights were only significantly correlated with weather parameters during the early part of the mating season, the trend changed thereafter probably due to depletion of sexuals in the nests as the season progressed. This information improves our understanding of ant nuptial flights and offers a tool to improve forecasts of *O. longinoda* flights, enabling easier collection of mated queens to stock ant nurseries that supply ant colonies for IPM-programs.

Key words; African weaver ant, sexuals, nuptial flight, weather, entomophagy, Tanzania

3.1 Introduction

Mating is the key process of reproduction. Most ant species mate during or after nuptial flights, where males and queens (sexuals) liberate from their natal nests to find mating partners from other colonies (Kaspari *et al.* 2001; Depa 2006; Levin *et al.* 2008; Peeters and Molet 2010; Gomez and Abril 2012). The copulation between unrelated sexuals (Depa 2006) provides an opportunity for genetic mixing and is an important part of the life history of most ant species (Dhami and Booth 2008). Therefore, observations on the behavior of ants in relation to their nuptial flights are of basic interest and could bring a broader understanding of ant life histories and ecology (Dunn *et al.* 2007).

It is crucial that conspecific ant colonies are able to time their flight with neighbouring colonies to ensure genetic mixing. This requires either communication between colonies or some common flight triggers. Weather parameters have been suggested to trigger the flights of several ant species (Depa 2006; Gomez and Abril 2012). For example, the mating flight of *Pheidole sitarches* was reported to take place on days with moderate rain and an overcast sky (Wilson 1957). Kaspari *et al.* (2001) indicated that temperature and rainfall were abiotic factors of importance when several ant species were timing their flights in the Neotropics. The swarming behavior of the species, *Atta vollenweideri*

FOREL is highly associated with rainfall and a temperature increase (Staab and Kleineidam 2014). Further, Dhimi and Booth (2008) indicated that the nuptial flight of *Solenopsis invicta* generally occurred after one or two days of rain and at temperatures between 21-33 °C and RH of above 80%.

In the case of the two extant *Oecophylla* species (*O. smaragdina* and *O. longinoda*), mating behavior is not only of basic interest, but also important in an applied context. These ants are used in biological control programs in the tropics where they protect crops against pests, resulting in increased crop yields and/or higher fruit quality (Way and Khoo 1992; Barzman *et al.* 1996; Peng and Christian 2007). Furthermore, they may be utilized as protein food (Sribandit *et al.* 2008; Offenbergs 2011; FAO report 2013) or feed (Cesard 2004) and in this way contribute to food security (Offenbergs and Wiwatwitaya 2009). In both types of utilization, it is important for farmers to obtain right queen colonies, but it can be difficult to find the maternal queen in wild colonies (Peng *et al.* 1998). Therefore, ant nurseries, where live ant colonies can be reared, are under development (Rwegasira *et al.* 2014). Ant nurseries need to collect newly mated queens' right after their nuptial flight to stock the production of larger colonies (Rwegasira *et al.* 2015). A reliable prediction of *Oecophylla* mating flights via identification of weather based triggers may reduce the costs of collecting such queens.

The mating season of *Oecophylla spp* was reported to coincide with rainy seasons (Way 1954; Vanderplank 1960; Offenbergs and Wiwatwitaya 2010; Peng *et al.* 2013; Rwegasira *et al.* 2015), and studies on *O. smaragdina* showed that their mating flight takes place early in the morning (Renkang Peng, unpublished data). However, the timing of flights in *O. longinoda* had never been reported. Moreover, there exists little knowledge about the mating behavior of *O. longinoda*. Weather parameters have long been suggested to

trigger *O. longinoda* mating flights but specifics on which parameters and to what extent have never been elucidated. Whether the flights occur at a limited part of the day as seen in *O. smaragdina* or extended across range of time over the day had never been established.

In the present study, we observed the mating behaviour and the timing of the nuptial flight of *O. longinoda*. In particular, we tested for correlations between weather parameters and nuptial flights and did extensive observations to identify the time of the day when flights occur. The presented results have improved our understanding of weaver ant life history and ecology and they provide a tool to predict *O. longinoda* flights, making the collection of queens for ant nurseries easier, cheaper and more reliable.

3.2 Materials and Methods

3.2.1 Study area

Studies were conducted at Naliendele Agricultural Research Institute (NARI) ($10^{\circ} 21' 22.49''$ S, $40^{\circ} 09' 57.05''$ E and 140 Meters above sea level) about 10 km from Mtwara town in the southern part of Tanzania. The Mtwara region has a unimodal wet season, with regular rains from November/December to April/May. The annual rainfall ranges from 810 to 1090 mm. Mating behavior and flight events were recorded by visual inspection and by two web cams. The study period covered 2012/13 and 2013/14 mating seasons, and included 25 colonies (18 colonies under visual observations in fields and 7 colonies on close observation under webcam).

3.2.2 Timing of nuptial flights

Two cameras (IP Edimax Camera; Edimax Technology Co., Ltd) were placed close to nests to monitor flight events from December, 2013, to the end of February, 2014 (during the second season of observation). The cameras focused on nests of seven ant colonies kept on potted mango and cashew seedlings, set on tables outside a residential house, for easy surveillance and security. The colonies were introduced three months prior to monitoring, to allow for nests building and establishment. One camera focused on a single nest at a time, and the second camera monitored at least six nests at a time. The focuses of the cameras were turned to other nests after the release sexuals. The cameras recorded timing of preparation for and occurrence of flights.

3.2.3 Pre- and post-flight behavior on nests

Colonies containing mature sexuals i.e. imago winged males and winged queens with green gasters, were identified and selected for monitoring. Two colonies were monitored in situ at sunrise (between 06:30a.m. and 08:30) and sunset (from 03:00 to 06:30 p.m.) during the first mating season. Preliminary records showed increased levels of nest surface activity during these times. Another 25 colonies, located at three sites approximately 1 km apart, were monitored in the evenings only during the second season.

During monitoring, we estimated the number of males and queens aggregating on the outside surfaces of the nests and; the numbers dispersing from the nests on days when flights occurred. We also recorded (on flight days), the duration of each aggregation, as the time between occurrence of first two sexuals and departure of all sexuals from the nest surface and; the duration of each take off event as the time between taking off of the first and the last sexual.

Finally, we compared the number of sexuals aggregating on nest surfaces; number of sexuals aggregating in the mornings between days with and without flight in the following evenings and number of sexual aggregating in the evenings on days with and without flight; the duration of aggregations and take off between small and large flights (small flights occurred when less than 50 sexuals dispersed per colony and big flights occurred when at least one colony released more than 50 sexuals). We also determined whether ants abandoned or continued to occupy the nests after the release of sexuals. The presence of workers and the physical condition of the nests were recorded three days after a flight had taken place. As data were not normally distributed and without variance homogeneity, statistical comparisons were based on non-parametric Mann-Whitney tests performed with GenStat software.

3.2.4 Association of weather parameters and mating flights

Occurrences of mating flights were closely monitored on six nests in each of the 18 ant colonies containing mature sexuals, from November to March, 2013/14. Monitoring was done in the evenings, when mating flights occur. Weather data were collected from the Tanzania Meteorological Agency (TMA) at Mtwara airport located approximately 3 km from the experimental sites. Daily total rainfall, daily average temperature, average minutes with sunshine per hour of the day, average wind speed, daily average relative humidity, cloud cover and average air pressure were collected. Additionally, moon phase was recorded as the percentage of the moon visible on a particular day (disregarding cloud cover).

We recorded flight days (days with at least one flight) in both seasons. One flight was recorded when at least one nest in the colony released sexuals. Thus two flights were

recorded if nests from two different colonies released sexuals. Monitoring periods were divided into days with small flights and days with at least one big flight.

Logistic regression analysis was used to determine association between weather parameters/moon visibility and occurrence of nuptial flights. Also logistic regression was performed to determine the relationship between male aggregations in evenings and occurrence of nuptial flight. Data were analysed using JMP 11.1.1 software (SAS 1995).

3.3 Results

3.3.1 Timing of nuptial flights

We recorded 56 mating flights in 25 ant colonies during the study period, of which 15 and 41 occurred in the first and second season, respectively. We also recorded 28 flight days, 11 in the first season and 17 in the second season. In some cases, flights occurred from several colonies on the same day. In addition, we recorded 25 flights by using cameras that focussed on seven colonies kept outside a residential house during the second season. The remaining flights were recorded visually in the field. Of all 56 flights, 54 (96.4%) started between 18:25 and 19:00 hrs, whereas only two flights (3.6%), started between 17:00 and 18:00 hrs. At no other time of the day were any flights observed.

Throughout the mating season, males aggregated on the outside of the nest surface in the mornings and evenings only, even on days without flights. Queens were almost exclusively seen on nest surfaces on days with flights (see below). Queens aggregated 3-7 minutes before the start of the take-off. No aggregation was recorded by cameras during other periods of the day.

3.3.2 Pre- and post-flight behavior on nests

On days with flights, there were unusually high activities of workers as well as males and queens on nest surfaces and on the leaves near the nests, one to two minutes before the first sexuals took off. More than 90 % of the flights happened from nests composed of old leaves. Due to the hectic movements in and out of the nests, the entry holes expanded and increased, and the silk between leaves were broken. Workers were observed to escort the sexuals for the take off.

