METHODS FOR ENHANCING WEAVER ANT (OECOPHYLLA LONGINODA LATREILLE) (HYMENOPTERA; FORMICIDAE) POPULATIONS FOR SUSTAINABLE CONTROL OF INSECT PESTS OF CROPS

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A THESIS SUBMITTED IN FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY OF SOKOINE UNIVERSITY OF AGRICULTURE. MOROGORO, TANZANIA.

2016

EXTENDED ABSTRACT

Weaver ants (*Oecophylla* spp) are eusocial insects used in pest management. Studies were conducted in citrus field in Tanga and Morogoro regions to determine occurrence of sexual, efficient methods for trapping mated queens, population structure and optimizing queen rearing methods as prerequisite for transplanting weaver ants population. Result of a study conducted between 2011 to 2013 showed that sexuals occurred in nests for a prolonged period with peaks between February and April. Numerically, males were more abundant than queens (6:1). Average number of males per nest was 87 (± 54) and for the queens was 17 (± 6). Harvesting of two nests per colony per month did not affect the number of nests per colony (treatment effect, $F_{1.31}$ = 1.87, P = 0.8; time effect, $F_{11.21}$ = 8.96, P < 0.0001, treatment x time $F_{11.21}$ = 1.18, P = 0.36).

Results also showed that out of 34 collected queens 73% were from leaf traps, 24% from paper traps and 3% from search and catch. However when light trap, was included it accounted for 91% of 236 collected queens.

The collected mated queens were reared in the screen house using three methods; limited access to water, Continuous direct access to 5 mls of water Continuous indirect access to 15 mls of water. Results showed that queen survival was independent of all the three tested rearing methods ($\chi2$ = 2.0; df = 2, 48; P = 0.36). Rearing methods significantly affected the total numbers of brood (F = 4.85; df = 2, 48; P = 0.012) with higher numbers recorded on continuous direct and indirect access to water methods.

Results also showed that colony size varied significantly. One colony of *O. longinoda* covered 3 to 30 citrus trees in an area between 27 m^2 to 270 m^2, Average number of nests

per colony ranged from 18 (± 6 SE) to 142.5 (± 17.5 SE). The highest numbers of major and minor workers per nest was 1064.5 ± 252.9 and 2365 ± 895.9 respectively. Weather parameters had effects on the numbers of major workers, minor workers and winged queens.

DECLARATION

I, Rozalia Gration Rwegasira 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 not been submitted for a degree award to any other institution.

_________________________ ______________________

Rozalia Gration Rwegasira Date

(PhD Candidate)

The above declaration is confirmed by;

_________________________ ______________________

Prof. Maulid Mwatawala Date

(Supervisor)

Co-supervised by Joachim Offenberg

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ACKNOWLEDGEMENTS

I would like to thank my employer the ‘Regional Administrative Secretary (RAS)’ for Morogoro Region for granting me permission to pursue this study. My sincere gratitude goes to the Danish International Development Agency (DANIDA) who funded my studies through project No. 10025AU. Many thanks go to my supervisors Prof. Maulid Mwatawala (PhD) and Joachim Offenberg, PhD, for their technical support during the period of my studies. Their advices, comments, constructive criticisms and moral support were highly valuable.

I am grateful to several organizations I have interacted or associated with during research inclusive of; Muheza District Council in Tanga, Mlingano Research Institutes in Tanga, the Swedish and Denmark Embassies in Dar es salaam Tanzania and Aarhus University in Denmark. Many thanks go to Semngano village authorities in Muheza District, in Tanga for allowing me to conduct my experiments in their field. I thank Semngano village farmers; Ramadhani Mwahimu, Yassin Abdubakari and Juma Alli for offering their fields for my research. The Extension Officer Mr. Jimmy Mhina was instrumental in guiding the farmers on weaver ant maintenance throughout the studies.

I would also like to thank the Mzinga Cooperation Authority in Morogoro region for allowing me to carry my studies in their fields. I am indebted to Mr. John Kusolwa for his technical support in the field activities and for enduring a lot of bites from weaver ants. Tiba Xsaverina is acknowledged for her assistance in laboratory work and screen house activities. My friends and colleagues William George, Halfan H. Nene and Nassor R. Abdulla (Tanzanian Team) and Issa Ouagoussounon, Rosine Wargui Brisso and Florence Anato (Benin Team) were a constant source of encouragement in this endeavour. The technical assistance from Gabor L. Lövei (PhD) of Aarhus University, Denmark was fundamental in writing and publishing the scientific papers in peer reviewed journals and Dr. Renkang Peng for supporting field experiments. Finally I would like to thank my family for being a strong source of inspiration and encouragement throughout the course of the studies.

DEDICATION

I would like to dedicate this work to the Almighty God for keeping me alive, my parents Mr. and Mrs. Stephen Shiyo for good upbringing and my family 'Rwegasira's family' for their moral support.

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LIST OF ABBREVIATIONS AND SYMBOLS

%	Percentage
±	Plus or minus
°C	degree centigrade
a.s.l.	Above sea level
cm	Centimetre
DANF	Days After Nuptial Flight
DANIDA	Danish International Development Agency
Dr.	Doctor
FAO	Food and Agricultural Organization
g	gram
JMP	Jump
km	Kilometres
log	logarithm
m	Meters
MANOVA	Multivariate Analysis of Variance
n	Number
P	Probability
PhD	Doctor of Philosophy
RAS	Regional Administrative Secretary
RC	Rearing Condition
SE	Standard error
Spp	Species
SUA	Sokoine University of Agriculture
USD	United states Dollars
X^2	Chi-square

CHAPTER ONE

1.0 GENERAL INTRODUCTION

1.1 Background

Weaver ants (*Oecophylla* spp) are eusocial insects of the family Formicidae (order: Hymenoptera) along with the related wasps and bees. Currently, there are two species recognized, *Oecophylla longinoda* (Latreille) occur in a wide band across equatorial Africa and *Oecophylla smaragdina* (Fabricius)occurs in Sri Lanka and much of India through Indo-China and Southern China to Indomalayan region, Northern Australia and Melanesia. Cole and Jones, (1948), cited by Ross *et al*., (2010). They live exclusively on trees and shrubs, where they nests on fresh plant leaves. The ants are highly territorial and aggressive, feeding on other small animals and honeydew (Peng *et al*., 1999). Hence they are often used as natural enemies against destructive crop pests, like cashew sucking pests and fruit flies.

Oecophylla spp. have been used in controlling pests in orchards for more than seventeen hundred years. Colonies of *O. smaragdina* were sold in markets in China as pest control agents, as early as 304 AD. Huang and Yang (1987), cited by Krag (2010). Recent research in Australia has revealed that *O. smaragdina* can increase economic benefit compared to commonly used pesticides (Peng and Christian, 2005b; Peng *et al*., 2004). Crops, like cashew, mango, citrus, coffee, cocoa and coconut, benefit from *Oecophylla* ants as biological pest controllers (Van Mele, 2008; Way and Khoo, 1992). According to Van Mele (2008) the use of weaver ant for pest management in Mango orchard increases the profits by 50% compared to conventionally management.

Mango and cashew are attacked by a long list of pests of which fruit flies and heteropteran bugs are regarded as most important. Mango is commonly attacked by various combinations of five indigenous species of fruit flies including *Ceratitis cosyra* (Walker), *C. fasciventris* Bezzi, C. *rosa* Karsch, *C.anonae* Graham and *C.* c*apitata* (Wiedemann*)* (Lux *et al*., 2003). These fruit flies cumulatively cause annual mango yield losses of up to 40%, which fluctuated broadly among location, season and between mango varieties. Damage to fruits such as mango in East and West Africarisen from 40 to 86% in smallholder farms with the arrival of *Bactrocera dorsalis* (Hendel) (under the synonym of *Bactrocera invadens* Drew Tsuruta & White)an invasive fruit fly species of Asian origin (Lux *et al.,* 2003).

Cashew pests have been a major problem affecting productivity of African cashew in terms of qualities and quantities of marketed nuts. Cashew sucking insect pests, including *Helopeltis anacardii* (Miller), *H. sochoutedenii* (Reuter), and *Pseudotheraptus wayi* (Brown), are the second most serious biological constraints to cashew production and productivity after the powdery mildew, *Oidium anacardii* (Noack) in East Africa, particularly Tanzania. Yield losses associated with *P. wayi*on potentially harvestable nuts have been estimated at 30-65 % (Stathers, 1995) and 80% Martin *et al.* (1997); Mitchell (2000); CABI (2005); Maniania (2009) and Nyambo (2009) cited by Econyu *et al*. (2013).

Recent research in Benin showed that the abundant weaver ant populations on mango drastically reduced damage from fruit flies (Van Mele and Vayssieres, 2007). Similar research findings from East Africa suggest that weaver ants impart significant reduction in damages caused by cashew sucking pests (Stathers, 1995). Also the use of weaver ants reduces the damaged caused by bug (*Amblypelta lutescens lutescens)* on cashew plantation (Peng *et al*., 2005).

Additionally, *Oecophylla* ants are reported to be used as a human food source in a multitude of countries including Thailand, India, Myanmar, Borneo, Philippines, Papua New Guinea, Australia, Congo and Cameroon (Bristowe, 1932; DeFoliart, 2010 as cited by Offenberg, 2011). In Thailand *O. smaragdina* is considered a delicacy and has been eaten by humans for centuries. Imago as well as brood are used in a variety of Thai dishes and are easily obtained on many local markets throughout the country during the ant harvest season (Sribandit, 2008). Larvae and pupae are preferred over imago and the queen caste preferred over the worker castes and males. The ants are used as ingredients in soups, salads and fried dishes and sometimes eaten raw together with spices as a snack (Cesard, 2004; Sribandit *et al*., 2008; Offenberg, 2011; Van Itterbeeck *et al.,* 2014; FAO report, 2013). In Chhattisgarh, India, traditional healers believe that regular intake of *O. smaragdina* prevents rheumatism – a view shared by practitioners of traditional Chinese medicine (Chen and Alue, 1994; Oudhia, 2002 cited by Sribandit*et al*., 2008). The Indian healers also prepare oils in which they dip collected ants. After 40 days oils are used externally to cure rheumatism, gout, ringworm or other skin diseases, or else as an aphrodisiac (Oudhia, 2002 cited by Sribandit *et al*.,2008).

1.2 Colony Structure

Weaver ants (*Oecophylla*spp*)* are predominantly arboreal (Holldobler, 1980; Way 1954a), but sometimes venture onto the ground to forage, or between trees when canopies do not interconnect (Jackson, 1988). Their colonies are among the largest in the ant family. Way (1954a) observed one colony of *O. longinoda* inhabiting 151 nests, scattered throughout 8 coconut and 4 clove trees, and covering an area of 800 m^2**.** He estimated that this colony contained 480 000 worker ants, and 280 000 broods. Vanderplank (1960), and Holldobler and Wilson (1978) also reported populations of mature colonies in the range of 100 000 to 500 000. Peng *et al*. (1998b) reported that in a mango orchard, one colony of

O. smaragdina occupied two to 30 trees with 25 to 153 nests. Likewise, Majer (1976) estimated that colonies could contain several millions of ants.

The number of ants in one nest on average varies between 4000 and 6000 individuals, and the whole colony can have as many as 500 000 adult workers. The ant colony is like an extended family with many nests and individuals who all know each other and who work closely together in a certain area. Paul and Nguyen (2007) found that numbers of nests in one colony depend on many different factors such as availability of food and level of disturbance, but it can reach up to 100. These nests may be found in over 15 trees and over an area bigger than $1000m^2$.

Each colony of *O. smaragdina*has one egg laying queen (which cannot be replaced) but may consist of several hundred nests and cover a territory of up to one hectare (Crozier *et al*. 2009; Höldbler and Wilson, 1983; 1990). According to Peeters and Andersen (1989) and Peng *et al.* (1998) there are multiple mated queens (pleometrosis) in *O. smaragdina* colony during the early colony establishment, but suggested that only one queen succeed in mature colonies.

Plate 1.1: Weaver ant Mother queen

(**Source** Rwegasira Rozalia, 2015)

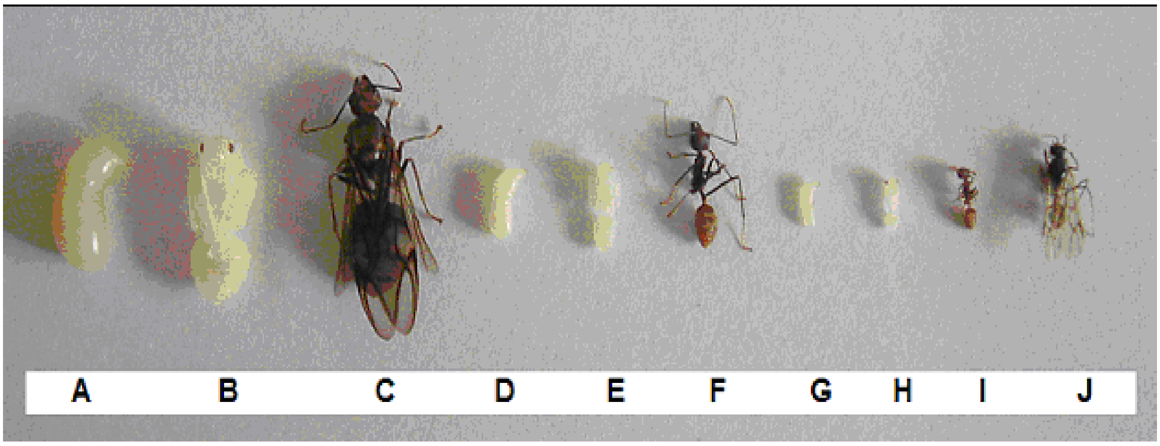

Plate 1.2: Different life stages and forms of weaver ants. A – queen larva; B – queen pupa; C – : winged queen; D –male or large worker larva; E – male or large worker pupa; F – large worker; G– small worker larva; H – small worker pupa; I – small worker; J – male.

Source: (IITA-CIRAD, 2008).

The castes *of Oecophylla* spp consists of mother queen/queen laying eggs (Plate 1.1), winged queen which is the new produced queen, major and minor workers (female) and males (Plate 1.2). The two workers caste shows a clear division of labour with the minors staying inside the nest (caring for brood) and the majors workers performing outdoor tasks (foraging and defence). In North Queensland, the growth of a colony is highest during the wet season, when temperature, humidity and food availability (after flowering and leaf flushing of the trees and subsequently increased abundance of prey) are most suitable. Brood production then peaks, following a lag of about a month, worker numbers increase to reach the maximum in March. During the wet season, colonies tend to spread out throughout their territory and then to construct fewer and bigger nests during the dry months (Lokkers, 1990). While larvae are present at all times of the year, pupae are absent for three months in the cooler and drier season. The continuous presence of larvae may be favoured by selection to be able to weave at all times (Lokkers, 1990).

Established wild ant colonies produce thousands of winged queens (flying ants) each year, which individually leave their colony and start new colonies alone. The mortality rate is high (>99%) during the first six months of initial colony establishment (Vanderplank, 1960; Greenslade, 1971) because of high intra- and interspecific competition and disease. For new survivors, it takes 18 – 24 months before the colony is ready to be transplanted into orchards (Vanderplank, 1960).

1.3 Mating Season

The males and winged queens are observed in the colony after attaining the age of 18 months (Vanderplank, 1960). Larger colonies consist mostly of sterile wingless females forming castes of "workers", "soldiers", or other specialized groups. Nearly all ant colonies have some fertile males and fertile females (queens). The males and new queens are found in the separate nests although occasionally both are found in the same nest. The winged individuals of *O. longinoda* emerge after heavy rainfall and are most frequently encountered between November and March in Zanzibar (Vanderplank, 1960). Peters and Andersen (1989) found three aggregations of de-alate queens of *O. smaragdina* in March 1987 from two locations in the coastal region of the Northern Territory, Australia. Populations of colonies of *O. smaragdina* increased from March to November 1995, and started to decline in December after two heavy rainfall events (Peng *et al*. 1999b). In the Mekong Delta of Vietnam, a lot of queens of *O. smaragdina* are found in the rainy season from July to October (Van Mele and Cuc, 2007).

Colony reproduction in ants typically occurs through the foundation of a new nest by a single mated queen (haplometrosis), but sometimes involves several cooperating queens (pleometrosis). Pleometrosis enables successful colony foundation under adverse conditions (Rissing and Pollock, 1988), but it is not necessarily followed by polygyny once the colony has become established. Richards (1969) observed a group of 11 mated queens together with many eggs and larvae on a window ledge. During the initial period, when a nest cannot yet be built (because the first generation of larvae are too young to produce silk), the physical presence of several large queens serves as an effective shelter for the brood lying on the leaf. Later in the genesis of a colony, the construction of a leaf nest is clearly essential (Peeters and Andersen, 1989). Cooperative colony foundation appears to have advantages specific to the leaf-nesting habit of *O.smaragdina***,** and in particular overcomes the difficulty faced by a single queen constructing a nest. Once a viable young colony has been established, the ecological benefits of cooperation ceases, and it is in the genetic interest of an individual queen to eliminate her rivals so as to monopolize reproduction (Peeters and Andersen, 1989).

1.4 Queen Rearing and Ants Nurseries

Winged queens emerge from their original nest for mating flight during the rainy season. Captured mated queens of *Oecophylla* spp, can be reared in the laboratory using match-boxes (Vanderplank, 1960). During this period queens do not eat, they are sprinkled with water daily. Queens drink water from the droplets. The life cycle of *O. smaragdina* colony starts with a mated queen find a sheltered site between leaves of a tree or shrub and laying a batch of about 35 eggs within 5 - 10 days after de-alation / shedding her wings (Lokkers 1990). Further brood development strongly depends on temperature. At 30°C Lokkers (1990) found larvae emerging from about day eight. Pupae follow after 17 days and the first adult workers appears after 27 days. *Oecophylla* ant-colony rearing under ambient tropical conditions showed that it took 18 to 24 months for a young colony with founding queens to become mature (emergence of sexual forms) (Vanderplank 1960; Peng *et al*., 1998).

1.5 Justification

Weaver ants have been used successfully in controlling various pests of crops. Successful control of pests requires well-distributed and stable populations of weaver ants in the field. The use of weaver ants in a pest management involves collection of ant colonies from the wild and subsequent introduction into crop fields. Establishment and perpetuation of the introduced colonies depend on the presence of mother queen. However, in large colonies with more than 100 nests, it is always difficult to locate the mother queen nest because it is often hidden in less accessible sites (Vanderplank, 1960). Mortality of fertilized weaver ant's queen is higher during initial time of colony establishment. Also the time taken for the colonies to be ready for use to a new field is very long, it takes about 18 to 24 month to mature. Among the perceived causes for mortalities of weaver ants include unsuccessful mating, poor rearing conditions, limited food supply and harsh environment like heavy rains and irrational spray of pesticides. Only limited research has so far been conducted to alleviate these problems. Peng and Christian, (2005) suggested that modification of the environment to promote fast breeding and elimination of competing ants would largely promote a quick build up in population of Asian weaver ant species *O. smargidna.* However little is known about the African weaver ant species *O. longinoda*. Reliance on colony collection from the wilderness for introduction may be uncertain. This study intended to overcome this uncertainty through artificial rearing of colonies from newly mated queens.

Little is known about the mating season, mating success of the queens, as well as distribution of different growth stages of *O. longinoda.* Environmental /weather parameters that trigger mating flights for *O. longinoda* are also not known. Knowledge on weaver ants behaviour would be useful in mapping population dynamics of weaver ants, identification of period of appearance of sexuals in the colonies (in order to set traps for collecting mated queens), identification of best methods of collecting and rearing newly mated queens for ant nursery establishment in order to reduce mortality of fertilized queens, having colonies with mother queen will ultimately lead to sustainable pest management in cashew, mangoes and other fruit crops.

1.6 Objectives

1.6.1 Overall objective

To develop methods for collecting and rearing fertilized queens in order to boost weaver ants populations in the field.

1.6.2 Specific objectives

i. To define the population structure of weaver ants across seasons in bimodal rainfall zone of Tanzania.
ii. To establish techniques for obtaining fertilized ant queens through collections
iii. To determine suitable rearing conditions for fast perpetuation of weaver ant colonies in nurseries;

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CHAPTER TWO

Occurrence of Sexuals of African Weaver ant (*Oecophylla longinoda* Latreille) (Hymenoptera: Formicidae) under a bimodal Rainfall Pattern in Eastern Tanzania

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Appearance of sexuals in African weaver ants

Abstract

The African weaver ant, *Oecophylla longinoda,* is being utilized as a biocontrol agent and may also be targeted for future protein production. Rearing of mated queens in nurseries for colony production is needed to cater for such demands. Thus, newly mated queens must be collected for use as seed stocks in the nurseries. To collect mated queens efficiently it is important to identify when sexuals occur in mature colonies. We studied the occurrence of sexuals in *O. longinoda* colonies for two years in Tanga, Tanzania, a region characterized by a bimodal rainfall pattern. We found that *O. longinoda* sexuals occurred almost throughout the year with abundance peaks from January to April. Production of sexuals appeared to be triggered by rainfall, suggesting that populations in areas with long rainy periods may show prolonged mating periods compared to populations experiencing extended dry periods. The bimodal rain pattern may thus cause a low production of queens over a long period. The average yearly production of queens per tree and per colony was estimated to be 449 and 2753, respectively. The average number of queens per nest was 17. Worker abundance declined from January to March with minimum by the end of this period, being inversely proportional to the production of sexuals. In conclusion, mated queens may be collected almost throughout the year, but most efficiently by the onset of the long rainy season when the majority disperse.

Key words: *Oecophylla longinoda*, sexuals, bimodal rainfall, dispersal flights, Tanzania.

2.0 Introduction

Weaver ants (*Oecophylla* spp.) are highly territorial and aggressive, with a diet consisting of other small animals, honeydew and nectar (Hölldobler, 1983; Peng *et al*., 1999). Hence they are used as natural enemies against destructive crop pests such as cashew-sucking pests and fruit flies (Way & Khoo, 1992; Peng *et al*., 1999; Van Mel *et al.,* 2007). Weaver ants (*Oecophylla spp*) have been known for their utility as pest controllers in orchards for more than seventeen hundred years, although the weaver ant Oecophylla is the first written record of biological control, dating from 304 A.D. (Himender Bharti, 2011). Recent research in Benin showed that abundant *O. longinoda* populations on mango can drastically reduce damage from fruit flies (Van Mele and Vayssieres, 2007). Similar research findings from east Africa suggest that *O. longinoda* imparts significant reduction in damages caused by cashew-sucking pests (Stathers, 1995; Olotu *et al.*, 2013). Additionally, *Oecophylla* spp. are a human food source in many countries in tropical Asia, Australia, Congo and Cameroon (De Foliart, 2008 and references therein as cited by Sribandit *et al*., 2008; Offenberg, 2011; FAO report, 2013; Van Itterbeeck *et al.,* 2014). In addition larvae, pupae and adult stages of both the worker and the reproductive castes are sold as food but queen brood (larvae, pupae and virgin queen) are the most expensive (Sribandit *et al*., 2008). Due to this dual utilization of *Oecophylla* spp., there is an increasing demand for developing weaver ant management methodologies.

One of the challenges of utilizing *Oecophylla* for biocontrol is obtaining enough colonies for large-scale transplanting into plantations (Ouagoussounon *et al.,* 2013), and this is also important for harvesting adequate amounts of queen larvae for human consumption. Therefore, there is a need for developing rearing methods. A key issue in this respect is knowledge of the phenology of sexuals as it is imperative to collect queens immediately after their nuptial flight, because newly mated queens suffer large mortalities during the founding stage (Offenberg *et al*., 2012).

Some important details regarding *Oecophylla* reproduction have already been elucidated. Wild *Oecophylla* colonies produce thousands of new winged virgin queens each year that individually leave their natal colony, mate and start new colonies (Vanderplank, 1960; Hölldobler and Wilson, 1990; Van Itterbeeck *et al.,* 2014). The investment in sexuals increases with colony productivity, but the investment in reproductive females is proportionately much larger than the investment in males (Hasegwa, 2013).

Way (1954) found the release of winged sexuals of *O. longinoda* in Zanzibar coincided with the beginning of prolonged periods of high humidity and frequent rainfall. Mated queens were commonly found on vegetation from February to March and from May to October. Nuptial flights were observed in Zanzibar after heavy rains in November and March(Vanderplank 1960). Sexual production and timing of mating flights is similarly linked to rainfall and restricted to limited periods of the year for *Oecophylla* spp. (Vanderplank, 1960; Peeters and Andersen 1989; Peng *et al.,* 1999; Van Mele and Vayssieres, 2007; Sribandit *et al*., 2008).

Here we have determined when *O. longinoda* colonies produce sexuals under a bimodal rainy season in Eastern Tanzania, and estimated the yearly production of new queens.

2.1 Materials and Methods

The study was conducted in citrus orchards in the Muheza District, Tanga Region in eastern Tanzania (S 06o47’12.3”, E 37o39’01.7”, altitude 501 m). The rainfall in this region is bimodal, with a long rainy season from mid-March to May and a short rainy season from November to December with averages of 1,200 mm and 650 mm, respectively. Thirty-five *O. longinoda* colonies were mapped in two adjacent citrus orchards that were similar with respect to cultivation, tree age and size. To optimize the conditions for *O. longinoda*, and to mimic their management as part of a biocontrol program, host trees belonging to the same colony were connected by ropes. This facilitated the movement of ants within colonies and discouraged workers from moving between trees on the ground. The latter may expose *O. longinoda* workers to attacks from *Pheidole megacephala*, which was present in some parts of the study area. *P. megacephala* is a highly aggressive invasive ant species that is considered to be the most efficient and widely distributed enemies of *O. longinoda* (Perfecto and Castineiras, 1998). We applied management actions to reduce the *P. megacephala* populations largely following recommendations by Seguni *et al*. (2011), and the *O. longinoda* colonies were provided supplementary food twice a month during the dry season consisting of grounded fish and a 25% sucrose solution. The amount of fish and sucrose was not measured as the effect of feeding was not a topic of this investigation.

Twenty colonies from one of the citrus orchards were assigned to a nest sampling program and the remaining 15 colonies from the other orchards served as controls on the impact of removing nests. *O. longinoda* nests are assemblages of leaves woven together by major workers using silk produced by mature larvae. To determine the periods when sexuals were produced, one nest was sampled randomly from a randomly chosen colony every two weeks for two years from October 2011 to September 2012 (hereafter referred to as the first year) and October 2012 to September 2013 (hereafter referred to as the second year). Nest sampling was a two-step process, with the first step being an inspection of the randomly selected nest for the presence of brood, workers and the egg-laying queen. If the egg-laying queen was found in the nest, it was returned back to respective colony. If there were both brood and workers present the second step was placing it in a plastic bag and storing it in a freezer. The ants were then sorted into queens, males, workers, and immature forms. The latter were further sorted according to their developmental stage. The caste of eggs and 1^{st} instar larvae could not be determined but were assumed to be workers. The numbers of each caste and developmental stages were counted and the wet biomass weighed on a four digit weighing balance (0.0001g).

Artificial queen nests (rolled leaves fixed with a rubber band) were created on ant-free trees to monitor mating flights. After a mating flight founding queens settle in such nests (Peng *et al.,* 2013), thus the presence of queens in the artificial nests indicated that a mating flight had occurred. The artificial nests were inspected by looking inside each nest every two weeks as well as each dry day following rain.

To assess the impact of nest harvesting on colony size, the number of nests in each colony was counted every six weeks. Daily rainfall data were obtained from Mlingano Agricultural Meteorological Station located at Muheza, approximately 7 km from the study area.

2.1.1 Data analysis

Repeated measures MANOVA was used to test the effect of nest harvesting on colony size by comparing the number of nests per colony between the sampled colonies and control colonies. Data were log transformed to obtain normal distribution and variance homogeneity. All analyses were performed with JMP version 10a statistical Discovery, SAS Institute.

2.2 Results

2.2.1 The effect of nest harvesting on colonies production

The harvesting rate of two nests per colony per month did not affect the number of nests per colony (treatment effect, $F_{1.31} = 1.87$, $P = 0.8$; time effect, $F_{11.21} = 8.96$, $P < 0.0001$, treatment x time $F_{11.21} = 1.18$, $P = 0.36$) (Fig. 2:1).

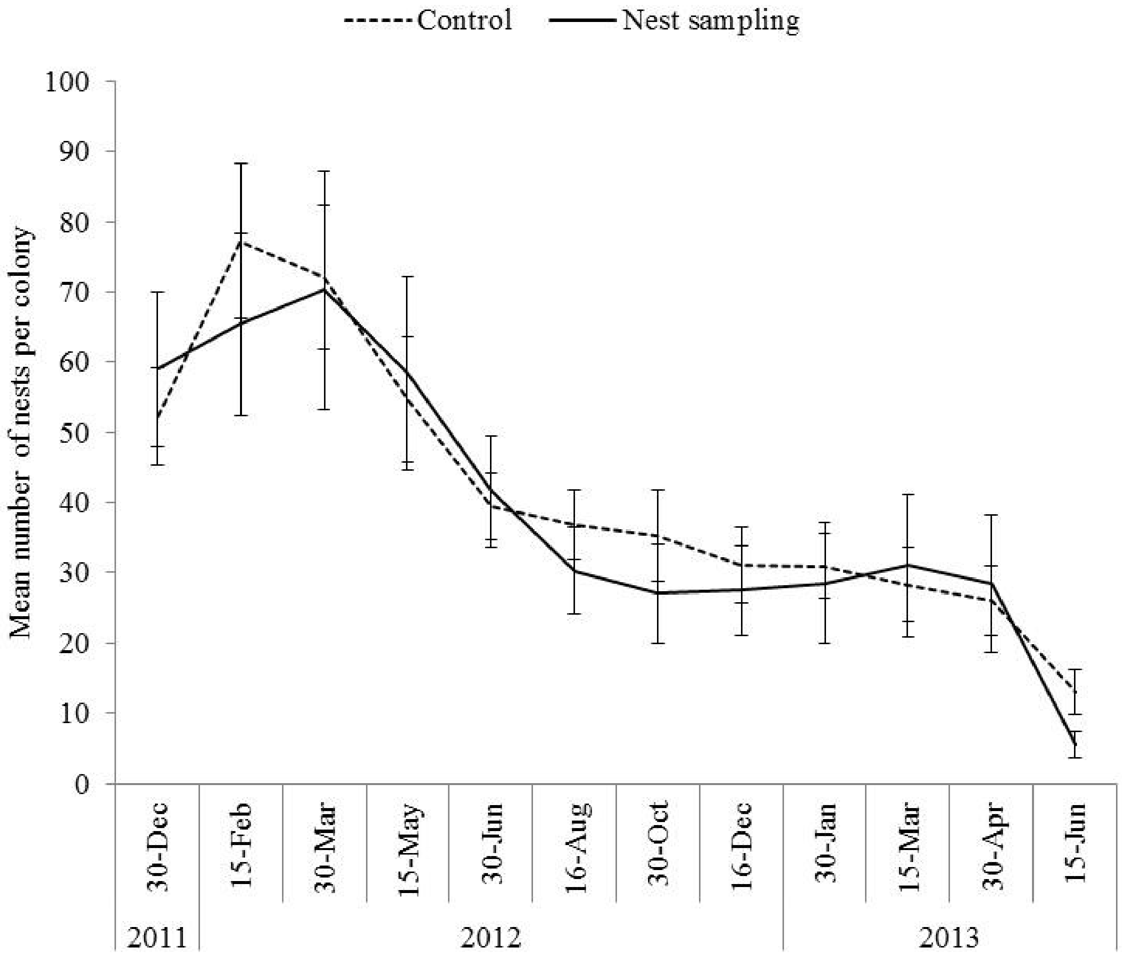

Figure 2: 1: Number (± SE) of nests per colony of *O. longinoda* throughout the sampling period

2.4.2 Presence of sexuals

The presence of larvae, pupae and adults of sexuals varied among years, being far more common in the first than the second year. Adult males or queens were found in at least one of the two years (Fig. 2:2). Sexuals were produced throughout most of the year but were not present for short periods (mid-December 2012 and August/September, 2013).