About 3.7 times more males aggregated in the evenings, on days with flights compared to days without flights. There was no significant difference on number of males that aggregated in the mornings between days with and without flights on the following evenings (Table 3.1). Queens did not aggregate on nest surfaces in the mornings and showed almost no aggregation on evenings without flights. However, queens aggregated in the evenings with flights (Table 3.1)

Male aggregations lasted longer on days with big flights than days with small flights, but the flight event itself was shorter (Table 3.2). More than 80 percent of the nests from which flights occurred were abandoned within three days after the flight. The abandoned nests were in a bad condition, with large holes in the silk constructions.

Table 3. 1. Numbers of aggregating males and queens on days with and without flights

Males aggregation in the evening		Test statistic
Days with flights	Days without flights	Mann-Whitney test, U = 14, p<0.001
135.8 ± 34.5 SE, n=5	36.9 ± 4.7 SE, n=35	
Males aggregation in the morning		Mann-Whitney test, U = 47.0, p= 0.099
Days with flight in the evening	Days without flight in the evenings	
19.0 ± 4.5 SE, n=5	11.3 ± 1.2 SE, n=35	
Queen aggregation in the evenings		
Evenings with flight	Evenings without flight	Mann-Whitney test, U=4.5, p<0.001
47.0 ± 38.3SE, n=5	0.91 ± 0.11 SE, n=35	

Bold values indicate the total number of colonies

Table 3. 2. Duration of male aggregation and take off duration in relation to the size of flights

	Mean (SE), n	Days	Test statistic
	Days with big flights	Days with small flights	
Duration of males aggregation (min)	88.1 (5.09), 22	28.2 (1.50), 14	Mann-Whitney test, U = 4.5, P < 0.001
Take off duration (minutes)	12.1 (2.0), 23	19.2 (1.4), 18	Mann-Whitney test, U=4.5, P < 0.01

Bold values indicate the total number of colonies

3.3.3 Relationship between weather parameters/moon visibility and mating flights

Our results showed that days with flights had higher relative humidity, higher percentage of the moon visible and fewer hours of sunshine (Fig. 3.1a-c).

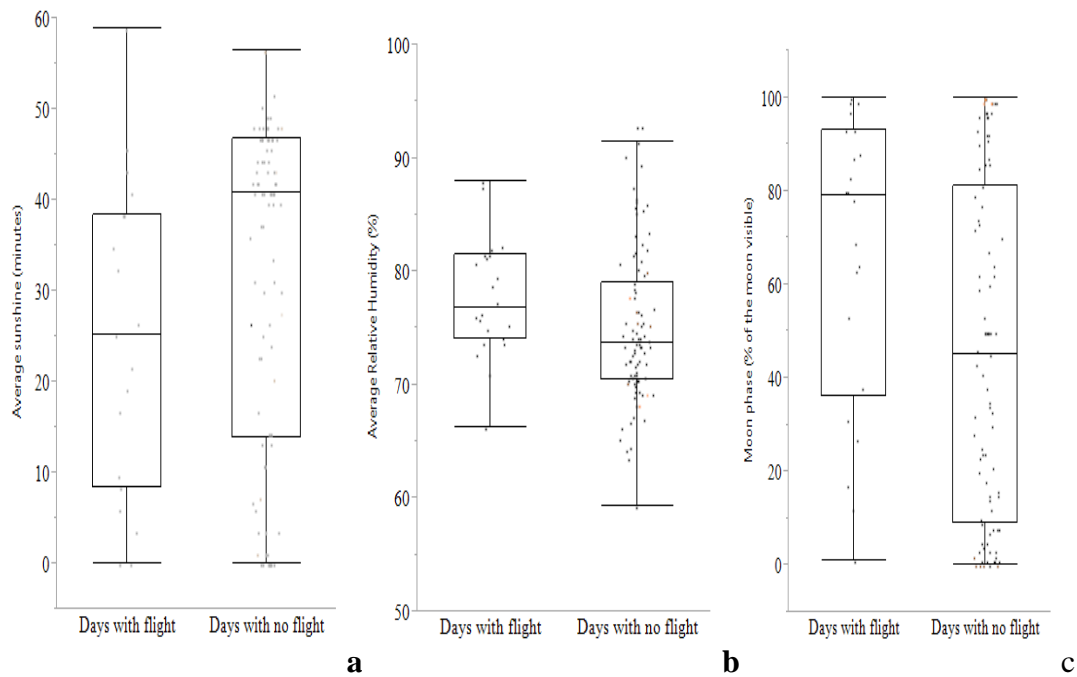


Figure 3. 1. Box plots showing relationships of; a; sunshine (average minutes per hour of the day), b; relative humidity and c; moon phase, between days with and without mating flights. N and p-values are given in Table 3

A unit increase in relative humidity increased the probability of flight occurrence by 12 % ($\exp(\beta) = 1.12$; $p = 0.03$) while a unit increase in percentage moon visible significantly increased the likelihood of flight occurrence by 2 % ($\exp(\beta) = 1.02$; $p = 0.02$) (Table 3.3). However, an increase in sunshine hours during the day, significantly decreased the likelihood of predicted flights ($\exp(\beta) = 0.97$; $p = 0.05$). Results in Table 3.3 showed that, rainfall, air pressure, temperature, wind speed and cloud cover had no effect on occurrence nuptial flight for *O. longinoda*.

Table 3. 3. Logistic regression results showing relationship between weather parameters /moon phase for *O. longinoda* nuptial flights at Naliendele, Tanzania

Parameters	Estimates	Std Err	(χ^2)	Prob.	Exp (β)
%Moon visibility	0.017	0.007	5.54	0.02	1.02
Average sunshine hours	-0.028	0.014	3.75	0.05	0.97
%Relative Humidity	0.109	0.050	4.68	0.03	1.12
Rainfall (mm)	0.012	0.009	1.76	0.18	1.01
Cloud cover	-0.003	0.170	0.00	0.98	0.99
Wind speed m/sec	-0.141	0.161	0.77	0.38	0.86
Air Pressure	-0.224	0.134	2.80	0.09	0.79
Temperature ($^{\circ}$ C)	-0.207	0.210	0.98	0.32	0.81

* The analysis in Table 3.3 compared days during mating season which included 22 and 91 days with and without flights, respectively

Furthermore, increase in relative humidity on two or one day before the flight significantly increased the likelihood of flight to take place (Table 3.4). Additionally, flight was significantly associated with increase in relative humidity, increase in cloud cover and decrease in sunshine hours in a day after the nuptial flight (Table 3.4).

Table 3. 4. Influence of weather parameters on *O. longinoda* nuptial flights

Days before and after a flight day	Parameters	Estimates	Std Err	(χ^2)	Prob.	Exp (β)
Two days before a flight	%Relative humidity	0.103	0.037	7.50	0.006	1.108
	%Relative humidity	0.112	0.050	4.87	0.027	1.118
One day before a flight						
	%Relative humidity	0.076	0.037	4.26	0.038	1.078
One day after a flight	Sunshine hours	-0.035	0.015	5.42	0.019	0.965
	Cloud cover	0.314	0.124	6.38	0.011	1.368

*Weather parameters showing significant differences between days up to (one and two days before a flight and one day after a flight) compared to all other days during mating season excluding flight days. The analysis included only weather parameters which showed significance differences. Probabilities (Prob.) are based on Logistic regression analysis under Generalized Linear Model. In this analysis, there were 22 and 91 days with and without flights, respectively

Finally an increase in male aggregation in the evening was significantly associated with occurrence of nuptial flight $\exp((\beta)) = 1.04$, $p = 0.02$).

3.4 Discussion

Our results showed that flights take place just before sunset. Assessment of occurrence of flights should, therefore, be done in the evenings. Also, searches for mated queens should ideally take place during nights or mornings after flights before too many queens fall prey to natural enemies. Results also signify that since flights occur at nearly sunset, light trapping may be used as a means to collect queens as previously suggested (Rwegasira *et al.* 2015). This information will facilitate collection and trapping of newly mated queens for rearing in ant nurseries. Time of flight for *O. longinoda* is in contrast to a closely related *O. smaragdina*, which are known to fly at sunrise (Peng *et al.* 2013).

As with many other ant species, *O. longinoda* started their mating flights first; by aggregations of males around nest openings, followed by aggregations of queens soon after. Following aggregations, sexuals took off from the nests but the actual mating was not observed in this study. Males aggregated both in mornings and in evenings, but they only flew in the evenings, where aggregations were also more pronounced. It is puzzling that males aggregated but never flew in the morning. We hypothesise that that evening flights are a trait derived from original morning flights and that morning aggregations are a relic from this earlier behavior. The closely related *O. smaragdina* fly at sunrise (Peng *et al.* 2013).

We found that on days with big flights, durations of aggregations were longer, but the durations for taking off of sexuals from the nests were shorter. As more traffic of sexuals is needed on days with big flights, aggregations may last longer. The take off period, on the other hand, may be shorter as the stimuli may be strong on such days. This effect may further be reinforced if already dispersing sexuals use pheromone calls to stimulate more individuals to join the flight, as this would further shorten hesitation time.

The aggregation behavior displayed by sexuals on the nests may also inform ant collectors about the probability of a flight. If more than usual number of males or queens aggregate on nest surfaces in the evening, then a flight is likely to take place, and therefore queens should be collected during the night or the next morning.