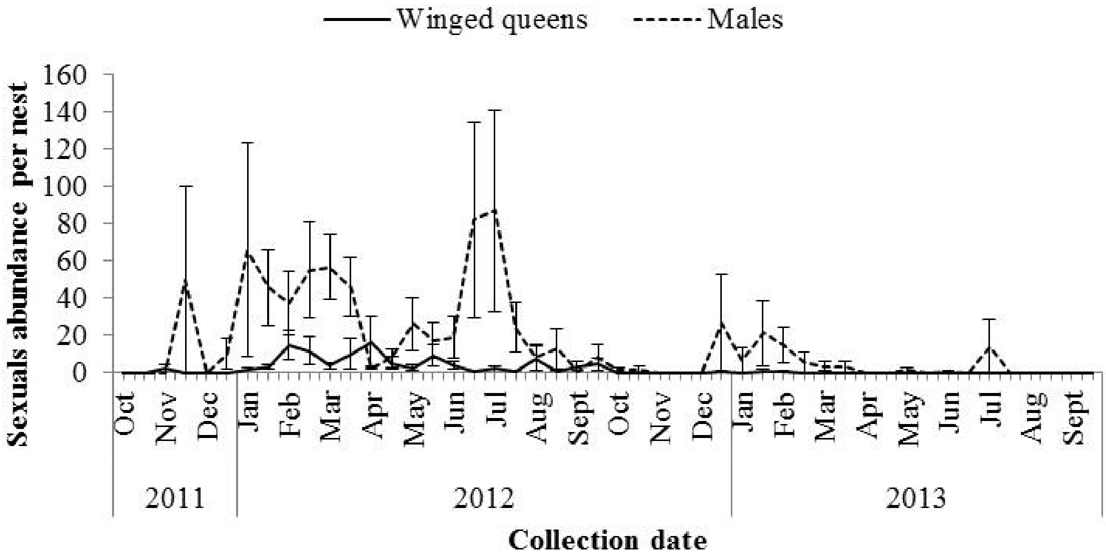

Figure 2: 2: The mean abundance (±SE) of winged queens and males of *O. longinoda* per nest

The largest proportions of sexuals were recorded between January and April during both years (Fig. 2:3). A lower proportion of sexuals was recorded the second year compared to the first year. Production of immature sexuals occurred between November and December, which coincided with relatively high rainfall. During the dry months of January and February, the proportion of sexuals continued to increase until the onset of rains in March (Fig.2:3). At this time the proportion of the wet biomass of sexuals decreased, suggesting that dispersal flights had commenced. Similarly during the dry period of June/July 2012, there was a second smaller build-up of sexuals that declined at the onset of rain in August.

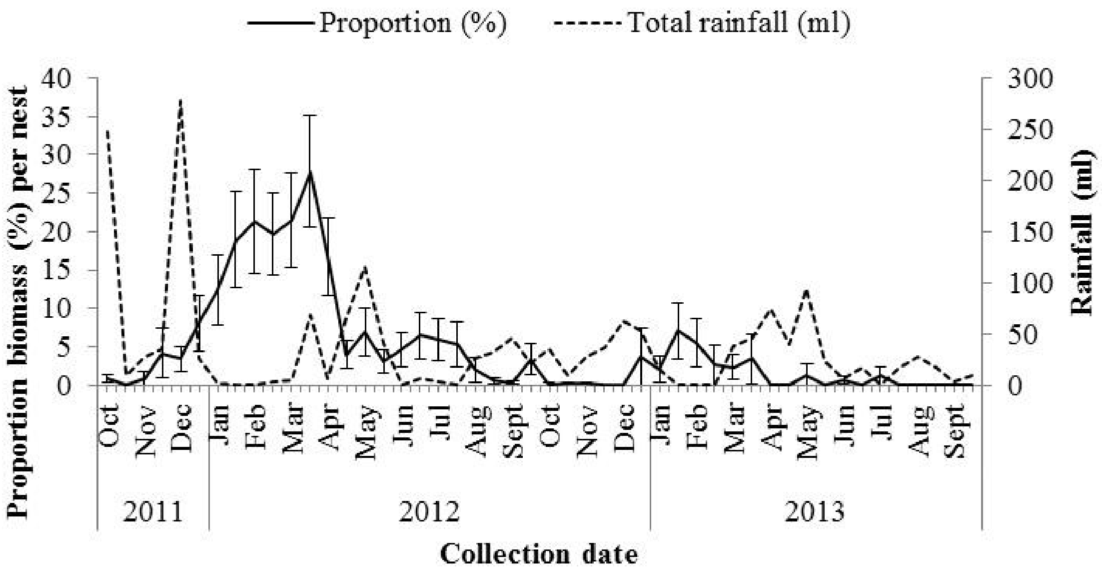

Figure 2.3: The mean proportion (±SE) of wet biomass contributed by *O. longinoda* sexuals per nest, and rainfall in Tanga, Tanzania. Rainfall is given as the sum of the daily rainfall totals between each sampling date

The presence of queens in artificial nests indicated six dispersal flights were recorded on both years. These occurred from 29th March to 30th June in 2012 and from 2nd April to 30th August in 2013. The sexuals (all stages) from January to March accounted for 25% of the total biomass found in the nests in the first year and less than 10% the second year.

Numerically, males were more abundant than queens (Fig. 2:2) (6:1 males to queens, in the first year and 37:1 in the second year). In the second year new queens were only recorded three times with an average of less than one queen per nest. The average number of males per nest was 87 (± 54) whereas the average number of queens was 17 (± 6). In 2012, there were several peaks in the production of both males and queens. Peaks of males and queens were out of phase with the time between peaks being approximately one month, with new batches being laid only after the prior batch had matured and dispersed. Therefore, the number of queens produced per year equals the sum of the queens observed during the peaks. From January to October 2012 there were six peaks averaging 14.7, 16.7, 9.2, 2, 7.3 and 4.8 queens per nest in each peak, giving an annual average of 54.4 queens per nest. In the same period, the mean number of nests per ant-occupied tree was 8.3 (± 0.3) and the mean number of nests per colony was 50.6 (± 3.1). Thus, an average ant-occupied tree and ant colony produced 449 and 2753 queens respectively. The average number of trees per colony ranged from 5 (± 2) to 21 (± 9).

2.4.3 Dynamics of workers abundance

Workers abundance fluctuated seasonally, declining from January to March, when it reached an annual low (Fig.2:4) and was inversely proportional to the sexual biomass (Fig. 2:3). Worker abundance was greater the first year than the second year. The highest and lowest average number of workers per nest was 3,632 (± 1,041) in November 2011 and 581 (± 112) in March 2013.

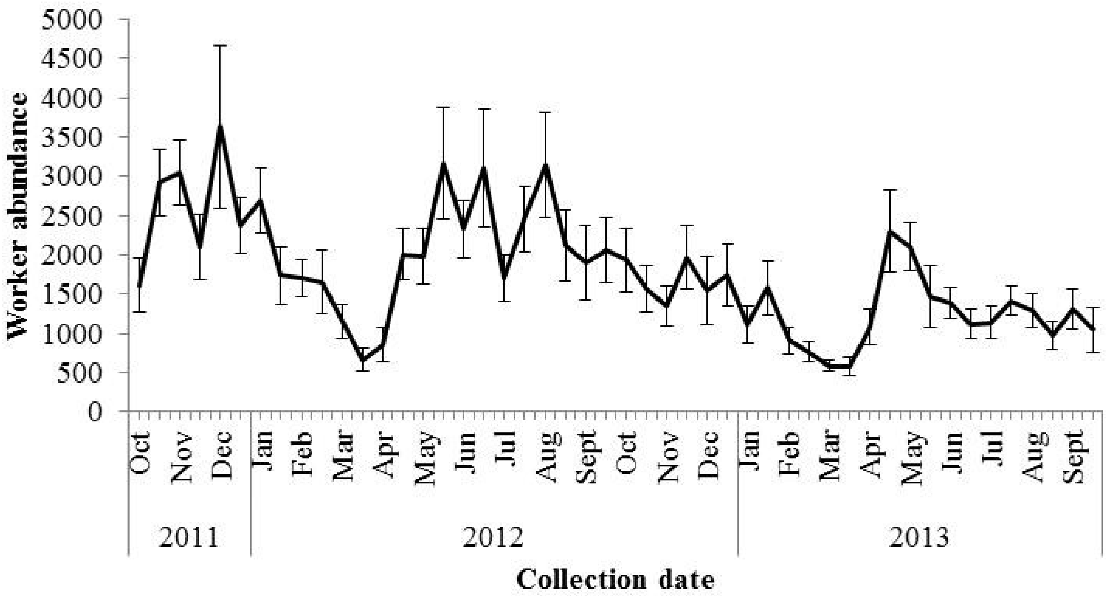

Figure 2.4: The mean abundance (±SE) of *O. longinoda* workers per nest

2.5 Discussion

2.5.1 The effect of nest harvesting

We examined the population structure and dynamics of *O. longinoda* colonies to quantify the production and dispersal of sexuals. The destructive sampling of nests had no effect on the number of nests per colony, which indicates that colony size would not be affected by nest harvesting. However, there was an approximately six-fold decrease in number of nests of both groups from the beginning to the end of the study. This decline was probably caused by invasions of *P. megacephala*, a major enemy of *O. longinoda* (Seguni *et al.,* 2011), which invaded the study area in the second year. This population decline may in turn have resulted in the highly skewed sex ratio bias in the second year, where almost all sexuals produced were males. It is well known that smaller ant colonies invest mainly in males, rather than the larger, more costly queens (Hölldobler and Wilson 1990; Hasegwa 2013). This suggests that only the first year of data reflects the production of sexuals in healthy, well performing colonies. It also highlights that *Oecophylla* colonies (maintained for the harvest of founding queens) need protection from *P. megacephala.*

2.5.2 Presence of sexuals

We identified that the production of sexuals takes place almost throughout the year, which suggests that mated queens may be collected from nests most of the year. If the regular fluctuations of the abundance of sexuals throughout the year reflect dispersal flights, the collection of founding queens via artificial nests may also be possible most of the year, but collection efforts may show higher returns from February to April when the abundance of queens peak in the nests.

Sexuals were found in the nests for much longer periods in this study than have been reported for *Oecophylla* spp. elsewhere. The winged individuals of *O. longinoda* have been shown to emerge from their nest in November and in March in Zanzibar (Vanderplank, 1960). Van Mele and Vayssieres (2007) reported that queens of *O. smaragdina* were found from July to October in the Mekong Delta of Vietnam while in the Northern Territory of Australia queens were found in March (Peeters and Andersen, 1989). However, these studies monitored mating flights and not the presence of sexuals inside nests. Sribandit *et al*. (2008) reported that queens are harvested from nests for a period of 4-5 months in Thailand. In Darwin, Australia queens can be found in nests for only a few months during the rainy season (M. G. Nielsen, R. Peng and J. Offenberg, unpublished data).

The abundance of sexuals in nests would be expected to decline simultaneously if such a decline was caused by dispersal flights. It is unclear why male abundance peaks occurred when queen abundance was low as a temporal asynchrony would make it difficult for males and queens to locate each other for mating. One possibility is that males first fly to other colonies where they are adopted into nests containing queens, and mating subsequently takes place in the nest (Vanderplank 1960). The already mated queens would then leave their nest on a “founding” flight, rather than on a mating flight. However, ongoing observations on mating flights (N. Halfan, G. Rwegasira, M. Mwatawala & J. Offenberg, unpublished data) do not support Vanderplank’s hypothesis.

The decline in the number of workers reconciled with a simultaneous increase in the proportion of sexuals. It appears worker production stops during the periods when sexuals are produced and is initiated again once the sexuals disperse from the nest. Similar results have been found with *O. smaragdina* in Darwin Australia where the wet biomass of the nests content was rather constant, but the ratio of sexuals to workers fluctuated seasonally (Christian Stidsen, unpublished data).

Rainfall appeared to play an important role in *O. longinoda* mating, because we found it to be very closely related with both the production of sexuals and the timing of dispersal flights. Production of sexuals commenced at the start of the rainy season (October/November) after the long dry season. This is supported by (Vanderplank, 1960; Peeters and Andersen 1989; Peng *et al.,* 1999; Van Mele and Vayssieres, 2007; Sribandit *et al*., 2008) who also found that the production of sexuals and the timing of mating flights to be restricted to limited periods of the year for *Oecophylla* spp. Approximately one month after the rains began, the first adult sexuals emerged. One month is approximately the time taken for *Oecophylla* to develop from egg to adult (Vanderplank 1960).

In Asia, *O. smaragdina* larvae are collected as a protein source for human consumption or as animal feed (Offenberg 2011, FAO report, 2013). In Thailand it is mainly the virgin queens that are targeted for harvesting (Sribandit *et al*., 2008). Therefore the protein harvest is compatible with the use of *Oecophylla* for biocontrol efforts, because only the workers control pest insects (Offenberg and Wiwatwitaya, 2010). Our study has shown that a similar practice does not seem feasible in Tanga as the number of queens in the nests was very low. We found an average of fewer than 17 queens per nest, whereas nests in Thailand and Australia contain hundreds of queens (J. Offenberg, unpublished data). In an extreme case in Thailand, 1.2 kg of queens was found in a single nest (J. Offenberg, unpublished data). However, the harvest rate of two nests per colony per month was not detrimental to the colonies. Thus, a limited amount of ant protein may be harvested sustainably.

2.6 Acknowledgements

This study was funded by DANIDA through project DFC No. 10-025 at Sokoine University of Agriculture. We are grateful to Semngano village leaders and farmers for allowing us to conduct our experiment in their village. Thanks to the village agricultural extension officer Mr. Jimmy Mhina for guiding and supervising the farmers. Thanks to John Kusolwa for his assistance in the field work and Miss Saverina Tiba for assisting in the Laboratory work. Lastly our sincere gratitude to Dr. Renkang Peng for his technical advice during this study especially on mapping weaver ants colony and Lars Bjørnsbo of ECO style A/S for providing spinosad.

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CHAPTER THREE

Comparing different methods for trapping mated queens of weaver ants (*Oecophylla longinoda* Latreille) (Hymenoptera: Formicidae)

This work was supported by [DANIDA] under grant [Project 10-025 AU]

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Trapping of mated queens of African weaver ants

Abstract

The predatory efficiency of African weaver ants *Oecophylla longinoda* and their utilization in protein production is a function of ant abundance. Reliable control of insect pests in tropical crops is achieved when ant populations are constantly high. Transplanted populations of weaver ant colonies containing egg-laying queens are more stable than those without. Achieving such stability through collection of colonies established in the wild is usually difficult because of uncertainty in locating the nest containing the egg-laying queen. In this paper, we investigated four methods that may be used to collect mated queens that subsequently can be used to stock ant nurseries. The catch efficiencies of (i) leaf traps, (ii) paper traps (both types providing a refuge for founding queens), (iii) random search for queens, and (iv) light trapping, were compared. Light trapping was the most efficient method to collect queens followed by leaf traps, random search and, lastly, paper traps. Light trapping and random search, though, required the presence of a person throughout the ant's mating season (several months), whereas this was not required when using leaf and paper traps.

Key words: *Oecophylla longinoda*, trapping, mated queen.

3.0 INTRODUCTION

Weaver ants (*Oecophyla* spp. Latreille) live on trees and shrubs, where they nest in fresh plant leaves (Crozier, Newey, Schluns and Robson, 2009). Being generalist predators, *Oecophylla* spp. can significantly reduce the abundance of several insect pests of tropical crops (Adandonon, 2009; Peng, Christian and Gibb 1995, Peng and Christian 2004, Peng, Christian and Reilly, 2011; Way and Khoo 1989). Furthermore, *Oecophylla* ants are targeted as mini livestock for sustainable protein production, as they are used as food by humans and animals (Ce´sard, 2004; FAO report, 2013; Van Itterbeeck, Sivongxay, Praxaysombath and Van Huis, 2014; Offenberg 2011; Sribandit, Wiwatwitaya, Suksard and Offenberg, 2008). In both cases, a stable population of weaver ants is needed, and therefore augmentation and introduction are often practiced where weaver ants exist in low numbers or are completely absent. Transplanted colonies containing an egg laying queen are more stable than those without queens (Renkang Peng, person communication, August, 2011). Hence, identification of the nest harbouring egg-laying queen is usually an important component of augmentation or introductions to new areas. Each colony has one egg laying queen, but may consist of several hundred nests and cover a territory of up to one hectare (Crozier *et al.,* 2009). This makes it difficult to locate nests with a mother queen.

Established weaver ant colonies produce thousands of winged queens and males each year, which during the rainy season leave their colonies for mating (Peng, Mogens, Offenberg and Birkmose 2013; Vanderplank 1960; Peeters and Andersen, 1989; Van Mele and Vayssieres, 2007). According to Vanderplank (1960), the newly mated queens alight on plants, shed their wings and seek a suitable refuge and establish their colony. This can be on folded leaves or overlapping leaves of ant free trees (Peng *et al.,* 2013). Due to the difficulties in obtaining refuge, more than 99% of queens die within the first six months of initial colony establishment (Vanderplank, 1960). Consequently, developing efficient trapping techniques for mated queens and protecting them during colony establishment is important for practical use of *Oecophylla* spp. in biocontrol, and the captured mated queens could eventually be reared in nurseries in screen houses before being used in the field for pest management or protein production for human consumption.

Several insect trapping methods exist, some of which are applicable to mated weaver ant queens. For example, mated queens of *O. smaragdina* can be collected from artificial nests made of leaves (Peng *et al*., 2013). Sexuals of fire ants (*Solenopsis invicta* Buren) can be trapped using an airplane equipped with two nets Marking, Diller, Hill, Blum and Hermann (1971) or nylon tulle netting (Gary, 2011). Queens of *Curvispinosus* spp are known to seek light after mating flights that occurred the previous night, (Kennedy, 1948).

Due to the difficulty in locating egg-laying queens in existing colonies, as well as securing refuge for newly mated queens, the need for developing methods for the collection of mated queens of *O. longinoda* after nuptial flights is imperative. Trapped mated queens can be used to stock ant nurseries and to spare users from the hustle of collecting them in the wilderness, where the certainty of getting an egg-laying queen usually is meagre. In the current study, we tested different methods that may be used to trap mated queens of weaver ants, thereby reducing their mortality after their nuptial flights. Four methods inspired by preliminary observations of the settling behaviour displayed by *O. smaragdina* in Thailand (Joachim Offenberg; unpublished data) were tested; the use of (i) rolled leaves where queens can seek refuge, (ii) paper tubes where queens can seek refuge, (iii) random searching for queens during the mating season, and

(iv) light to attract queens after their nuptial flight. Here, we report on the effectiveness of the tested techniques.

3.1 Materials and Methods

The study was conducted in a citrus orchard in the Tanga region of eastern Tanzania (S 06^{o}47'12.3", E 37^{o}39'01.7", 501 m.a.s.l) from 2011 to 2013 and in a citrus plantation in the Morogoro region in eastern central Tanzania (S 06^{0}53'27.6", E 037^{0}36'44.5", 516 m.a.s.l) from 2012 to 2014. Both regions are under a bimodal rainfall patterns with a long rainy season from mid-March to May and a short rainy season from November to December. The average rainfall is 1200 mm and 650 mm for the two seasons, respectively, in the Tanga region, and 1800 mm and 600 mm for the two seasons in the Morogoro region.

3.1.1 Queen trapping in Tanga

In an area of approximately one hectare of citrus inhabited by 15 weaver ant colonies, 20 citrus trees without resident weaver ant colonies or other ant species were randomly identified within the plantation. These trees were protected against established ant colonies via application of a sticky barrier around the tree trunks in order to avoid ant predation on founding weaver ant queens. On these trees, three different queen trapping methods were tested: (i) leaf traps, (ii) paper traps, and (iii) Search and catch of queens via random search. Leaf traps were made by rolling up living leaves and tying the rolled leaf with a rubber band at the middle. This produced a leaf tube open in both ends, where queens could enter to seek shelter and protection against predators.

Paper traps were made of brown hard paper (Manila type) cut into small pieces (6cm x 5cm) and rolled to produce a tube of2 cm in diameter. The tube was covered with nylon adhesive tape to prevent it from rolling up and to protect it against rain. As with the leaf traps, both sides were left open to allow water to pass through and to allow queens to enter from two directions. Fifty traps of each type were mounted on every tree with ten traps in each of five directions. Thus, a total of 1000 traps of each type were used, with 100 traps per tree. In both years of the study, traps were mounted on trees in early January. After that, all traps were inspected to collect queens at the mid and at the end of each month from mid-January to the end of August and, additionally, during the dry season whenever intermittent showers occurred (as queens are known to disperse in association with rainy weather). During inspections, which took place between 8 and 11 am, all queens hiding in the traps were collected, and the type and direction of the occupied traps were registered. During each inspection of traps, each of the 20 trees was also searched randomly to find naturally settled queens and queens still searching for a nesting site, i.e. queens found outside the traps (hereafter called search and catch). The time used to prepare and inspect the traps and the time spent on search and catch were recorded and held against the number of queens collected via each method. Two months after the mounting of the traps, the fate of each trap (intact, damaged or fallen from the tree) was observed to determine their durability. At the same time, all lost or damaged nests were replaced by new ones, and traps occupied by other arthropods (often spiders) were cleaned.

3.1.2 Queen trapping in Morogoro

A similar experiment was conducted in a citrus plantation in Morogoro in an area of approximately one hectare inhabited by 20 weaver ant colonies. In this case, however, paper traps were replaced by light trapping, as the initial data from Tanga showed low efficiency of the paper traps (see results) and since it was observed that founding queens dispersed from their maternal colonies at sunset and were attracted to light sources

(Rozalia Rwegasira and Halfan Nene, unpublished data). Three trapping methods were compared: (i) leaf traps, (ii) light trapping and (iii) search and catch. Leaf trapping and search & catch were conducted as in Tanga, except that 100 leaf traps were mounted in each of 16 citrus trees, totalling 1600 leaf traps, and that traps were inspected once a week and on each day following a night where a nuptial flight was observed. Traps were mounted on trees in mid-November in both years. Light trapping was conducted by placing a 60 Watt light bulb near the middle of the plantation. The light was only switched on at sunset and only on nights where nuptial flights were observed (in 2012/2013 ten mating flight were observed while only three were recorded in 2013/2014). On those nights, all queens attracted by the light bulb were collected by hand between 7 and 9 pm. As the queens mate during their nuptial flight, which takes place at sunset shortly before the light trap was turned on, we determined whether the queens attracted to the light had mated before being trapped. To test this, 22 of the queens collected in this way were randomly selected and allowed to rear brood in a screen house. Queens that were able to produce viable eggs (developing into larvae and older brood stages) were considered mated, as eggs produced by unmated weaver ant queens do not hatch (Joachim Offenberg, unpublished data). As in Tanga, the number of queens caught by each method was divided by the time spent collecting them, to compare each method's efficiency.

3.1.3 Data analysis

At each site and for each season, the average number of queens collected per tree by each method (except light trapping) was calculated and compared with Wilcoxon/Kruskal Wallis tests performed in JMP version 10. Also, the number of intact nests per tree after the two month period was compared between leaf and paper nests with a Wilcoxon test. A G-test was performed to test if the queens collected in the traps showed any preference for cardinal direction. Based on the time expenditures described above, the average cost of obtaining one queen via the different methods was found by using a casual labour salary of 0.39 USD/h (=3.12 USD/day).

3.2 Results

3.2.1 Tanga

Mated queens were collected from the end of March throughout June in 2012, and from early April to the end of July in 2013 (Fig.3:1). Number of queens was collected during rain events in the dry season. Thirty-four and 27 queens were collected in Tanga in the first and second mating season, respectively. Of the 34 queens collected in first season, 73% were from leaf traps, 24 % from paper traps and 3% from search and catch. A similar trend was observed in the second season, where 80% came from leaf traps, 16% from paper traps, and only 4% from search and catch.

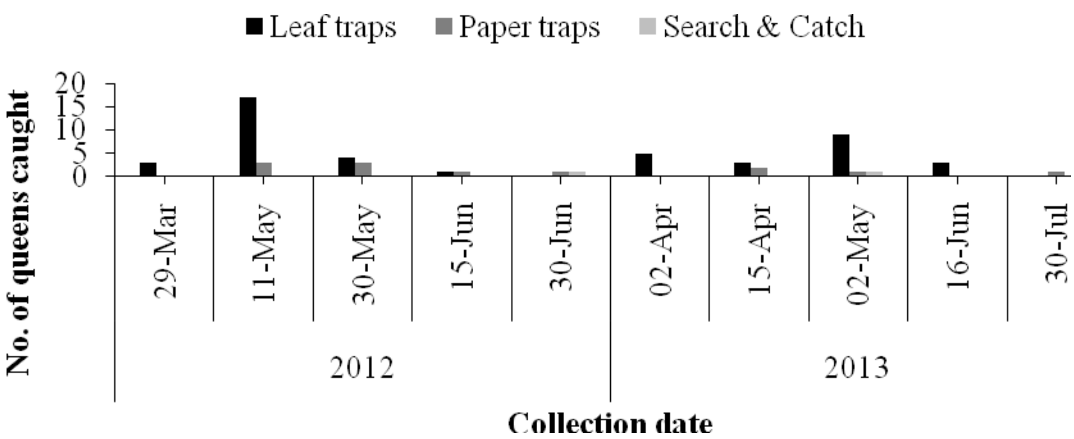


Figure 3.1: Number of queens caught by date using different techniques in Tanga during the ants mating season in 2012 and 2013. Only dates where at least one queen was collected are shown; no queens were found during the dry season (after rainfall events).

The highest numbers of queens collected on one day was 17 in the leaf traps, 3 by paper traps and 1 by search and catch (Fig.3:1). In 2012, the seasonal average number of queens (±SE) collected per tree was 1.2 (± 0.32) in the leaf traps, 0.35 (± 0.13) in the paper traps, and 0.1 (± 0.07) from the search & catch method. In 2013, the corresponding figures were 0.85 (± 0.33), 0.45 (± 0.20) and 0.05 (± 0.05). In 2012, averaged over the season, the leaf trap method caught significantly more queens per tree than the two other methods, that were not significantly different from each other, whereas, in 2013 the leaf and paper traps were statistically equally efficient, and significantly more efficient than the search and catch method (Table 3:1).

Table 3.1: Pair wise statistical comparisons of the seasonal trap efficiency (number of occupied traps per tree) of leaf and paper traps in Tanga

Trap types	P-Value 2012	P-Value 2013
Search & catch versus paper traps	0.12	0.21
Paper traps versus leaf traps	0.001	0.09
Search & catch versus leaf traps	<0.0001	0.009

The queens did not show any preference for the cardinal orientation of the traps they occupied ($G = 1.71.$, $P = 0.19$). Thus, it does not seem to be important on what side of a tree the traps are positioned.

After two months in the field, paper traps were more durable than the leaf traps ($P < 0.0001$, $n = 20$). On average, there were 47.2 (± 1.58) intact paper traps per tree, whereas there were only 29.0 (± 1.58) leaf traps. Thus, 94% of the paper traps were functioning after two months compared to only 58% of the leaf traps (Table 3: 2). The proportion of damaged traps was 0.6 and 13.2% for papers and leaf traps, respectively, while the corresponding proportions of traps fallen to the ground were 5 and 27%.

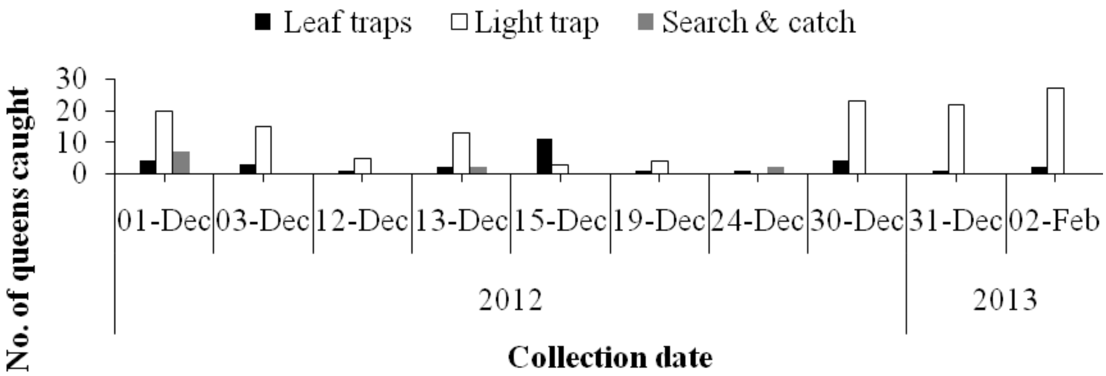
Table 3.2: The fate of traps used for trapping mated queen in Tanga two months after their mounting on trees. Intact traps were functioning, undamaged traps still mounted on trees. Damaged traps were non-functioning traps present on the trees and dropped traps were traps that had disappeared from the trees

Tree no.	**Intact traps**		**Damaged traps**		**Dropped traps**	
	Paper traps	Leaf traps	Paper traps	Leaf traps	Paper traps	Leaf traps
Average no. of traps per tree ± SE	47.20 ± 0.55	28.95 ± 2.83	0.3 ± 0.18	6.6 ±1.72	2.5 ± 0.57	13.45 ± 2.35
Percentage	94.4	57.9	0.6	13.2	5.0	26.9

3.2.2 Morogoro

In the first and second season, 173 and 236 queens were collected, respectively. Of the 173 queens collected in 2012/13, 48% were from light traps, 33% from leaf traps and 19% from search and catch (Fig. 3:2a). In the second season, 91% of the collected queens were caught from the light trap, 8% from leaf traps and 1% by search and catch (Fig.3:2b). The highest number of queens collected in one day was 161 by the use of light and only 11 by leaf traps and 7 by search and catch (Fig.3:2). Similar to the results from Tanga, more queens were collected per tree from leaf traps than by search and catch ($P = 0.016$, $n = 16$ and $P = 0.017$, $n = 16$, in the first and second season, respectively). The mean number of queens collected in leaf traps and via the search and catch method in the first season was 1.89 (± 0.62) and 0.69 (± 0.35), respectively. In the following season, the corresponding Fig. were 1.19 (± 0.0.39) and 0.19 (± 0.14).