The present study showed that periods within the mating season with less sunshine and high relative humidity increased the probability of mating flight to take place. As also observed among other ant species, weather parameters were associated with flight events (Staab and Kleineidam 2014). Days before, with, and after flights were all associated

with high humidity, which may be adaptive for founding queens as they and their initial brood are in the early phase of colony founding, fully exposed to the environment and may suffer desiccation. Not until the first larvae reach the last instar can they produce the silk needed to construct a nest that can protect the founding colony against desiccation (Hölldobler 1983). Thus, incipient colonies are especially prone to desiccation in the period immediately following the mating flight. This is also supported by lower sunshine hours in flight day, higher cloud cover and lower sunshine hours found on days following a flight. It was observed that if wind increased during male aggregations, the workers started to escort males back into the nests. The low wind speed on days with flights may also be adaptive, as the sexuals may not be strong fliers and may need calm conditions if males use pheromones to call the queens as seen in other species (Peeters and Molet 2010).

In the present study, flights were not correlated with air pressure or rain. This is in contrast to *O. smaragdina*, where flights were highly associated with days with high air pressure and no rain (Nielsen et al. unpublished data). However, the two species fly at different times of the day, with different weather parameters.

Interestingly, flights were also associated with full moon periods. In contrast to *O. smaragdina*, *O. longinoda* fly in the evenings. Thus, the newly mated queens must search for suitable nesting sites during the night, probably with the aid of moonlight, explaining why flights are related to full moon phases. In another study, *Oecophylla* ants showed profound visual orientation (Dejean 1990). The association between flight and full moon must, however, be taken with caution as the observed flights only covered a total of five full moon phases in the two seasons. As such, more observations covering more moon phases would be required for a more robust statistical analysis.

Combining the information given above may provide ant queen collectors with a tool to fine-tune their forecasts of mating flights for a more or less precise timing hence more efficient and effective collections. This will save time and costs, resulting in a cheaper production of *O. longinoda* colonies. This again may facilitate the implementation of the use of weaver ants in biocontrol programs and protein production (Offenberg 2011; Offenberg 2015).

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CHAPTER FOUR

4.0 Foraging behavior and Preferences for Alternative Supplementary Feeds by the African Weaver Ant, *Oecophylla longinoda* Latreille (Hymenoptera: Formicidae)

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Abstract

Weaver ants, *Oecophylla spp*, are effective predators that control a wide range of insect pests in multiple crops when maintained at high population. Supplementary feeding, particularly during reduced food availability is one of management practices that maintain and boost weaver ants' populations. Experiments were conducted between September and October 2013, January and February, 2014 to determine the type of food preferred by weaver ants, *O. longinoda*. Twenty colonies of *O. longinoda* were provided with four types of food to determine their feeding preferences. These included anchovy, chicken intestine, fish intestines and earthworms. We examined food preferred by ants based on; weight of the food removed and activity of the ants on foods. Furthermore, we examined foraging behavior of ant workers on anchovy food (fresh-ground and dry-ground) in nine *O. longinoda* colonies. Thereafter, small and large particles of dried-

ground anchovy were tested. The results showed that, *O. longinoda* preferred anchovy to other foods provided. However, the results of Analytical Hierarchy Process showed that earthworm and fish intestine were the most accessible food types by farmers, as determined by availability, affordability and applicability. We observed more ants on fresh-ground as opposed to dry anchovy; similarly, large particles were more easily removed by ants than was the case with small particles. Thus, during reduced food availability, farmers in the study area should use earthworms and fish intestines feeds to supplement *O. longinoda* colonies. Fresh moist anchovy or dry anchovy of large particle sizes can be used where available.

Key words: African Weaver Ant, citrus, cashew, food preference, anchovy, Tanzania

4.1 Introduction

Two species of weaver ants, *Oecophylla longinoda* Latreille and *Oecophylla smaragdina* Fabricius are generalist predators that protect crops against insect pests (Way and Khoo 1991; Peng and Christian 2007; Van Mele 2008; Materu *et al.* 2014). The use of *Oecophylla* as a biocontrol agent can increased fruit yield and quality (Barzman *et al.* 1996; Peng and Christian 2005; Olotu *et al.* 2013a). The ants prey directly on insect pests and obtain energy from honeydew produced by homopterans or from plant nectaries (Way and Khoo 1992). Crop protection is therefore more successful when there is a high and stable population of weaver ants. For instance, Stathers (1995) revealed that, cashew trees colonized by high number of *O. longinoda* (>500 foraging *O. longinoda* workers) recorded lower damages by coreid bugs; *Helopeltis anacardii* Miller and *Pseudotheraptus wayi* Brown than those recorded with few *O. longinoda* (1-20). Adequate palm protection is realized when two or more nests of *O. longinoda* are found in the palm crown (Way 1953) or when 60-70% of the palms are colonized by *O.*

longinoda (Way and Khoo 1992). As Sporleder and Rapp (1998) reported, the population of *P. wayi* sinks to zero after a long and stable occupation of palm trees by *O. longinoda*.

In addition to biocontrol, *Oecophylla* is used as a valuable source of food for humans (Sribandit *et al.* 2008), contributing directly to food security (Offenberg and Wiwatwitaya 2010) and also serve as a feed for song birds in Indonesia (Césard 2004). Populations of weaver ants in crop fields are, however, not stable, as they can commonly drop to very low levels in the field, resulting in inadequate crop protection. This can be caused by many factors one of which is movement of colonies to non-agricultural fields. Different management practices are being developed in order to maintain, boost, and expand the existing colonies to optimum levels. These management practices include artificial nests, (Offenburg 2014), the use of pesticides that are less harmful to the ants, protection of ants from competitors like black ant *Dolichoderus thoracicus* (Smith), facilitation of colony expansion by using strings and poles to connect trees (Van Mele and Cuc 2000; 2007) and maintenance of ground vegetation to control *Pheidole* spp (Way and Khoo 1992; Seguni *et al.* 2011). Furthermore, technologies of rearing weaver ants in nurseries are being developed (Peeters and Andersen 1989; Ouagoussounon *et al.* 2013)

Social insect populations are negatively affected when food is scarce (Dusstour and Sympson 2012). Weaver ant colonies may even move among trees in search of forage (Van Mele and Cuc 2007). The goal of biocontrol is to have large and stable colonies; thus food supplementation may help to boost population size.

The feeding preferences of *Oecophylla longinoda* are not well known. In Vietnam, farmers provide fish and chicken intestines to *O. smaragdina* as supplementary foods during scarcity (Van Mele and Cuc 2000). Food supplementation (Van Mele and Cuc 2000; Lim 2007) or feeding (Offenburg and Wiwatwitaya 2010) of weaver ants is reported to increase weaver ant populations. Furthermore, an increase of populations of *O. longinoda* was reported on fed colonies (Abdula *et al.* 2015). However, weaver ants consume food in order of preference. *O. smaragdina* prefer mealworm to fish, honey or weaver ant formula (Lim 2007).

The practice of food supplementation for *O. longinoda* is limited by inadequate knowledge on food preference (based on cheap local available feeds), forms or states as well as particle sizes. Therefore, the objective of this study was to determine the type of food preferred by weaver ants in terms of form and size. A good understanding of food preferences by weaver ants and their foraging behaviour is essential for effective management of *O. longinoda* during food scarcity.

4.2 Materials and Methods

We conducted experiments at Naliendele Agricultural Research Institute (NARI), Mtwara Region, in Southern Tanzania ($40^{\circ} 09' 57.05''$ E, $10^{\circ} 21' 22.49''$ S, 140 m asl). The region has a unimodal rainfall pattern, starting from November/December to April/May, with a single peak in January. The annual rainfall ranges from 810 to 1090 mm, whereas mean temperature ranges from 23 °C in July to 27 °C in December. Relative humidity ranges from 79% in October to 87% in March.

The study was approved by the Directorate of Research and Postgraduate Studies (DRPG) of SUA, a body responsible for monitoring and evaluating compliance to ethical

conduct of staff and students undertaking research. The research complied with Code of Conduct for Research Ethics of Sokoine University of Agriculture (SUA) available at www.drpgs.suanet.ac.tz. Food preferences were tested for 10 days in orchards colonized by weaver ants. The tests were conducted for two seasons, between September and October 2013 (dry season), and between January and February 2014 (rainy season). Two orchards one of cashew *Anacardium occidentale* L. and another of orange *Citrus sinensis* L were selected. In each orchard, colonies occupied by at least 40% per tree (Peng *et al.* 2008), extending to at least two trees were selected. Each tree had between 5 and 25 nests. Each colony was provided with four types of foods; (i) earthworm (ii) chicken intestine (iii) fish intestine and (iv) anchovy. The intestines and anchovy were fresh and prepared by chopping and grinding. The Intestines and anchovy were ground with the use of local grinding equipment made up of woods. Earthworms were dug out of wet soils, near water ponds, or irrigated fields. About 6 g of each food type were placed in a 0.01x0.1 m bowl, set on a feeding platform. Ants could access the bowls by crawling through a guiding stick. Feed bowls were placed equidistant from the middle of the feeding platform. Feeds and water were provided *ad libitum* throughout the experimental period. All the food types were tested in 10 colonies in each orchard. Preferences were determined by i) counting all foraging workers observed on food station and inserting their mouths into a food type and ii) weighing the amount of each food removed by the ants. Counting started 60 minutes after more than one forager had discovered each food type. Thereafter, the weight of the remaining food in each bowl was determined. The amount of food type removed by the ants was determined by establishing the difference in weight between the food supplied and the food which remained in the bowl. In each case, the weight loss due to evaporation was deducted. Weight loss due to evaporation was determined in the control food types that were inaccessible by ants.