A



B.

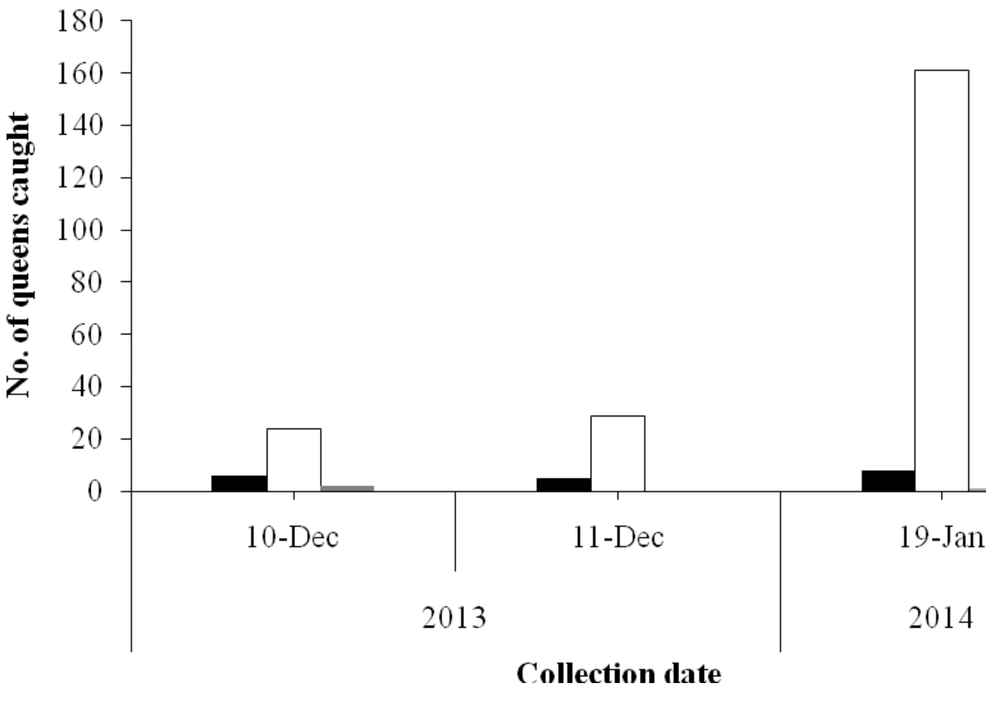


Figure 3: 2: **Number of queens caught by date using different trapping techniques in Morogoro during the ant's mating season in (A) 2012/13 and (B) 2013/14. Samplings were conducted weekly and whenever nuptial flights were observed (see methods). Only dates where at least one queen was collected was shown.**

Of the 22 queens tested for queen fertility, 68% laid eggs that developed into either imagines or pupae, whereas 18% laid eggs that did not hatch and 14% did not lay eggs at all. Thus, a minimum of 68% of the queens caught at the light trap had mated before being caught via this method.

3.2.3 Time/cost efficiency

Time expenditure and related costs for the collection of queens by the various methods are summarised in Table 3:2. Based on the overall average, it took almost 5 minutes (0.1 h) to collect a queen when using the light trap. Second most efficient were leaf nests, where an average investment of 48 minutes (0.8 h) was needed to collect a queen, followed by 120 minutes (2 h) investment when searching randomly for queens. The time needed to prepare the paper traps combined with a low catch rate led to a high time investment per collected queen, as, in this case, almost 420 minutes(7 h) were needed per caught queen. Thus, light was by far the most efficient method followed by the use of leaf nests.

Table 3.3: Time expenditures and costs of catching mated queens using different trapping techniques at the Tanga and Morogoro sites. A salary of 0.39 USD per h was used to convert time investments into costs

Location	Year	Trapping method	Total time spend (hrs.)	Number of queens caught	Average time spend per queen (hrs.)	Cost per queen (USD)
Tanga	2012	Leaf traps	25.2	25	1.0	0.4
		Paper traps	41.0	8	5.1	2.0
		Search and catch	10	1	10	3.9
	2013	Leaf traps	26.2	20	1.3	0.5
		Paper traps	42.3	4	10.6	4.1
		Search and catch	10	1	10	3.9
Morogoro	2013	Leaf traps	11	25	0.4	0.2
		Light trap	8	97	0.1	0.0
		Search and catch	12.8	11	1.3	0.5
	2014	Leaf traps	9.6	20	0.5	0.2
		Light trap	9	209	0.0	0.0
		Search and catch	1.4	4	0.9	0.4
Location	**Year**	**Trapping method**	**Total time spend (hrs.)**	**Number of queens caught**	**Average time spend per queen (hrs.)**	**Cost per queen (USD)**
Overall		Leaf traps	72.1	90	0.8	0.3
		Paper traps	83.3	12	6.9	2.7
		Search &catch	34.2	17	2.0	0.8
		Light trap	17.0	306	0.1	0.04

3.3 Discussion

3.3.1 Efficiency of trapping methods

In the Tanga experiment, leaf traps were the best method for trapping mated queens followed by paper traps, and the number of mated queens collected by the search &catch method was very low in both years. This confirmed Vanderplank's (1960) proposition that due to the difficulty in obtaining refuge, more than 99% of the mated queens of *Oecophylla* spp.die during the first six months of colony establishment. Shortage of refuge is one of the major mortality factors of mated queens. As they search for a safe refuge, *O. longinoda* may be attacked by birds, lizards, spiders and other species of ants, e.g. *P. megacephala*. Similar observations were reported by Peng *et al*. (2013) on *O. smaragdina*. Many mated queens of *O. smaragdina* were collected from artificial leaf nests at Darwin in Australia after nuptial flights (Peng *et al*., 2013) and, unlike previous experience with a search and catch method, the use of artificial leaf nests increased the numbers of collected mated queens by 97%. Likewise, in absence of artificial nests, the lives of mated queens were in danger.

The results from the Morogoro experiment indicated a great potential to increase survival of mated queens if light traps were used. Collection of mated queens using light has never been tested with *Oecophylla* species before, but has been reported for different species of ants, e.g. *Curvispinosus* spp Kennedy (1948). Since the mating flight of *O. longinoda* occurs during sunset, there is great potential for collecting mated queens using light traps. Such an approach may be less appropriate for *O. smaragdina,* whose flights occur at sunrise, though a smaller fraction of queens are still looking for shelter in the evening and are attracted to light (Joachim Offenberg; unpublished data). Our results further show that the majority of the queens attracted to the light had already mated before 7 pm, as almost 70% of the queens collected at the light trap were able to produce viable offspring. During the study, no queens were observed around the light on days without mating flights. Thus, the light trapping technique requires a continued presence of an ant queen collector during the entire mating season. Light traps were not only the best method for large scale collection of mated queens, but also the most time and cost efficient method. Light trapping is time efficient, as it requires almost no preparation compared to preparing and mounting the leaf and paper traps. One challenge with light trapping was that it required a collector to be present and ready to collect queens whenever mating flights occurred. In contrast, the artificial nests did not require permanent attention during the mating season, as the traps could be checked whenever it is was convenient. Thus, light traps can only be used in places that are reachable during the night time and where electric current is accessible, but in places far from an electricity source and at less reachable places the leaf trap method becomes the best alternative. Alternatively, both method, light and leaf trap, could be used in the same place to optimize collections.

The two rainy seasons in Tanzania can be considered an added advantage, as they increase the chances of collecting mated queens in a year. In Morogoro, the mated queens were collected during the short rainy season, while in Tanga they were collected during the long rainy season. Other studies (Rwegasira *et al*. 2014; Vanderplank, 1960; Peters and Andersen, 1989; Van Mele and Vayssieres, 2007; Peng *et al*. 2013) also support that the collection of newly mated *Oecophylla* queens is restricted to rainy seasons.

3.3.2 Trap persistence

Although the preparation of paper traps was time consuming, these traps lasted longer in the field. Paper traps did not easily fall off the trees because they were tied up with a nylon adhesive tape on small branches, while leaf traps fell off easily due to senescence. Thus, paper traps may be used in places where the mating season of weaver ants is not well known and more persistent traps therefore are desirable. In that case, high numbers of traps should be applied, as occupancy by other arthropods builds up over time, leaving fewer and fewer traps available for the ant queens. A higher trap density may also compensate for the relatively low catch rate of the paper traps. Conversely, leaf nests could be used in places where the mating flight season is well known.

3.3.3 Trap orientation preference

The cardinal orientation of traps on the trees did not affect their catch rate, suggesting that it is of no importance where traps are placed in trap-trees. Probably, queens are stressed to find safe shelter as fast as possible. Therefore, the first suitable shelter they find during their search will be accepted regardless of its orientation.

In conclusion, use of artificial traps increased the probability of catching mated queens collected. Light trap and leaf traps proved to be the most efficient methods of collecting mated queens because of less time investment. In Tanzania, the probability that a mated *O. longinoda* queen will find a safe refuge and subsequently survive to establish a new colony seems to be very low due to the presence of many enemies and predators, e.g. *P. megacephala*, birds, lizards, and *O. longinoda* workers from other colonies (Peng *et al*., 2013). Therefore, trapping and artificial rearing of mated queens will inevitably improve their survival. The use of light in collecting mated queens may be optimized by developing a light-embedded device or facility, which will allow the entry of mated queens but not their exit, thereby avoiding the need for the presence of a person at the time of collection. With less efficient, paper traps would make the best method of collection in places where mating flight is not well known, because of their longer persistence in the field.

3.4 Acknowledgement

This study was supported by DANIDA through the Project 10-025 AU. We are thankful to the personnel at Muheza District Agricultural Office in Tanga for their support in identifying research sites for our field activities. The Semngano village government and farmers are acknowledged for offering their fields for experimentation and assisting in taking care of traps and data collection. Mr. Jimmy Mhina of the Muheza extension department will always be remembered for his tireless support in supervising the day to day activities at Tanga study sites. Special thanks are to John Kusolwa for his tremendous support on data collection and assistance in field works.

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CHAPTER FOUR

Optimizing methods for rearing mated queens and establishing new colony of *Oecophylla longinoda,* Latreille (Hymenoptera: Formicidae)

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Methods for rearing mated Queens of *O. longinoda*

Abstract

Oecophylla spp are used as bio control agents for many types of insect pests. A large and stable population is essential for effective control of pests. Colonies of *Oecophylla* spp can be transplanted from wild habitats into orchards. Transplanted colonies can only survive in presence of mother queens. It is usually difficult to trace nests with mother queens in large colonies which may sometimes contain more than 100 nests. Therefore, the need to explore and develop methods for rearing newly mated queen in nurseries may not be overemphasized hence the current study. In the first experiment, we tested three rearing methods on queen survival and colony establishment. In the second experiment we compared different weaver ants feeding techniques on young colony growth. We observed that queens were best reared under continuous indirect access to water methods. First workers emerged earlier (32 days on average) in continuous indirect access to water and continuous direct access to water methods than on limited access to water (sprinkled) (38 days on average). Moreover, rearing mated queens under continuous indirect access and continuous direct access to water methods saved labour and time, because of limited attendance to the colonies. Availability of water, sugar solutions and different sources of protein throughout the time improved the growth of young colonies and the number of workers increased rapidly.

Key words; ant nursery; ant colonies; *Oecophylla longinoda;* queens; rearing methods

4.0 INTRODUCTION

Weaver ants (Hymenoptera: Formicidae) are social insects better known for forming remarkable nests on trees. Two species of weaver ants exist, the Asian weaver ants, *Oecophylla smaragdina* (Fabr.) and the African weaver ants, *O. longinoda* Latreille (Lokkers, 1986). The species share bio and ecological characteristics (Sribandit *et al.,* 2008). Both species have multiple benefits, for example they are used as bio control agents in tropical tree crops (Way and Khoo, 1992; Lim and Kirton, 2001; Van Mele, 2008; Peng and Christian 2005, 2010; Peng *et al*., 2004, 2010) source of income (Sribandit *et al.,* 2008), and food (FAO 2013,Offenberg*et al*., 2013, Sribandit *et al.,* 2008). The multiple benefits trigger great demand for *Oecophylla* spp. suggesting a need to sustain their populations.

More ants and nests of *Oecophylla* species are found during the rainy season (Van Mele and Cuc, 2007). Production of sexuals commences at the start of the rainy season (Vander plank, 1960; Peeters and Andersen, 1989; Peng *et al.,* 1999; Sribandit *et al*., 2008; Rwegasira *et al.,* 2014). The use of weaver ants in pest management involves collection and transfer of ant colonies into orchards. Establishment and growth of the introduced colonies depends on the presence of a mother queen. It is important, therefore, to locate the nest with the mother queen when sampling colonies. Some colonies are very large, extending to many trees with more than 100 nests, and it is always difficult to locate the nest with a mother queen, often hidden in less accessible sites (Vanderplank, 1960; Peng *et al.,* 1998). Furthermore, mortality rate of mated queens are usually very high (>99%) during the first six months of initial colony establishment (Vanderplank, 1960).

Peng and Christian (2005) suggested the modification of the environment to promote fast breeding and elimination of competing ants to promote a quick build up in population of

O. smaragdna. Colonies of *O. Smaragdina* are readily accepted artificial nesting sites in the form of plastic bottles, plastic cups, aluminum cans, test tubes and other types of transparent vials (Offenberg, 2014). There is usually one reigning queen in each colony (Greenslade, 1971, Hölldobler and Wilson, 1983, 1990). However, Peeters and Andersen, (1989) showed that during early colony establishment there may be multiple queens, but only single queens remain in mature colonies. The life cycle of an *O. smaragdina* colony starts with a mated queen finding a sheltered site for a first nest between leaves of a tree or shrub and laying a batch of about 35 eggs within 5 - 10 days after dealation / shedding her wings (Lokkers, 1990, cited by Crozier *et al*., 2010). Larvae of *O. smaragdina* emerging from about day eight, pupae follow after day 17 (Crozier *et al.,* 2010) and the first adult worker appeared after 28 days (Peng *et al.,* 1998; Crozier *et al*., 2010). In the vicinity of water (eg. a pond) queen brood production is sooner than elsewhere as queen brood believed to drink a lot of water to become large (Van Itterbeeck *et al*., 2014). Mated queens can also be reared in match boxes (Vanderplank, 1960) or in a cylindrical transparent plastic vial with a mango leaf (Offernberg *et al.,* 2012 and Ouagoussounon *et al.,* 2013) or in clove leaves, and they do not eat until the first workers appear (Way, 1954), rather they are provided with a few drops of pure water every day to allow the queens to drink (Peng *et al*., 2013).. Studies on rearing of *O. longinoda* are limited (Way, 1954; Vanderplank, 1960). Recent studies on *O. smaragdina* and *O. longinoda* showed that pupae transplantation on new mated queens before the emergency of first workers led to increase in the developmental stages of intrinsic brood (Ouagoussounon *et al.,* 2013; Peng *et al.,* 2013).