A sample of each food type was analyzed for nutrient compositions at the University of Dar es Salaam. The total carbohydrate, crude protein, total lipids (Fat) and vitamin A were determined according to the procedures described by Allen (1989). The total flavonoids were determined based on the procedures described by Bonvehi *et al.* (2001); the moisture content was determined gravimetrically after oven has dried at 105°C for 24 hours.

Furthermore, we used anchovy food to test for food forms and particle sizes that can be preferred by *Oecophylla longinoda*. Anchovy was used because it is processed in a standard form. We hypothesized that anchovy type (dry and fresh) affected the foraging behaviour of workers. We also hypothesized that the particle size of dry anchovy affects foraging behaviour of workers. The experiments were conducted between May and July 2014. The first experiment involved two different forms of anchovy, dried and fresh. This experiment was conducted for 10 days, with the observation starting around 0900 am each day. The anchovy was sun dried (27-29 °C) for 7 days before grinding. We used fresh-ground anchovy of approximately similar in size as the dried one. A Y- shaped feeding arena made up of wood was used as a feeding platform. A feeding bowl was placed on top of a board tied at each end of the Y shaped arena. This gave an equal chance for the workers to access each of the anchovy food type. Nine colonies were used. Each colony occupied at least two citrus trees. One bowl of each anchovy food was supplied per colony. The foraging behavior was assessed by counting workers carrying food particles from the source. The counting was done ten times at an interval of one minute (ten observations) every day per each colony for 10 days consecutively. In the end, we calculated the average number of foraging workers per minute per colony for a given food form.

The second experiment involved dried-ground anchovy of different particle sizes. The particles were measured by using laboratory test sieves (Wagtech International Ltd UK). Two particles sizes were selected; particles ranging from 0.5 to 1 mm in diameter (referred hereinafter as small particles) and; particles of 2 mm d (referred hereinafter as large particles). The experiment was conducted on six weaver ant colonies for 10 days using similar procedures for testing food types (above).

Thereafter, Analytic Hierarchy Process (AHP) (Saaty 1980) was used to determine the food type that would be accessed by the farmers. The set of evaluation criteria consisted of affordability, availability and applicability. The set of alternative options among which the decision was made consisted of four food types. The weights for each evaluation criterion were generated. The score for each criterion was assigned according to the pair wise comparisons of the options (on a scale of 1 – 9). Finally, the criteria weights and the options scores were used to compute the global score for a given option, as a weighted sum of the scores obtained with respect to all the criteria.

4.3 Data analysis

The analyses were performed by using JMP 10.00 software (SAS 1995). A non parametric one way ANOVA was used followed by Multiple Comparison-Wilcoxon Each Pair for counting forage workers and the amount of food removed under food preferences. Mann-Whitney tests were performed to compare the number of forage workers on dried and fresh anchovy; similar comparisons were done for small and large particle sizes.

4.4 Results

Nutrients composition of the feeds fed to the ants are presented in Table 4.1. Crude protein ranged from 47.7 % (from fish intestine) to 31.2 (chicken intestine). All tested feeds, except earthworms contained flavonoids. The highest amount of flavonoids was 0.013 mg/g.

Table 4. 1. Nutrients composition of the feeds fed to ants

Parameters	Food type			
	Anchovy	Fish intestine	Chicken intestine	Earthworm
Crude Protein (%)	44.6	47.68	31.2	45.6
Total carbohydrate (g/100g)	0.2	3.1	6.21	0.01
Fat content(g/g)	0.059	0.078	0.087	0.005
Moisture content(%)	89.4	52.7	78.4	92.9
Vitamin A(mg/100g)	4.5	3.4	5.6	0
Flavonoids(mg/g)	0.013	0.0002	0.001	0.00
Energy(kj/g)	9.711	11.414	9.553	7.834

The number of workers foraging on food types were significantly different ($p=0.05$) in both citrus and cashew orchards, during both dry and rainy seasons (Tables 4.2 and 4.3). More workers foraged significantly, ($p=0.05$) on anchovy than they did on other food types. However, in citrus during the dry season, the number of workers foraging on anchovy and chicken intestine were not significantly different ($p=0.05$).

Table 4. 2. The p-values for weaver ant counting and amount of food removed in citrus orchard

Food types	Citrus season	dry	Amount of food removed (P-values) (g)	Citrus season	rainy	ant (P-values)	Amount of food removed (g) (P-values)
Pairwise comparison		Weaver ant counting (P-values)	Amount of food removed (P-values) (g)	Weaver ant counting (P-values)	ant (P-values)	Amount of food removed (g) (P-values)	
Anchovy intestine versus Chicken intestine	0.054		0.042	0.0002		0.0011	
Anchovy versus Earthworm	0.021		0.108	0.0002		0.0011	
Anchovy versus Fish intestine	0.014		0.0018	0.0003		0.0011	
Chicken intestine versus Fish intestine	0.121		0.0095	0.0002		0.0011	
Chicken intestine versus Earthworm	0.68		0.77	0.004		0.0011	
Earthworm versus Fish intestine	0.33		0.014	0.0155		0.0011	

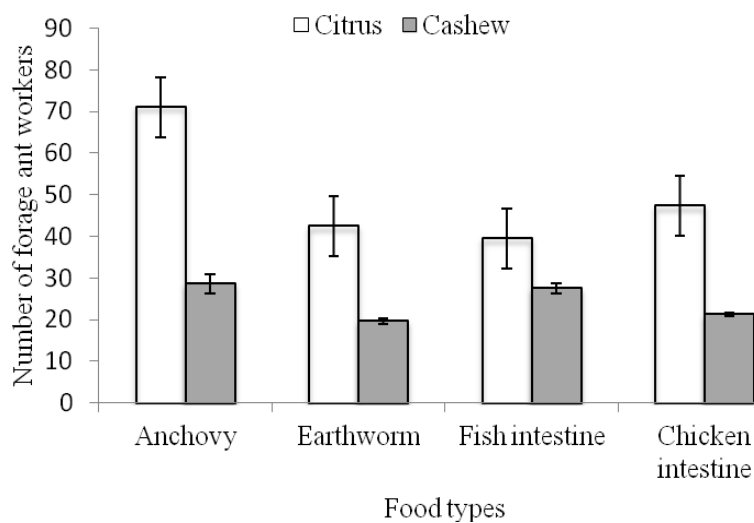
The P-values were based on weaver ant counts and the amount of food removed (g) /hour in 20 days between September and October 2013 and January and February 2014 in citrus orchard, Naliende, Tanzania. (Kruskal-Wallis Multiple Comparison-Wilcoxon Each Pair test)

Table 4. 3. The p-values for weaver ant counting and amount of food removed in cashew orchard

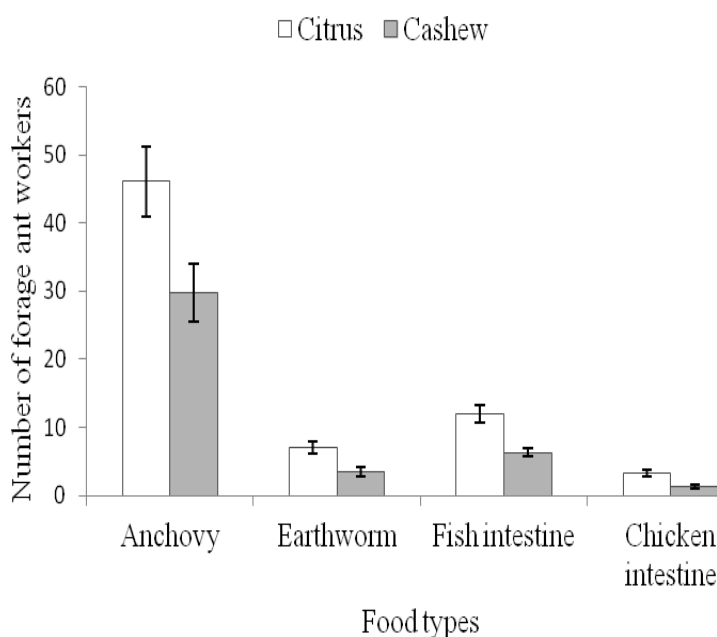
Food types	Cashew dry season	Weaver ant counting (P-values)	Amount of food removed (P-values) (g)	Cashew rainy season	Weaver ant counting (P-values)	Amount of food removed (P-values) (g)
Pairwise comparison						
Anchovy versus Chicken intestine	0.0013		0.51	0.0002		0.0002
Anchovy versus Earthworm	0.0006		0.817	0.0002		0.0002
Anchovy versus Fish intestine	1.00		0.86	0.0002		0.0002
Chicken intestine versus Fish intestine	0.0008		0.76	0.0002		0.0002
Chicken intestine versus Earthworm	0.068		0.84	0.023		0.0002
Earthworm versus Fish intestine	0.0003		0.92	0.005		0.0002

The p-values for weaver ant counting and amount of food removed (g)/hour in 20 days between September and October 2013 and January and February 2014 in cashew orchard, Naliendele, Tanzania. (Kruskal-Wallis Multiple Comparison-Wilcoxon Each Pair test)

The preference was the highest for anchovy and fish intestine and the least for earthworm and chicken intestines across both seasons and orchards except in citrus during the dry season (Figures 4.1 a and b).



a

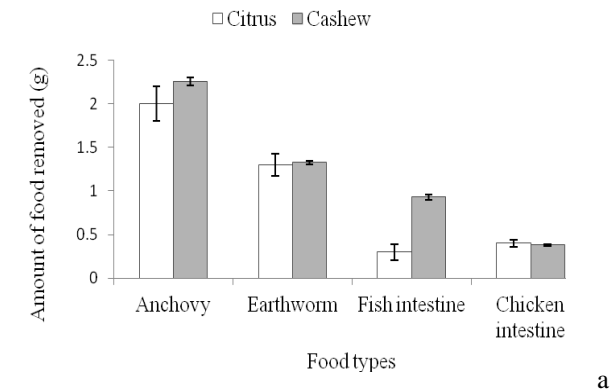


b

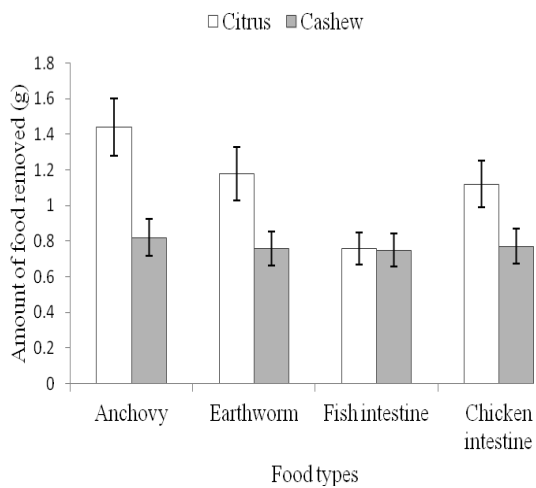
Figure 4. 1 a and b: Number of Weaver Ant workers at food sources supplied daily for 10 days during dry (a) and rainy (b) seasons at Naliendele Citrus and Cashew orchards

The quantities of food types taken by ant workers were significantly different ($p=0.05$) in citrus but not in cashew orchard during the dry season (Tables 4.1 and 4.2). In contrast, the quantities of food taken by ant workers in both orchards were statistically different ($p=0.05$) during the rainy season. Workers took significantly ($p=0.05$) more anchovy

than they did to other food types. In all situations, the preference was the lowest for chicken and fish intestine (Figs. 4.2a and b).



a

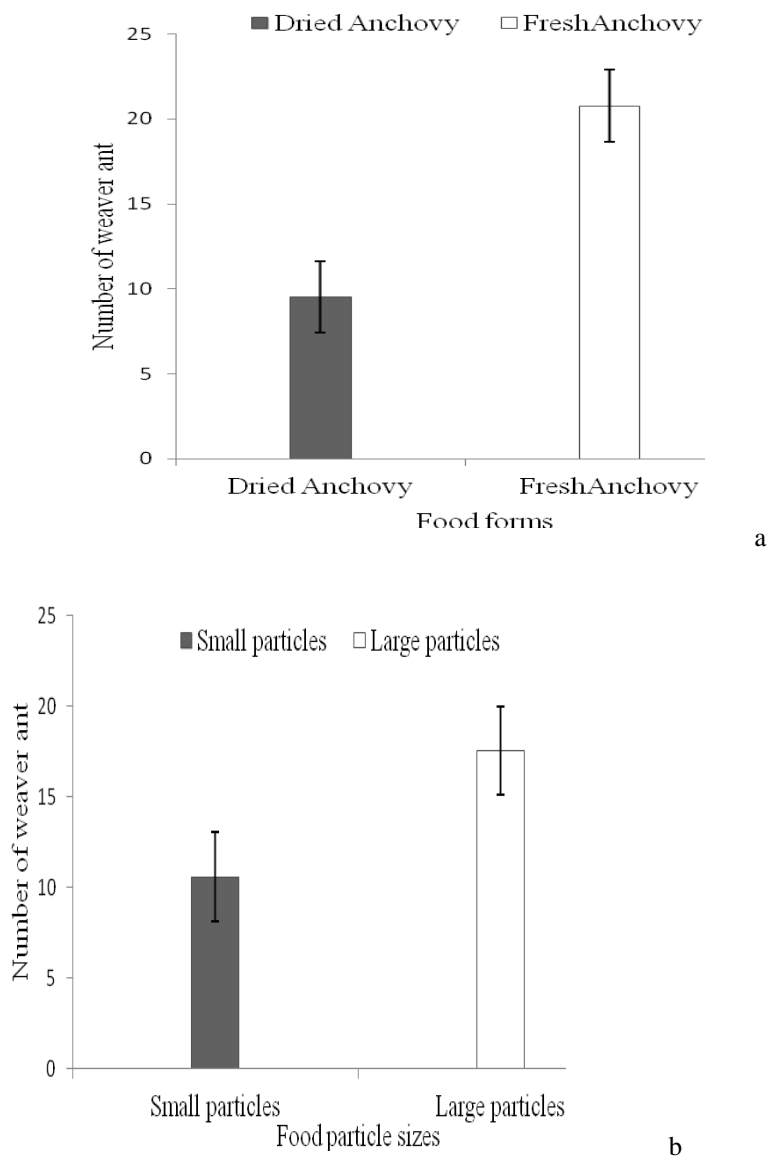


b

Figure 4. 2 a and b: The average amount of food removed by forage workers after 60 minutes foods supplied/day for 10 days during dry (a) and rainy (b) seasons at Naliendele Citrus and Cashew orchards

The results show further that more workers foraged on fresh-ground than they did on dried-ground anchovy (Fig. 4.2a). Similarly, more workers foraged on large particles than they did on small particles of ground-dried anchovy (Fig. 4.2b).

rainy season) at Naliendele Citrus orchard, Tanzania



Food forms (a): Wilcoxon test; N=90; Prob.=< 0.0001, Food particle size (b) Wilcoxon test; N=60;

Prob.=< 0.0001

Figure 4. 3 a and b: Number of weaver ant counting /10 minutes for 10 days between May and July, 2014 for the given anchovy food forms and different particle sizes at Naliendele Citrus orchard

The results of the AHP show that of the three criteria, earthworm ranked the highest followed by fish and chicken intestines (Table 4.4).

Table 4. 4: Analytical Hierarchy Process results on farmers' access to food types for weaver ants

Alternatives	Weights (Eigen Vector)*			Global score
	Affordability (0.539)	Availability (0.0845)	Applicability (0.126)	
Earthworm	0.59	0.63	0.41	0.42
Fish intestine	0.25	0.19	0.27	0.18
Chicken intestine	0.13	0.28	0.22	0.12
Anchovy	0.04	0.054	0.11	0.04

*Maximum Eigen Value = 0.24, CI = -1.09, CR = -0.27

4.5. Discussion

This study revealed that food preferences and foraging behavior by the *Oecophylla longinoda* can be influenced by food type, form, as well as particle size. Anchovy was highly preferred by *O. longinoda* in both citrus and cashew orchards during both dry and rainy seasons. The reasons for forage workers' preferences on anchovy to the other feeds are however not clear and could not be confirmed by this study. High preference for anchovy could probably be due to nutritional composition, particularly flavonoids or proteins. The foraging rate of *Pheidole megacephala* depends on the type of protein (Cornelius and Grace 1997). It has also been reported that the velvety tree ant, *Liometopum occidentale* Emery prefers anchovy to earthworm (Hoey-Chamberlain and Rust 2014).

Anchovy was the most preferred food across orchards and seasons but the order of preference for the other food types in both orchards varied between seasons.

Chicken intestine was the least preferred during the rainy season. This is probably due to the fact that individual ant workers more easily pick fish intestine particles and earthworm than chicken intestine. Chicken intestine became stickier and bound to the food bowl during the rainy season. Thus, the removal of chicken intestine by foragers was difficult. Foragers spent time trying to take sticky-bound food items but they often failed. A temporal change in foraging activities was observed when food became sticky and bound to the feeding bowl. At 15-30 minutes after food introduction, many ants were recruited and foraging activities increased with more ants observed on chicken intestine. However, as the food became stickier and bound to the feeding bowl, workers shifted to other food sources. It can be concluded that the nature of food at a particular time determines the foraging behaviour of *Oecophylla longinoda* workers, and thereby influences their preferences. Previous studies have showed that a large number of nest mates are recruited when ants are facing a non-transportable food items such as a shrimps (Cerdá *et al.* 2009), but, foraging shifts were observed when other food sources were present. According to Lim (2007), ants choose food types which are easier to transport, that is, requiring least energy to remove and transport.

More anchovy was removed by ants as opposed to other feeds across seasons and orchards. The probable reason for this could be the form that anchovy assumes after being ground. In the field anchovy became moist and grainy and could be removed without difficulty unlike the other food types. According to Hoey-Chamberlain and Rust (2014), the ease with which foragers are able to carry a particular type of food influences the amount of food to be consumed apart from food quality. However, inconsistency was

observed for the rest of foods across seasons or orchards. For instance, similar amounts of earthworm and chicken intestine were removed in citrus during the dry season. On the other hand, ants removed more chicken intestine than fish intestine in citrus orchard during the rainy season. Furthermore, similar amounts of food types were removed during the dry season in the cashew orchard. A possible cause of observed differences in food preferences across seasons could be colony needs at a particular time (Rust et al. 2000; Dussutour and Simpson 2012).