The occurrence of antagonistic ant species, poor rearing conditions, harsh environment like heavy rains, and irrational spray of pesticides are major causes of weaver ant mortality *in-situ* particularly in orchards and natural environments. Rearing colonies in the screen houses/laboratories could reduce the mortality rate during the initial phase of colony establishment. Mortality can also be reduced if antagonistic species of ants are prevented from foraging on the young *Oecophylla* colonies (Vanderplank, 1960).

Collecting colonies from the wilderness is laborious, time consuming and full of uncertainty. In this study we intended to overcome these problems by developing methods for rearing newly mated queens. We tested three rearing methods of mated queens, on continuous direct access to water, on continuous indirect access to water and limited access to water (sprinkled). We also tested the effect of frequency of feeding on the growth of weaver ant colonies.

4.1 Materials and Methods

Mated queens of *O. longinoda* were collected from citrus orchards in Tanga (6^{o} 49'S; 37^{o} 40'E) and in Morogoro Urban (5^{o} 18'S; 38^{o} 19'E) Tanzania, using leaf and light traps (Rwegasira *et al.,* 2015), in February, 2013 and January, 2014. The queens were transported to a screen house at the Crop Science Museum at Sokoine University of Agriculture (SUA).

4.1.1 Queen rearing

Oecophylla longinoda queens were reared under controlled environment in mesh-topped plastic vials (3 cm wide, 11.6 cm high) to test the effect of rearing methods on queens' survival and reproduction. Three methods were tested that included rearing queens in vials with (i) limited access to water, sprinkled once after every two days (ii) continuous direct access to 5 mls of water or (iii) continuous indirect access to 15 mls of water. In the latter method, ants were separated from water by a 10 cm thick cotton wool. Each rearing methods was replicated in 25 vials. A single mated queen was introduced into each vial.

After emergence of first workers each vial was opened and transferred into a plastic container (covered with wire mesh at the top). Young colonies were supplied with water, 20% sugar solution and fresh ground fish every two days. The temperature in the screen house ranged from 26.6^{0}C to 29.6^{0}C. We inspected vials at 10.00 am every day for 60 days and the numbers of workers emerged were counted after every 48 hours.

We recorded the number of surviving queens; time taken from nuptial flights to production of first egg(s), emergence of first larva (e), pupa (e) and workers. We also recorded numbers of individuals in each developmental stage 24 hours after first record; numbers of workers 24 hours on 60th day from nuptial flight.

4.1.2 Feeding techniques

The young colonies that were reared in vials were transferred onto the potted trees established in the screen house. A vial with a colony of five to ten workers was tied to a stem of a potted plant, 10 plants each of citrus, *Citrus sinensis* (L.) Osbeck, mango *Mangifera indica* L. and coffee *Coffea arabica* Linn plants were placed inside a basin with a shallow layer of water to restrict access by enemy ants. Ants were provided with water, sugar solution and ground fish (0.3 to 1.5 g) every two days during the first six months. Water and sugar solutions (20%) were soaked in a piece of cotton wool, while ground fish were placed on the leaves. After six months, each colony was provided with water and sugar (20%) solution *ad libitum* using Eppendorf tube. The colony was interchangeably supplied with either 5g of ground fish, 5 to 10 termites or 2 cockroaches after every two days. Data collected were, numbers of nests with workers, number of workers outside the nest after disturbances and numbers of empty nests.

4.1.3 Data analysis

Queen survival, total number of broods and developmental times were analyzed by using one way ANOVA Tukey- post hoc. Data were log transformed to obtain normal distribution and variance homogeneity. All other calculations were done using JMP version 11. We maintained unequal replicates due to mortality of queens during the experiment.

4.2 Results

4.2.1 Queen survived to produce workers

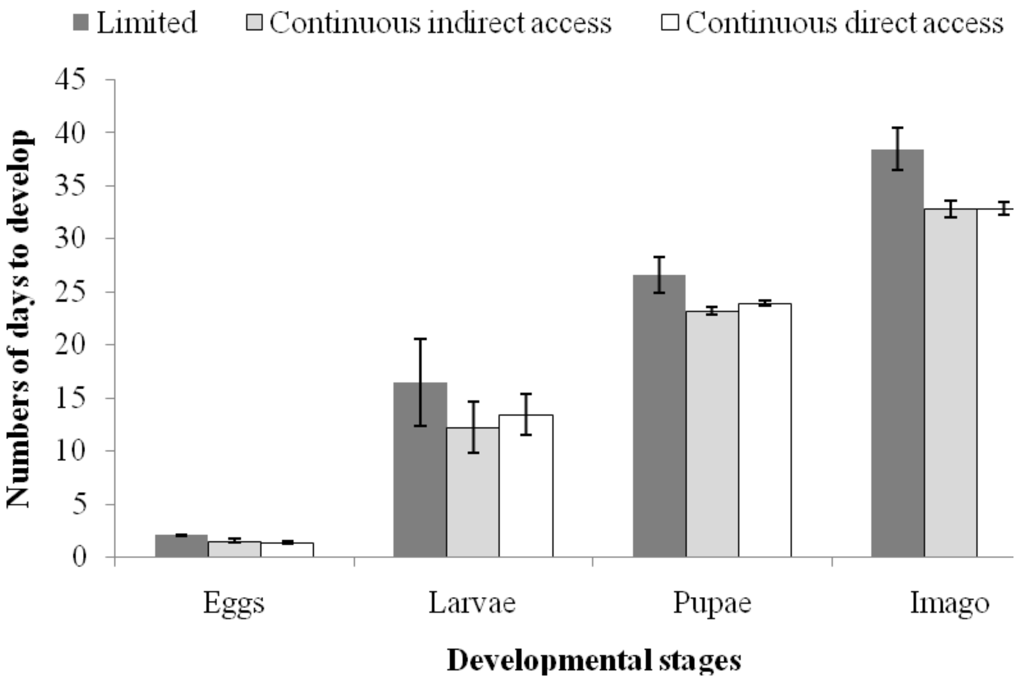
More than 50% of queens reared in each method produced workers. Thus, the queen survival was independent of all rearing methods used ($\chi 2 = 2.0$; df = 2, 48; $P = 0.36$). Despite of insignificant difference survival was lowest when queens were reared under continuous direct access to water (Table 4.1).

Table 4. 1: Queens who made up to production of first workers reared under different methods within 60 days from nuptial flight

Rearing method	Survive		Total
	No	Yes	
Limited access to water (sprinkled)	11 (44%)	14 (56%)	25 (100%)
Continuous indirect access to water	10 (40%)	15 (60%)	25 (100%)
Continuous direct access to water	12 (48%)	13 (52%)	25 (100%)
Total	33 (44%)	42 (56%)	75 (100%)

4.2.2 Developmental periods

All queens that produced workers did shed off their wings, except two. On average, queens shed off their wings a day from nuptial flights, while egg laying started after an average of two days. Rearing methods significantly affected time taken from nuptial flight to eggs production ($F = 5.2312$; df = 2, 62; $P = 0.0080$). Emergence of different stages of development was earlier on queens rearing under continuous indirect access and continuous direct access to water than queens reared under limited access to water (Figure 4.I).



Note: Methods refers access to water

Figure 4.1: Average number of days from mating flight to developmental stages using different rearing methods within 60 days of queens rearing

Our results further showed that rearing methods significantly affected number days from nuptial flights to emergence of first larvae ($F = 13.55$; $df = 2, 51$; $P = 0.0001$), first pupae ($F = 4.26$; df 2, 41; $P = 0.0208$), and first workers ($F = 7.125$; $df = 2, 39$; $P = 0.0023$). Significant differences in days to emergence of immature stages and workers were observed in all pairs except between continuous indirect access to water and continuous direct access to water methods (Table 4.2).

Table 4.2: Multiple comparisons by Tukey HSD on rearing methods on number of days from mating flight to developmental stages and number of workers produced within 60 days of queens rearing

Mean pair	Mean differences	SE of difference	Sig	95% CI Lower Bound	Upper Bound
Eggs					
Limited v Continuous direct access	0.67	0.22	0.0085*	0.147	1.185
Limited v Continuous indirect access	0.52	0.21	0.0393*	0.020	1.019
Continuous indirect v Continuous direct access	0.14	0.20	0.749	-0.338	0.632
Larvae					
Limited v Continuous direct access	0.292	0.056	<0.0001*	0.155	0.428
Limited v Continuous indirect access	0.192	0.058	0.0054*	0.050	0.334
Continuous indirect v Continuous direct access	0.099	0.056	0.190	-0.036	0.236
Pupae					
Limited v Continuous direct access	0.121	0.042	0.0183*	0.0178	0.224
Limited v Continuous indirect access	0.088	0.043	0.1227	-0.0185	0.195
Continuous indirect v Continuous direct access	0.033	0.042	0.720	-0.0705	0.1365
Imago					
Limited v Continuous direct access	0.148	0.044	0.0052*	0.040	0.256
Limited v Continuous indirect access	0.147	0.046	0.0077*	0.034	0.259
Continuous indirect v Continuous direct access	0.001	0.045	0.996	-0.109	0.111

Note: Methods refer to access to water

*The mean differences is significant at the 0.05 level

4.2.3 Developmental stages

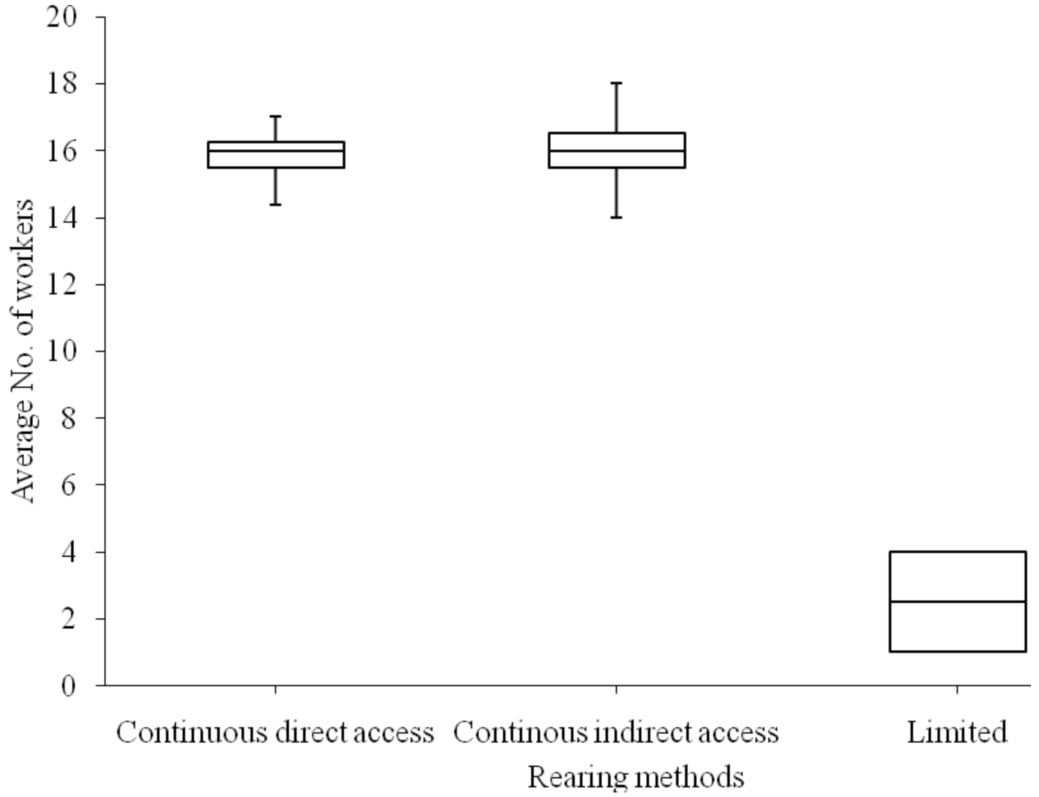
The numbers of brood produced decreased with advancement in developmental stages, from eggs to pupae under all rearing methods although the highest decrease was observed when queens were reared under limited access to water method (Table 4.3). Rearing methods significantly affected the total numbers of brood (F = 4.85; df = 2, 48; P = 0.012). Higher numbers of broods were recorded on continuous direct and indirect access to water rearing methods (Table 4.3).

Table 4.3: Average numbers of brood per queen observed on different rearing methods within 60 days after nuptial flight

Method	No. of eggs (SE)	No. of larvae (SE)	No. of pupae (SE)	No. of first workers (SE)
Limited access	10.56 (1.58)	2.92 (0.55)	1.36 (0.30)	1.2 (0.38)
Continuous indirect access	14.64 (1.14)	5.36 (0.96)	3.16 (0.63)	1.6 (0.30)
Continuous direct access	9.96 (1.32)	3.52 (0.57)	2.24 (0.50)	1.7 (0.45)

Note: Methods refer to access to water

After the emergence of first workers the numbers of workers continued to rise. Rearing methods significantly affected the average number of workers per queens observed on day 60 from nuptial flight (F= 92.3548; df = 2, 6; P = 0.0001). Number of workers was high on continuous indirect access and continuous direct access to water. (Figure 4.2)



Note: Methods refers to access to water

Figure 4.2: Number of workers per queen observed on day 60 from nuptial flights reared on different methods

Post hoc Tukey HSD showed differences in total number of broods were observed on all pairs except between continuous direct access and limited access to water on total numbers of broods.

Table 4.4 : Multiple comparisons by Tukey HSD on rearing methods on total number of broods and number of workers observed within 60 days of queens rearing

Mean pair	Means Differences	SE of difference	Sign	95% CI Lower Bound	Upper Bound
Total numbers of broods					
Continuous indirect access v Limited	8.32	2.93	0.017*	1.23	15.41
Continuous indirect v Continuous direct access	7.44	2.93	0.037*	0.34	14.53
Continuous direct access v Limited	0.88	2.93	0.951	-6.21	7.97
Number of workers					
Continuous indirect access v Limited	13.50	1.13	<0.0001*	10.1	16.9
Continuous direct v Limited	13.25	1.13	<0.0001*	9.76	1.73
Continuous direct access v Continuous indirect	0.25	1.13	0.97	-3.23	3.73

Note: Methods refer to access to water
*The mean differences is significant at the 0.05 level

Also significant differences in number of workers were observed on all pairs except between continuous indirect access and continuous direct access to water on day 60 from nuptial flight (Table 4.4).

4.2.4 Feeding techniques

Newly formed colonies were raised in the screen house for one year. They were raised on small potted trees and fed on protein, sugar solution and water. During that time the numbers of workers observed to forage outside the nest were recorded. We observed that the number of workers found outside the nest after disturbance increased with time on all plants species used as hosts in the screen house (Figure 4.3).