The quantities of food taken did correspond with the number of foraging workers, except for fish intestine in citrus orchard. We recorded more foraging workers on fish intestine than on chicken intestine and earthworm but the amount of food removed was smaller. Sometimes more ants visit a particular feed but remove less (Neff et al. 2011).

In this study, we observed higher foraging activities on ground-fresh than on dry anchovy. These results support previous studies whereby three ant species, *Linepithema humile* Mayr, *Anoplolepis custodiens* F. Smith and *Crematogaster peringueyi* Emery foraged more on liquid or moist food bait than on dry food bait (Nyamukondiwa and Addison 2014). Similarly, more activities for the ant, *L. lumile* were recorded on a 25 % sugar solutions or honey than was the case with solid based protein foods such as tuna (Baker et al. 1985). It can be argued that, *Oecophylla longinoda* prefers fresh, moist foods than dried solid particles.

However, fresh-ground foods become sticky after sometime, making it difficult for ant workers to remove them. Therefore, fresh-ground anchovy should be replenished to avoid stickiness; otherwise dried-ground anchovy should be used.

Forage workers easily collected and took large particles back to their nests in their mouthparts. However, they faced difficulties in collecting small particles and spent more time at the food bowl. A similar finding was reported for the fire ant *Solenopsis invicta* Buren (Neff et al. 2011).

Flavonoids have phytochemical properties against fungi, virus, and bacteria (Cushnie and Lamb 2005). They possess pharmacological activities such as antioxidant, anti-cancer and inhibition of tumor growth in mice (Shama 2006). They are considered to be an integral part of human diet (Arrabi et al. 2004). Therefore, flavonoid rich food types such as anchovy can be regarded as the best food to feed weaver ants. However, results of the AHP indicate that earthworm ranked the highest. This means farmers are more inclined to adopt the food type that is affordable and readily available. Earthworm is a cheap and widely available source of protein in the study area.

4.6 Conclusion

The results showed that all four tested feeds were removed by *Oecophylla longinoda* workers but anchovy was the most preferred. Considering the availability and affordability of the tested food sources, earthworms and fish intestine would be recommended as supplements during scarcity to boost weaver ants colonies on understanding that farmers preferred the less costly and sustainable option. All in all, fresh or dried-ground anchovy with particle sizes greater than 1 mm remains best choice if the availability and affordability is not subjects of concern.

4.7 Acknowledgements

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CHAPTER FIVE

5.0 Temporal abundance of African Weaver Ant, *Oecophylla longinoda*

(Hymenoptera: Formicidae) under unimodal rainfall pattern in Tanzania

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Abstract

Weaver ants, *Oecophylla spp.*, provide adequate protection to crops against insect pests when their populations are high and stable. A study was conducted in a cashew field in Tanzania from November, 2012 to October 2014 to determine temporal abundance of *O. longinoda*. We determined (i) the number of shoots with *O. longinoda* in a tree (established fortnightly) monitored at four cardinal points of a tree, using 1 m² wooden quadrats, (ii) the percentage of branches with ant trails per tree per month and (iii) the number of visible ant nests within the tree canopy per month. Furthermore, we assessed the association of weather and phenology with *O. longinoda* populations. More weaver ant populations were recorded during reproductive and vegetative phases of cashew. We recorded significant positive association between rainfall and temperature with the number of shoots with *O. longinoda* ($p < .001$), as well as relative humidity with the number of nests ($p < .001$). The percentage of shoots with *O. longinoda* was significantly associated with cashew growth phases. Rainfall and temperature negatively affected the number of nests. Consideration of cashew phenology and weather parameters can greatly enhance successful weaver ant augmentation strategies.

Key words: Cashew, phenology, *Oecophylla longinoda*, weather parameters, Tanzania

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5.1 Introduction

Weaver ants (Hymenoptera: Formicidae) are predatory insects that are widely used as bioagents for the control of insect pests in various crops (Way, 1953; Way & Khoo, 1992; Peng & Christian, 2007; Seguni, 1997; Olotu, du Plessis, Seguni, & Maniania, 2013a; Materu, Seguni, & Ngereza, 2014). The Asian weaver ant *Oecophylla smaragdina* (Fabricius) has been successfully used to control pests of various crops including cashew (*Anacardium occidentale* Linn). The African weaver ant *Oecophylla longinoda* Latreille has also been used as a biocontrol agent against several crop pests particularly coconut pests on which adequate information exists (Seguni, 1997; Seguni, Way & Van Mele, 2011). The knowledge on using *O. longinoda* against coconut pests could be extended to the African cashew production systems.

The Tanzanian cashew industry is valued at US\$70 million annually (Wakabi, 2004). However, cashew production is severely constrained by insect pests, particularly the coconut bug *Pseudotheraptus wayi* (Brown) and mosquito bug *Helopeltis anacardii* Miller (Topper, Grunshaw, Pearce, Boma, Stathers, & Anthony, 1998; NARI, 2007; Sijaona, 2013). A majority of cashew growers in Tanzania control insect pests using synthetic insecticides (Sijaona, 2013) albeit with negative consequences (Hajek, 2004; Tholkappian & Rajendran, 2011). *O. longinoda*, if maintained at optimum populations, can be a better alternative for the protection of cashew against attacks by insect pests. The costs of using weaver ants in cashew have been reported to be lower than the use of

synthetic insecticides (William, Hella, Lars, Offenber, Mwatawala, & Rwegasira, 2015a).

Weaver ants can offer adequate protection to crops when their populations are high and colonies are stable (Way, 1953; Way & Khoo, 1992; Sporleder & Rapp, 1998; Peng, Christian, Lan, & Binh, 2008). Studies on *O. smaragdina* showed that, more than 50% colonisation per tree is required to offer substantial protection to cashew crop (Peng, Christian, Lan, & Binh, 2008). Stathers (1995) reported that, at least 10 nests of *O. longinoda* per cashew tree are required for the control of *H. anacardii* and *P. wayi*. Conditions that favour colony stability and population build up should be maintained in order to sustain population levels that adequately offer protection against particular pests. Different weaver ant augmentation approaches have been recommended. These include moderation of weeding regimes to reduce weaver ant competitors such as *Pheidole megacephalla* (Seguni, Way, & Van Mele, 2011), the use of soft pesticides (Van Mele & Cuc, 2007), food supplementation during scarcity (Lim, 2007; Offenburg, 2015) and the provision of suitable artificial nesting sites (Offenberg, 2014).

Population size of weaver ants is related to host trees phenology and weather conditions (Holldobler, 1979). The ants depend on young shoots, evergreen trees and shrubs (Way 1954; Holldobler, 1979). Growing buds of new shoots provide nectar, which is a source of food for ants (sugar). Their nests are constructed using young leaves of the host tree (Way & Khoo, 1992; Van Mele & Cuc, 2007). New, flexible leaves can be easily folded to facilitate nests construction. It has been observed that the presence of nectar and new flexible leaves at some stages of cashew growth can influence ant distribution and abundance (Van Mele & Cuc, 2007)

Abiotic factors such as temperature, rainfall and relative humidity (RH) can also affect weaver ant abundances; similarly, weaver ant's queen might die under heavy rainfall (Peng *et al.*, 2008). Weaver ant, *O. smaragdina* prefers temperatures of between 26 °C and 34 °C and Relative Humidity (RH) of between 62 and 92 % (Van Mele & Cuc, 2007) the extremes of which are detrimental.

Limited knowledge exists on the abundances of *O. longinoda* in relation to cashew tree growth phases and weather conditions such as temperature, RH and rainfall. Such knowledge is crucial in maintaining high and stable populations of *O. longinoda* in the field (Rwegasira, R., Mwatawala, Rwegasira, G., & Offenber, 2014). Techniques for boosting ants' populations such as feeding (Nassor, Rwegasira, Jensen, Mwatawala, & Offenber, 2015) can be applied based on the knowledge of temporal abundances.

The objective of this study was to determine weaver ant abundance in relation to cashew phenology and weather conditions. Such knowledge will be used to improve the use of weaver ants in managing insect pests of cashew.

5.2 Materials and methods

Studies were conducted at the Naliendele Agricultural Research Institute, NARI (longitudes 40⁰ 09' 57.05" E, latitude 10⁰ 21' 22.49" S, 140 m asl), Mtwara, Tanzania from November 2012 to October 2014. Mtwara Region has a uni-modal wet season, with regular rains ranging from 810 to 1090 mm, which commences in November/December and ends in April/May. The average maximum and minimum temperatures are 27 °C and 23 °C respectively.

O. longinoda populations were monitored on 30 cashew trees, aged between 23 and 26 years, planted at a spacing of 12 x 12 m (an average of 69 trees/ha). The temporal abundance of *O. longinoda* was assessed each month, for each tree as (i) the number of shoots with *O. longinoda* (established fortnightly), which was monitored using 1 m² wooden quadrat at four cardinal points of a tree that is the east, west, southern and northern sides of cashew canopy, (ii) the percentage of branches with *O. longinoda* trails (branches with ant trails/total number of branches x 100) per tree (Peng *et al.* 2008) and (iii) the number of visible *O. longinoda* nests in a canopy (each tree examined for an average of five minutes).