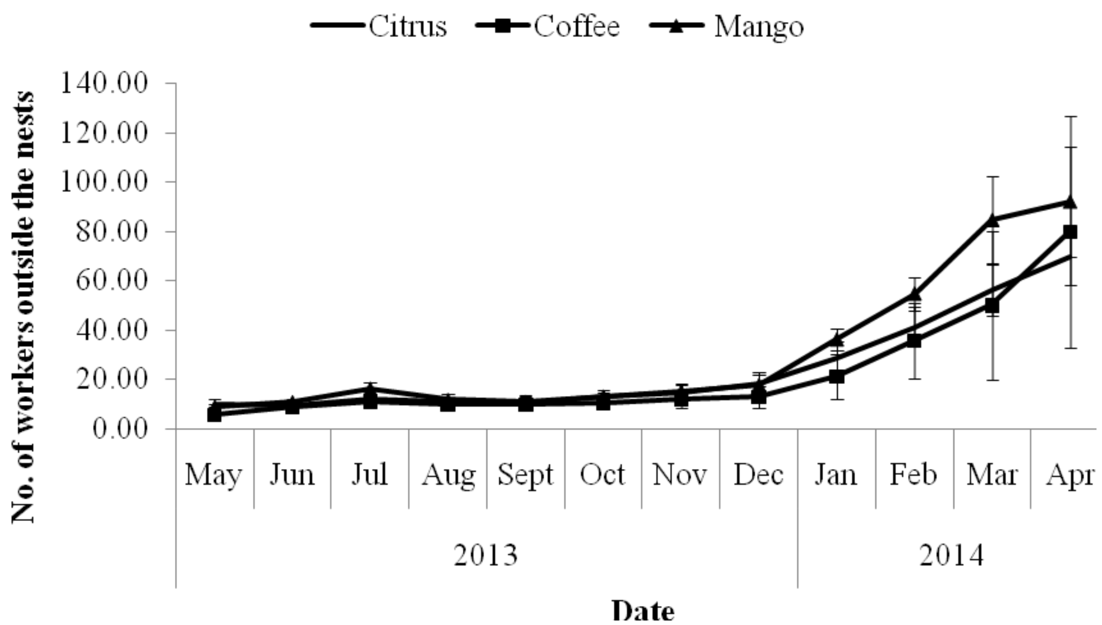


Figure 4. 3 : Average number of workers (±SE) outside the nests on potted plants in a screen house for a period of one year

The increase in the numbers of workers outside the nests was very low during the first six months, when colonies was supplied with fish as a source of protein, sugar solution and water after every two days. However, the increase was higher during the period after the first six months when colonies were supplied with *ad libitum* water and sugar solution and fish was supplemented with other sources of protein (Figure 4.3). This trend was similar in all host plant species.

4.3 Discussion

We tested different queen rearing methods with the aim of developing *O. longinoda* nurseries for supplying ant colonies to farmers. This study showed different rearing methods used did not affect queens' survival but rather production of workers. Mated queens reared on continuous direct access to water produced more workers, and their colonies grew fast. According to Van Itterbeeck *et al*., (2014) observed that in the vicinity of water (eg. a pond) queen brood production is sooner than elsewhere as queen brood believed to drink a lot of water to become large. Queens took longer time to produce first workers when reared on limited access to water methods, which slowed down development of the new colony. Queens reared under continuous indirect and direct access to water methods had high number of workers on 60 days from nuptial flight. These findings suggest that the queens needed water during the initial colony establishment in order to survive and raise broods properly. This is supported by sexuals productions commences at the start of the rainy season (Van Mele *et al*., 2007; Peeters and Andersen, 1989; Peng *et al.,* 1999; Rwegasira *et al.,* 2014; Sribandit *et al*., 2008; Vanderplank, 1960).Queens were observed drinking water on several occasions, either direct or through the cotton wool. Buckley *et al*., (2010) observed that Insects acquire water by drinking, from food and by the aerobic respiration and they loss it by excretion, via the cuticle and through the spiracles. With availability of water, queens were able to raise their brood until the formation of first workers without eating anything. Water positively affected production and rearing of weaver ants queens. The survivals of queens reared on continuous direct access to water were low due to drowning, despite of producing many first workers. As such, the survival of mated queens can be improved by modifying rearing methods on continuous direct access to water to protect queens from drowning. This study find that continuous indirect access to water was the best method for rearing mated queens of *O. longinoda* because there was higher number of numbers of first workers produce also queens survived to produce workers was higher.

Rearing mated queens on continuous indirect access to water and continuous direct access to water was less labour intensive, because once the queens were introduced, there was no need for frequent attendance. In contrast to queens reared under limited access to water sprinkled with water after every two days, which was costly in terms of time and labor. We observed that water is very important during initial colony establishment because it also prevent queens from desiccation. Therefore, on continuous indirect access to water is a recommended methods for rearing mated queens of *O. longinoda* under artificial rearing such as screen house.

The time spent by majority of *O. longinoda* queens to produce workers was similar to what was reported by Peng *et al.,* (1998) and Way (1954), with exception of queens which did not shed off their wings. According to Peng *et al.,* (1998), mated queen of *O. smaragdina* takes about 4 weeks to develop workers. They need favourable conditions to produce workers at a right period. Few queens that did not shed off their wings were observed on limited access to water method and some they took up to 60 days to producer first workers. This period is longer than what has been reported (Way 1954). It was observed that queens which shed off wings have higher capacity of caring for brood.

There was a disappearance of eggs and larvae laid by the queens that did not shed off wings and new eggs were laid after the disappearance of previously laid eggs and emerged larvae. The reason for disappearance of broods was not established but in the circumstances that no predator was observed it is most probable that such queens were cannibalistic eating their own eggs. We recorded more total number of broods on continuous indirect access to water than on continuous direct access to water and limited access to water methods. Although queens were able to access water on continuous direct access to water method, the total number of brood still remained low due to death of queen caused by drowning hence the number declined with time. General the number of broods decreased with advancing stages of development. Consequently there were low numbers of broods on the last stages.

Supply of water and sugar solution *ad libitum* improved growth of weaver ant colonies. Colonies responded to the availability of food, and less food reduced the rate of production of colonies. Reliance on one source of protein i.e. fish slowed down production of workers. Weaver ants often prefer different sources of protein (W. Nene, personal communication). Additionally, supply of water and sugar solution throughout the time increased rate of growth of *O. longinoda* colonies. Colonies survived and continued to lay eggs when food and water were adequately available. The use of piece of cotton wool to provide water and sugar solution was not very effective as it dried out fast. Conversely, the use of Eppendorf tube extended the availability of water and sugar solution, thus colonies were able to drink water/ sugar solution at any desired time. As the result there was an increase in the number of workers found outside the nests, which was the sign of strong colonies.

4.4 Conclusion

We conclude that ability of queens to produce broodsof *O. longinoda* and development of worker was good when reared on continuous indirect access to water method. Although different rearing condition did not show significant difference on queen survival but rather it affected the number of days taken by queens to produce first workers and the numbers of first workers produced. Provision of different sources of protein and unlimited supply of water and sugar solution increases the numbers of workers of the young colonies. Future studies should target to test the frequency and types of protein requirements throughout the initial colony developmental stages. In addition, the methods for transferring young colonies from nurseries to open field or orchards need to be developed and optimized.

4.5 Acknowledgement

This study was funded by DANIDA through project DFC No. 10-025 at Sokoine University of Agriculture. We are thankful to Semngano farmers in Tanga region and Mzinga cooperation in Morogoro region for allowing us to trap mated queens in their fields. We appreciate the assistance by Mr. John Kusolwa in trapping mated queens and Miss Saverina Tiba on the screen house activities. Dr. Renkang Peng (University of Darwin, Australia) and Dr. Joachim Offenberg (Aarhus University, Denmark) are acknowledged for the technical advice on queens rearing.

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CHAPTER FIVE

Seasonal population structure of African weaver ants *Oecophylla longinoda* (Latreille) (Hymenoptera: Formicidae) under bimodal rainfall pattern in Tanzania

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Weaver ant colony structure under bimodal rainfall pattern

Abstract

The ant colony of *Oecophylla* spp is like an extended family with many nests and individuals who all know each other and work closely together in a certain area. For effective control of insect pests; weaver ant population should be high and relatively stable throughout the cropping seasons. Citrus fields with naturally established weaver ant colonies were evaluated to determine the colony structure. One colony covered 3 to 30 trees, with up to 166 nests in an area of 27 m^2 to 270 m^2. The number of major and minor workers fluctuated throughout the season. There were highly significant difference in the number of major workers within a season ($F_{11.429}$ = 8.82, P< 0.0001*). Similarly, significant differences were observed on the number of minor workers ($F_{11.429}$ = 4.00, P < 0.0001*). The average number of workers was 1690.69 ± 79.79 and 1003.43 ± 42.13in the first year (2011/2012) and second year (2012/2013) respectively. The highest number of minor and major workers per nest was 2,365 and 1,065 respectively for both years while the lowest was 277 and 139 respectively. The poison regression results showed significant associations (df = 3, P<0.001) between relative humidity, temperature, rainfall and major and minor workers of *O. longinoda*. The regression analyses showed significant associations (df = 2, P <0.001) between number of leaves used to construct a single ants nest and number of workers and total numbers of ants.Positive correlations were observed among the numbers of leaves used to build single nest with numbers of total ants per nest (R^2 = 0.49, P<0.0001).

Keyword; *Oecophlla longinoda*, citrus, colony structure, nests, populations

5.0 Introduction

The ant colony is like an extended family with many nests and individuals who all know each other and work closely together in a certain area [Hölldobler and Wilson 1978]. Itcontains one mother queen and two distinct castes, the major workers which do virtually all the foraging, care for the queen, defending the colony and assist in care of the broods and minor workers which remain inside the nest and nurses for the broods family [Hölldobler and Wilson 1978].The nests are usually scattered across trees [Hölldobler and Wilson 1990; Lokkers, 1990]. These size differences are controlled by structure and density of foliage. In their study, Paul and Nguyen [2007] reported that the number of nests in one colony depended on various factors such as availability of food and level of disturbance, but it could reach up to 100 in 15 trees and over an area bigger than 1000 m^2. Way [1954] observed one colony of *O. longinoda* inhabiting 151 nests, scattered throughout 8 coconut and 4 clove trees, and covered an area of 800 m^2. Also according to Peng *et al*. [1998] one colony of *O. smaragdina* in mango orchard occupied 2 to 30 trees with 25 to 153 nests. Vanderplank [1960] and Hölldobler and Wilson [1978] observed that mature colonies of *O. smaragdina* contained 100 000 to 500 000 workers in nests made of leaves fastened together by larval silk. Conversely, Lokker, [1990] observed that the number of ants in one nest varied between 4 000 and 6 000 individuals and the size of *Oecophylla* nests varied from a single folded leaf to hundreds of leaves. Way, [1954] observed that a single palm frond could be utilized as a nest with two sides joined with silk.

For effective control of insect pests weaver ants population should be high and relatively stable throughout the cropping seasons. According to Peng and Christian [004] mango trees with abundant weaver ants were damaged by insect pests than trees with fewer, or without, weaver ants. Ant abundance is usually estimated indirectly by counting; (i) ant trails [Peng *et al*. 1998], (ii) ants on selected plant parts [Way and Khoo 1991; Way and Bolton 1997; Blüthgen *et al.,* 2004] or (iii) ant nests [Peng *et al.,* 1997; Olotu *et al.,* 2013; Wargui 2015]. Nest counts, however, may not always reflect current ant population levels because counts may include abandoned nests, and nest size (and therefore probably ant numbers) may vary [Lim 2007]. Although weaver ants have been used for year in controlling insect pests, knowledge about *O. longinoda* colony and distribution of brood and workers within a season are not well known. This limited the ability to estimate the actual or potential ant population an essential determinant of whether the pest control in a crop would be assured. In the current study we established the colony structure of *O. longinoda* in citrus fields, determined the nest size in relation to number of leaves, distribution of workers within the season, and the ratio of brood to workers. This knowledge will help farmers to determine timing of boosting *O. longinoda* populations.

5.1 Materials and Methods

Studies were carried out in citrus fields in Muheza district (S 06^{o}47'12.3", E 37^{o}39'01.7", 501 m a.s.l.) in Tanga Region. The area is characterized by and received long rains from mid-March to May and short rains from November to December. A total of 35 colonies of *O. longinoda* were identified and mapped within the selected citrus fields.

5.1.1 Colony size

Fifteen colonies were selected randomly in citrus field of about one hectare. The number of trees per colony and the number of nests per tree were counted and recorded after every six weeks, for 17 months from December, 2011 to April, 2013.

5.1.2 Assessment of nest assessment

The assessment was carried in 20 mapped colonies for a two year cycle from October, 2011 to September 2012 and from October 2012 to September 2013. Twenty nests were harvested twice a month (mid and end of calendar month). Nests were harvested during the day between 10:00 am to 13:00 pm. Each time, we harvested all twenty nests from a single in a systematic rotation. Each nest was placed in a separate plastic bag that was sealed and brought to Sokoine University of Agriculture entomology laboratory for further observations. In the laboratory the nests were refrigerated overnight to kill the insects. Each nest was subsequently dissected and sorted into respective castes. The castes included workers, queen and males. We counted and recorded numbers leaves used to construct each nest were counted and recorded. We also recorded number of larvae, pupae and imago of minor workers, major workers, winged queen and males; fresh mass of each broods and adults were weighed (using precision balance of four digit weighing balance, 0.0001g). We used number and the weight of each caste to determine the content of nests. We then estimated, based on the number and mass of the weaver ants in the nest, the variation in ant population within a season. Brood to adult ratio was calculated using brood biomass over adult biomass.

5.1.3 Data analysis

Poisson regression was used to determine the association between weather parameters and numbers of minor workers, major workers and winged queens. Predictor variables were temperature, rainfall and RH. Relationship between numbers of leaves used to construct a single ant nest and numbers of workers/total numbers of ants was also determined. One way analysis of variation (ANOVA) was used to test the effect of season on the colony size, distribution of workers, brood to adult ratio and colony size. Data were log transformed to normalize the distribution and variance homogeneity. Data were analysed using GenStat (VSN International, UK) and JMP 12 (SAS Institute).

5.2 Results

5.2.1 Colony size in citrus field

We observed significant differences ($F_{14.161} = 9.94$, $P < 0.0001$) on the numbers of nests among colonies during the assessment period. Similarly, numbers of trees occupied by a colony differed significantly ($F_{14.161} = 6.3$, $P < 0.0001$) among the studied colonies. One colony of *O. longinoda* covered 3 to 30 citrus trees in an area between 27 m^2 to 270 m^2, Average number of nests per colony ranged from 18 (± 6 SE) to 142.5 (± 17.5 SE) (Fig. 5.1 and 5.2).