In each quadrat, we recorded the numbers of shoots, as well as shoots with newly flushed leaves, shoots with mature leaves, and shoots with flowers or fruits. Finally, we recorded phenological stages of cashew, that is, the presence of mature leaves only, newly flushed leaves, flowers and fruits or nuts. We divided the growth phases of cashew, as dormancy, vegetative or reproductive.

Data on rainfall (mm), temperature (°C) and relative humidity (%) were obtained from a meteorological station at NARI, located about 1km from the study site.

5.3 Data analysis

The poisson regression analysis was used to determine the association between weather parameters, growth stage and the number of shoots with *O. longinoda* as well as the number of nests. Predictor variables were temperature, rainfall, and relative humidity. Correlation between *O. longinoda* population, nest counts and the number of shoots with weaver ants was determined by the Spearman correlation, because residuals were not normally distributed. Chi square test was used to test dependency of weaver ant

populations on different growth phase of cashew trees. Data were analysed using Genstat (VSN International, UK.)

5.4 Results

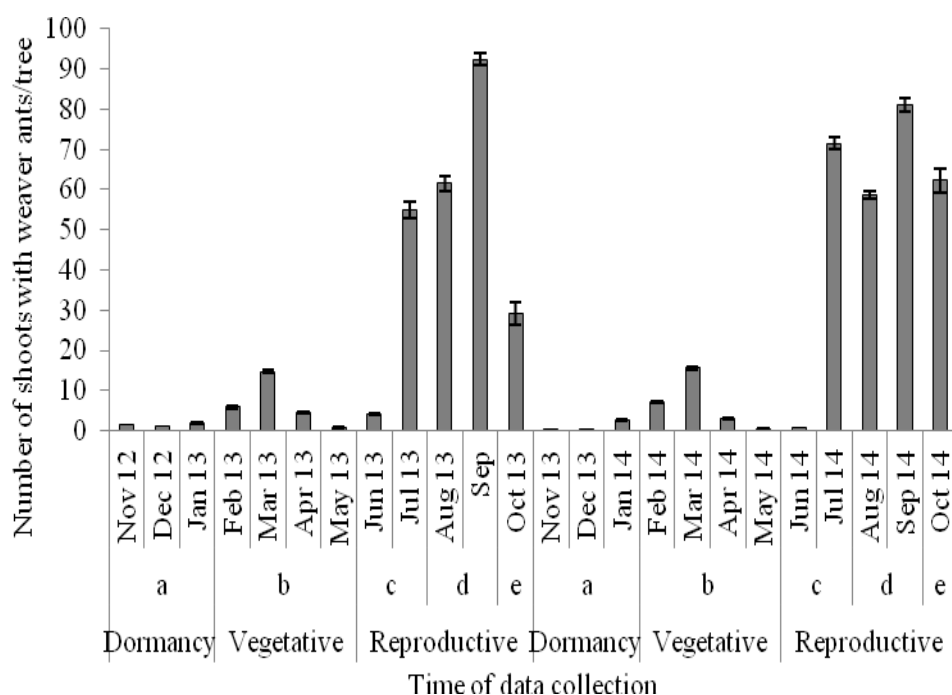
5.4.1 Cashew phenology

Results showed that the dormancy stage extended from November to January; and this phase was characterized by more than 95 % of shoots with mature leaves. The vegetative phase spanned from February to May, and was characterized by new leaf flushes for vegetative growth (flushes ranged from 20 to 80 % per tree). The reproductive phase spanned from June to October, and was characterized by new flush leaves, flowers, fruits and nuts.

New leaves started to flush in June, followed by bud bursting and flowering in June/July. Flushes, flowering and nut setting/fruitlet continued from July/August to September/October. The peak for nut harvest spanned from September to October.

5.4.2 Number of shoots with weaver ants per tree

The average number of shoots colonized by *O. longinoda* ants ranged from one to 92 per tree. The highest number of colonized shoots was 92, recorded in September. The numbers of colonized shoots were higher during the dry season, from July to October, and were the lowest from November to June (Figure 5.1).



Where; **a**=>95% mature leaves, **b**=generally 10-50% leaf flushes, **c**=>80% leaf flushes and flowering, **d**=>90% leaf flushes, flowering and fruiting and **e**=flowering and fruiting in figure 5.1, 5.2 and 5.3.

Figure 5. 1. Average number of shoots with weaver ants per tree (n=30) recorded from November, 2012 to October, 2014 at Naliendele site, Mtwara, Tanzania

The Poisson regression results showed significant associations ($df = 5$, $p < 0.001$) between relative humidity, rainfall, temperature and cashew phenology with the number of shoots colonised by *O. longinoda*. Relative humidity negatively affected the number of colonised shoots, in contrast to other tested parameters (Table 5.1). A unit increase in rainfall, increased the number of shoots with *O. longinoda* by 0.6% ($e = 0.007526$, $p < 0.001$, $\text{Exp} [\beta] = 1.006$). A unit increase in temperature corresponded with a 6.7% decrease in the number of colonized shoots ($e = 0.06456$, $p < 0.001$, $\text{Exp} [\beta] = 1.067$). In contrast, an increase in relative humidity decreased the number of nests in a tree by 1.9 % ($e = -0.019232$, $p < 0.001$, $\text{Exp} [\beta] = 0.9810$). The numbers of colonized shoots were 5.5 times higher in the vegetative phase than was the case in the dormancy phase ($e = -$

1.7087, $p < 0.001$, $\text{Exp} [\beta] = 5.522$). Numbers of colonized shoots were higher during the plant reproductive phase than was the case in dormancy phase ($e = 4.0365$, $p < 0.001$, $\text{Exp} [\beta] = 56.63$).

Table 5. 1. Relationship between relative humidity, rainfall, temperature and phenology on number of shoots with *O. longinoda*

Parameter	Estimate	s.e.	t(*)	t pr.	Exp (β)
%RH	-0.019232	0.000941	-20.44	<0.001	0.9810
Rainfall	0.005726	0.000225	25.50	<0.001	1.006
Temperature	0.06456	0.00547	11.81	<0.001	1.067
Vegetative	1.7087	0.0503	33.95	<0.001	5.522
Reproductive	4.0365	0.0511	78.94	<0.001	56.63

Note: s.e. = standard error of estimates, t(*) = estimate/standard error, tpr. = probability, Exp (β) = exponent of estimate

5.4.3 Number of nests per tree

Temporal variations in numbers of *O. longinoda* nests per tree are summarized in Figure 5.2. Numbers of nests were highest during the vegetative phase, from March to May with the peaks in April and May. In contrast, numbers of weaver ant nests were lowest from November to January, during the dormancy phase in both years. The number of nests per tree ranged from 4 to 13.

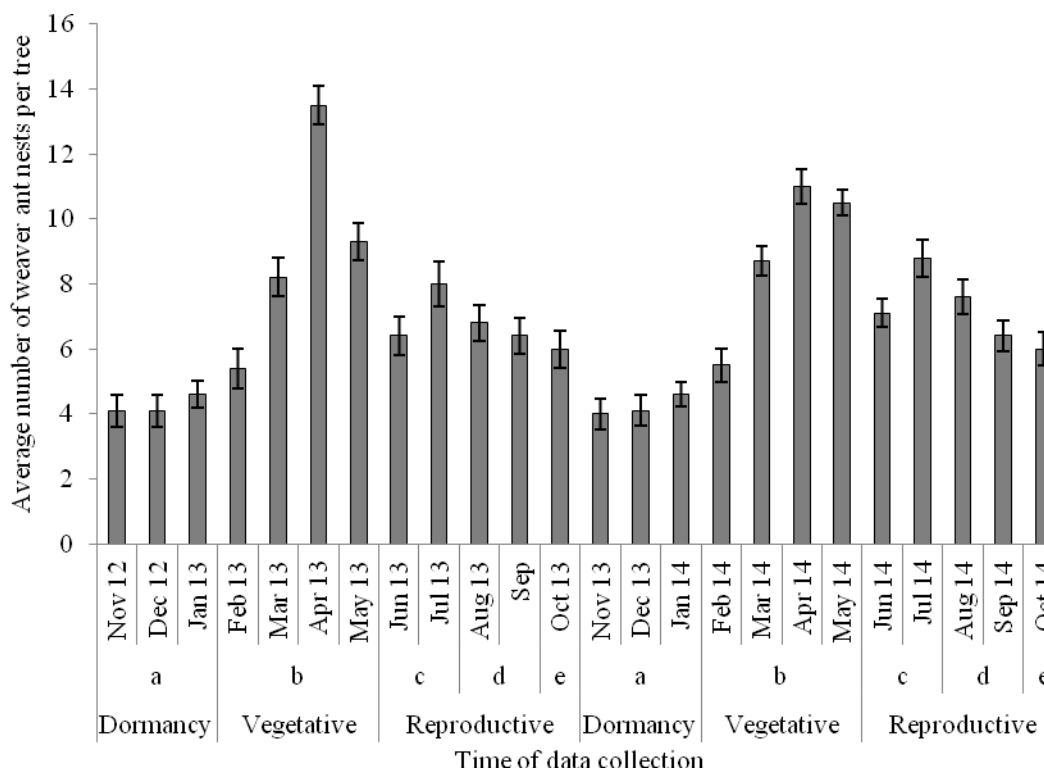


Figure 5. 2. Average number of weaver ant nests per tree (n=30) recorded from November, 2012 to October, 2014 at Naliendele site, Mtwara, Tanzania

We observed significant associations between weather parameters and phenology with the number of *O. longinoda* nests (df = 5, Chi Pr < 0.001). We recorded significant associations ($p < 0.001$) between rainfall and cashew phenology with the number of nests per tree. Rainfall was associated with low number of nests (Table 5.2). A unit increase in rainfall caused a 0.1 % decline in the number of nests ($e = -0.000995$, $p < 0.001$, Exp [β] = 0.999). A tree in vegetative phase had 2.08 times more nests than was the case in a dormant phase ($e = -0.7368$, $p < 0.001$, Exp [β] = 2.089) while a tree in reproductive phase had 1.34 times more nests than was the case in a dormant phase ($e = -0.232$, $p < 0.001$, Exp [β] = 1.341).

Table 5. 2. The association between weather parameters and cashew phenological development on total number of weaver ant nests

Parameter	Estimate	s.e.	t(*)	t pr.	Exp (β)
%RH	0.00055	0.00321	0.17	0.864	1.001
Rainfall	-0.000995	0.000223	-4.45	<0.001	0.9990
Temperature	-0.0493	0.0258	-1.91	0.056	0.9519
Vegetative	0.7368	0.0504	14.62	<0.001	2.089
Reproductive	0.2932	0.0721	4.06	<0.001	1.341

5.4.4 Percent of branches with weaver ants per tree

We recorded temporal variations in percentage of branches colonised by *O. longinoda* per tree (Figure 5.3). A lower proportion of branches was colonised by *O. longinoda* in November and December than it did in April and May (Figure 3). Generally, high percentages of branches were colonised by *O. longinoda* from January to October. The percentage of branches with *O. longinoda* varied between 51 and 97 (Figure 5.3).

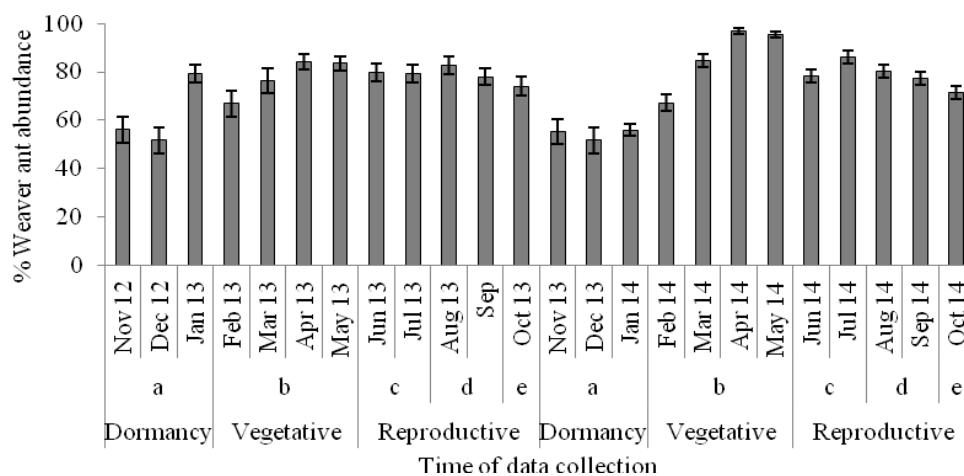


Figure 5. 3. Percentage of branches with weaver ants per tree (n = 30) recorded from November, 2012 to October, 2014 at Naliendele site, Mtwara, Tanzania

We observed a significant association between phenological development (flushing) of cashew and the percentage of branches colonised by weaver ants (Table 5.3). Higher populations of *O. longinoda* were significantly associated with the plant reproductive phase whereas medium populations were significantly associated with the dormancy phase ($\chi^2 = 16.25$, $p = 0.0003$, $n = 30$).

Table 5. 3. Results of Chi square test showing the percentages of weaver ant populations at different growth phases of cashew trees

Growth phases	Medium weaver ant population (50-70 %)	High weaver ant population (71-100 %)
Dormancy	66.7(100)	0.0(0)
Vegetative	22.2(25)	40(75)
Reproductive	11.1(10)	60(90)
Likelihood ratio	$X^2=16.25$, prob 0.0003*, n=30	

Numbers in brackets present percent population contributed by a corresponding growth phase

The percentage of colonised branches was significantly correlated with the number of nests ($p = 0.91$, $p < 0.0001$). However, there were no significant correlations between colonised shoots and the number of nests ($p = 0.23$, $p = 0.26$) as well as the percentage of colonised branches and the number of colonised shoots ($p = 0.27$, $p = 0.18$)

5.5 Discussion

Temporal fluctuations in insect populations are a universal rule, but vary widely across organisms. The underlying causes of population fluctuations also vary across organisms. This work revealed temporal variations in abundance of *O. longinoda* assessed by three different approaches. *O. longinoda* populations varied according to the phenological stage of cashew, with the number of nests, colonized shoots and the percentage of colonized branches primarily observed during vegetative and reproductive phases as opposed to the dormancy phase. Vegetative and reproductive phases had more flush leaves, tender shoots with corresponding essential nutrients such as nectar and suitable conditions for reproduction and perpetuation of *O. longinoda* population. In the previous studies, weaver ants were found to prefer flexible flush leaves for nest construction (Lokkers, 1990; Way & Khoo, 1992; Van Mele & Cuc, 2007; Van Mele, 2008). Normally ants get extra-floral nectar from flushing shoots, apples and nuts (Rickson & Rickson, 1998; Peng et al., 2008) as well as protein from corresponding high numbers of insects (Sijaona, 2013). During the vegetative phase, we observed a high presence of insect pests like *Hilda* spp and Aphids that produced honeydew, which is a source of food (sugar) for the ants (Van Mele & Cuc, 2007). A study by Peng et al. (2008) observed an increase in the population of antagonistic crazy ants (*Anoplolepis* spp) that negatively affected weaver ant populations.

The present study also revealed positive associations between weather and the number of shoots occupied by *O. longinoda*. Rainfall was inversely associated with the number of nests in a tree, while relative humidity was inversely related to the number of occupied shoots. Peng et al. (2008) observed reduced populations of weaver ants under harsh conditions including high rainfall. In the current study, temperatures ranged from 24 °C to 29 °C. Lokkers (1990) reported a negative brood development of below 21 and above 34 °C. In the current study, relative humidity (RH) ranged from 45 to 84 %, which was lower than that of from 62 to 92 % which was reported to be optimal for *O. smaragdina* (Van Mele & Cuc, 2007). On average in the current study, we recorded more than 50% of branches occupied by *O. longinoda* per tree, which implied adequate protection of the crop, as inferred from reports on *O. smaragdina* (Peng et al., 2008). The recorded number of nests in most trees ranged from 4 to 9 per tree, which was less than 10 nests per tree, which is the recommended number of nests that could offer the required protection of cashew (Stathers, 1995). The two methods of assessment were therefore at variance with one another. However, damage assessment was not part of this study hence a firm conclusion cannot be drawn.

We conclude that the presence of cashew shoots with new flush leaves, flowers or fruits greatly contributed to high *O. longinoda* abundances. Furthermore, abiotic factors such as temperature, rainfall and relative humidity regulated the weaver ant populations. We recommend that management practices such as supplementary feeding that can boost *O. longinoda* populations particularly during the dormancy phase are essential. Moreover, quantifying the effect of homopterans and crazy ants in relation to weaver ant abundance is important.

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CHAPTER SIX

6.0 General Conclusions and Recommendations

6.1 General Conclusions

The current study confirmed that, mating of *O. longinoda* queens takes place during a nuptial flight and does not take place within the nest, as previously suggested. The flight occurs during evenings just before sunset. Time from nuptial flights to shedding of wings and egg laying translates to hatchability of the eggs. Periods within the mating season with little sunshine, high relative humidity and low wind speeds are likely to trigger mating flights, especially in the first part of the mating season where more colonies contain sexuals in their nests. This information can make it easier to trap newly mated queens needed in ant nurseries. During reduced food availability, farmers in the study area can use various feeds such as earthworms and fish intestines to supplement food for *O. longinoda* colonies. Fresh moist anchovy or dry anchovy of large particle sizes can be used whenever available.

This study also established the variations in weaver ant population abundance in relation to cashew phenological development. More weaver ant population was recorded during reproductive and vegetative phases than in dormancy phases of cashew. The presence of cashew shoots with new flush leaves, flowers or fruits greatly contributes to high weaver ant abundances. Weather parameters such as temperature, rainfall and relative humidity tend to regulate weaver ant populations. Excessive rainfall and temperature negatively affected the number of nests, while relative humidity negatively affected number of shoots with weaver ants. Therefore, management practices such as supplementary feeding that can boost weaver ant populations particularly during dormancy phase are essential.

6.2 Recommendations

Based on the findings obtained during this study, the following are recommended:

- (i) Future studies should aim at understanding the effect of environmental conditions on egg hatching of the weaver ant, *O. longinoda*
- (ii) More data should be generated to confidently elucidate the true correlation between flights and full moon phases of *O. longinoda*
- (iii) Further studies should be undertaken to identify food compositions that are preferred by the weaver ant *O. longinoda*.
- (iv) Quantification on the effect of Hilda spp., Aphids and Crazy ant in relation to weaver ant abundance needs to be done.