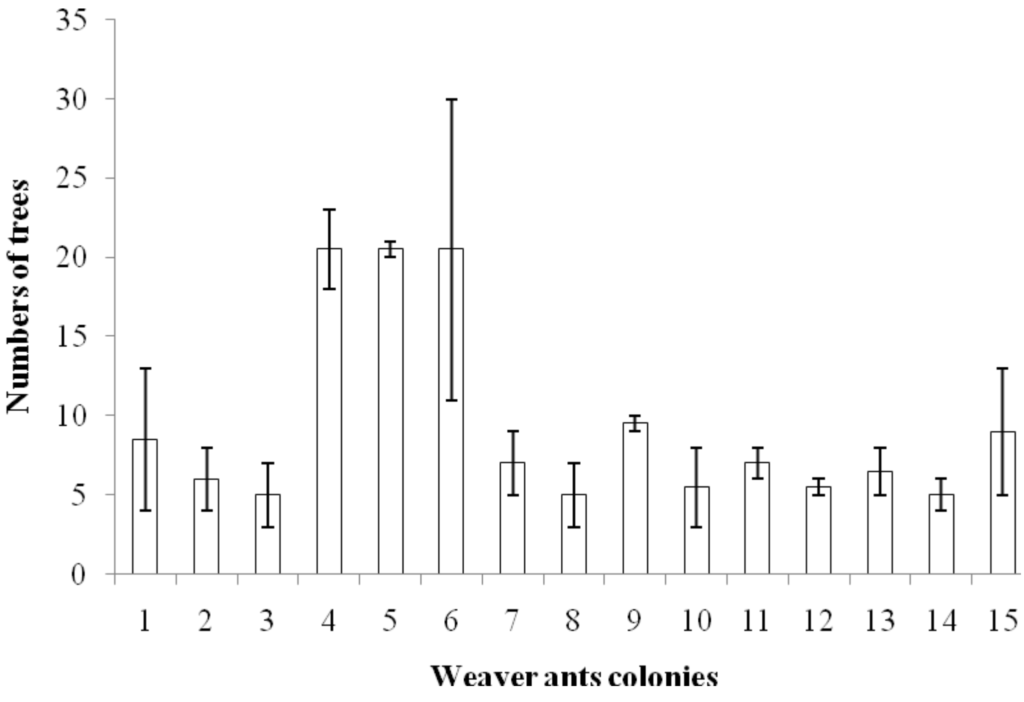

Figure 5.1: Average numbers of trees per colony in citrus field at Tanga Region north Eastern of Tanzania

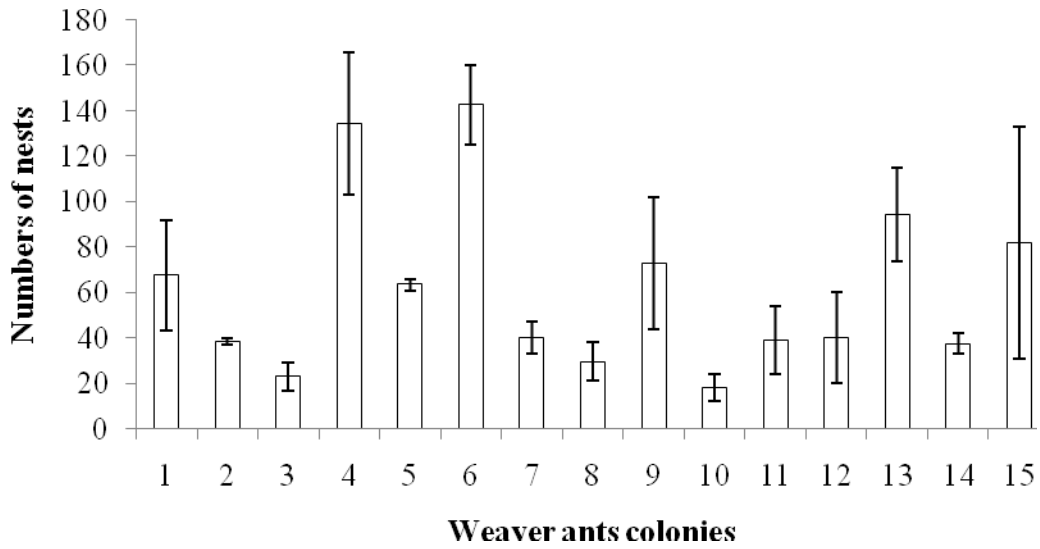

Figure 5.2: **Average numbers of nests per colony in citrus field at Tanga Region north Eastern of Tanzania**

5.2.2 Distribution of workers within the season

Numbers of major workers fluctuated significantly ($F_{11.429}$ = 8.82, P < 0.0001, LSD = 0.104) throughout the season. The highest numbers of major workers per nest was 1064.5 ± 252.9 recorded in August, 2012. Similarly, significant differences were observed on the number of minor workers ($F_{11.429}$ = 4.00, P < 0.0001, LSD = 0.615) within the season. Number of minor workers peaked 2365 ± 895.9 in December, 2011. The trends of fluctuation of major and minor workers were similar, but with high peaks for the former (Fig. 5:3 & 5.4).

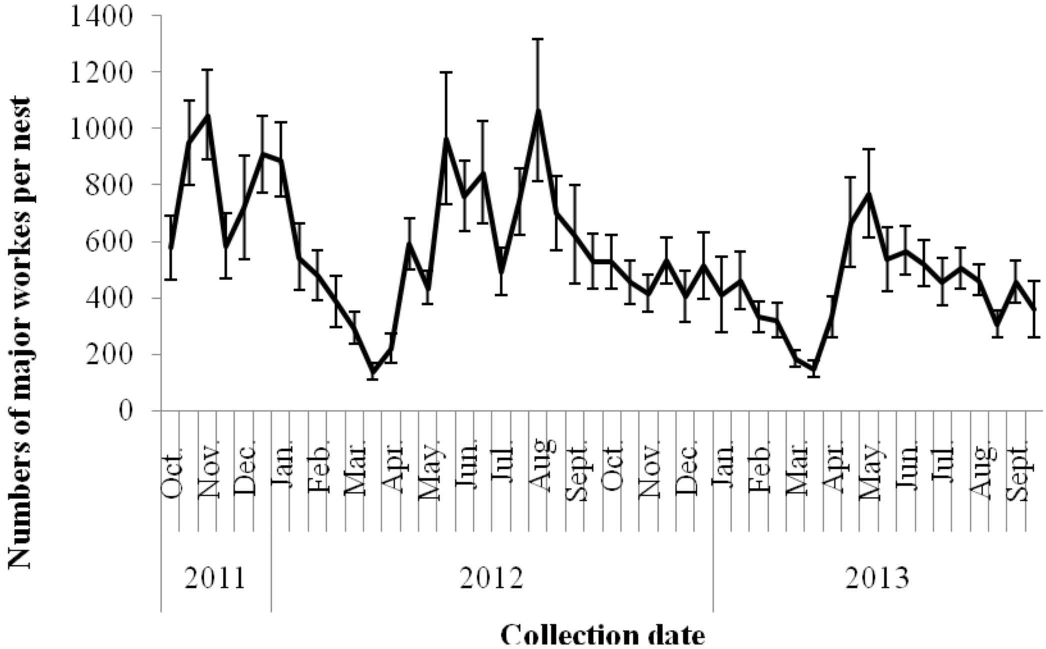

Figure 5.3: **The average numbers of major workers per nest over a season in a citrus field, Tanga Region, Tanzania.**

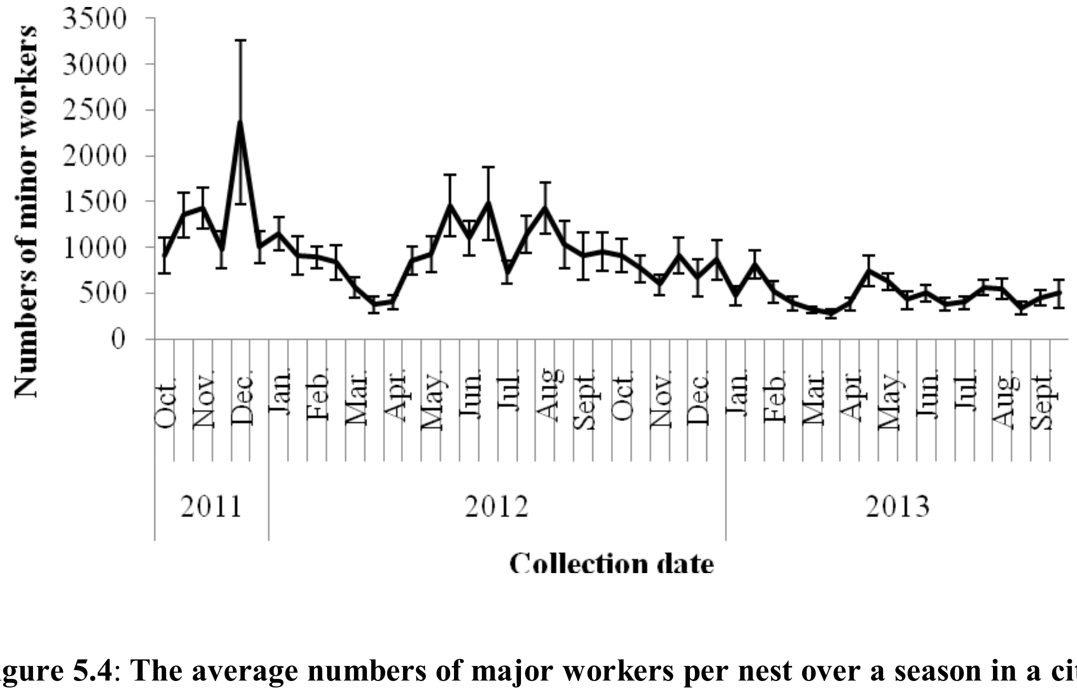

Figure 5.4: **The average numbers of major workers per nest over a season in a citrus field, Tanga Region, Tanzania.**

The numbers of workers declined from January to April for both years, with the lowest level observed in March. Generally, minor workers were more abundant than major workers in most of the time.

5.3.3Variation in fresh weight of weaver ants

Significant difference was observed on fresh weight of weaver ants during the assessment period ($F_{47,893} = 6.28$, $P < 0.0001$). The lowest weight per nest was observed in March for both year 2012 and 2013 (Figure 5.5). The highest fresh weight (± SE) per nest was 16.25 g ± 3.72 which was observed in August 2012 while the lowest was 1.60g ± 0.23 which were observed in March 2013.

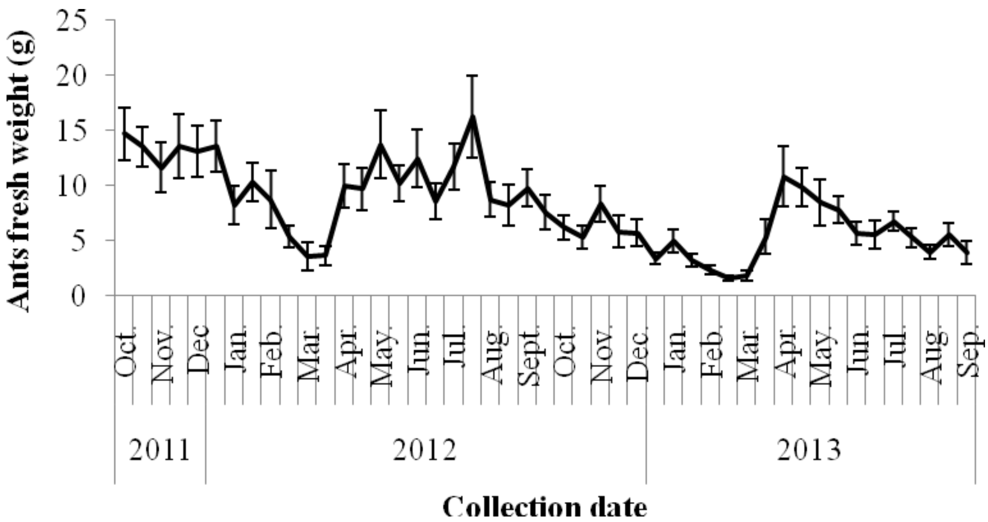

Figure 5.5: **Average fresh mass (g) ± se per nest in a citrus field in Tanga region, Tanzania**

5.3.4 Brood to adult ratio

A significant difference in brood to adult ratio was detected during the sampling period ($F_{47.893} = 2.77$, $P < 0.0001$*). The brood to adult ratio began to increase from April to June, 2012 and from March to June, 2013 with the highest ratio of 0.66 (± 0.12 SE) observed in April 2013 and the lowest 0.04 (± 0.01 SE) observed at the end of February and early March 2013 (Fig. 5.6).

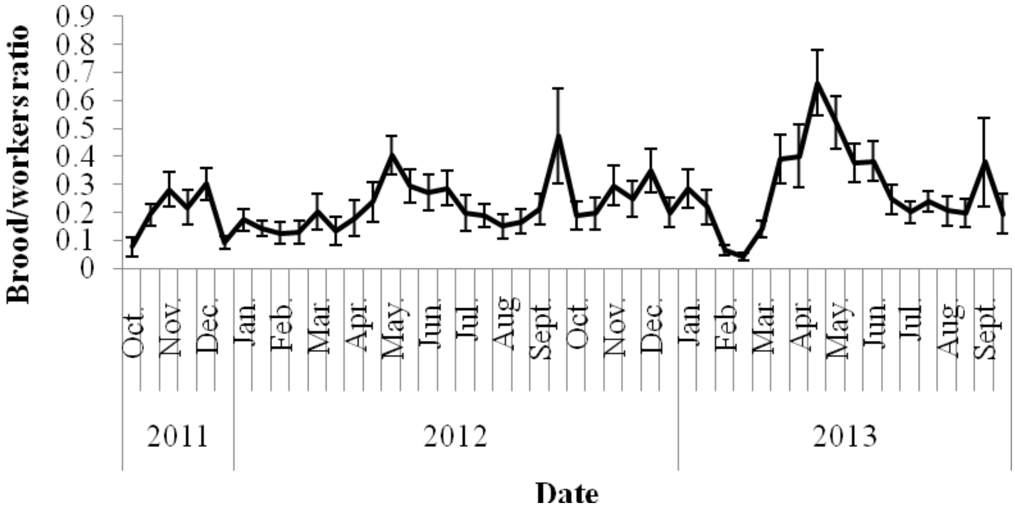

Figure 5.6: **Brood to adult ratios of weaver ants over seasons in citrus field Tanga, Tanzania**

5.3.5 Relationship between weather parameters and ants numbers

Major workers

The poison regression results showed significant associations (df = 3, P < 0.001) between numbers of *O. longinoda* major workers and relative humidity, temperature and rainfall. Temperature and rainfall negatively affected the numbers of major workers while RH positively affected the major workers. The abundance of major workers was negatively affected by temperature (e = 0.0062, P < 0.001, Exp [ß] = 0.93) and rainfall (e = 0.0008, P < 0.001, Exp [ß] = 0.99) (Table 5.1). On the other hand, relative humidity positively affected abundance of major workers (e = 0.0018, P < 0.001, Exp [β] = 1.021).

Table 5.1: Relationship between weather parameters and presence of major workers in the sampled nests

Parameters	Estimate	S.e	t(*)	tpr.	Exp [ß]
%RH	0.0210	0.0018	11.34	<0.001	1.021
Temperature ^{0}C	-0.0682	0.0062	-10.97	<0.001	0.934
Rainfall (mm)	-0.0008	0.0001	-6.06	<0.001	0.999

Minor workers

Significant associations (df = 3, P< 0.001) between relative humidity, temperature, rainfall and abundance of *O. longinoda* minor workers were observed. The population of minor workers decreased with increase in RH (e = 0.00395, P < 0.006, Exp [ß] = 0.99) and temperature (e = 0.00494, P < 0.001, Exp [ß] = 0.92) but increased with rainfall (e = 0.00218, P < 0.001, Exp [β] =1.002).

Table 5.2: Relationship between weather parameters and presence of minor workers in the sampled nests

Parameters	Estimate	S.e	t(*)	tpr.	Exp [ß]
% RH	-0.00395	0.00144	-2.73	0.006	0.996
Temperature ^{0}C	-0.0798	0.00494	-16.17	<0.001	0.923
Rainfall (mm)	0.00218	0.0000903	24.22	<0.001	1.002

Winged queens

Significant effects (df = 3, P < 0.001) of relative humidity, temperature, rainfall and numbers of winged queens were observed. An increased in temperature increased the number of winged queens (e = 0.00733, P = 0.114, Exp [ß] = 1.123) (Table 5.3). However abundance of winged queens was negatively affected by temperature (e = 0.0024, P < 0.114, Exp [ß] = 0.99) and RH (e = 0.024, P =0.14, Exp [ß] = 0.942).

Table 5.3: Relationship between weather parameters and presence of winged queens in the sampled nests

Parameters	Estimate	S.e	t(*)	tpr.	Exp [ß]
%RH	-0.0589	0.0241	-2.45	0.014	0.9428
Temperature ^{0}C	0.1160	0.0733	1.58	0.114	1.123
Rainfall (mm)	-0.00381	0.00241	-1.58	0.114	0.9962

5.3.6 Relationship between total numbers of ants and numbers of nest leaves

Positive correlations were observed among the numbers of leaves used to build single nest with numbers of total ants per nest ($R^2 = 0.49$, $P<0.0001$) (Fig. 5:7). The correlation was not very strong.

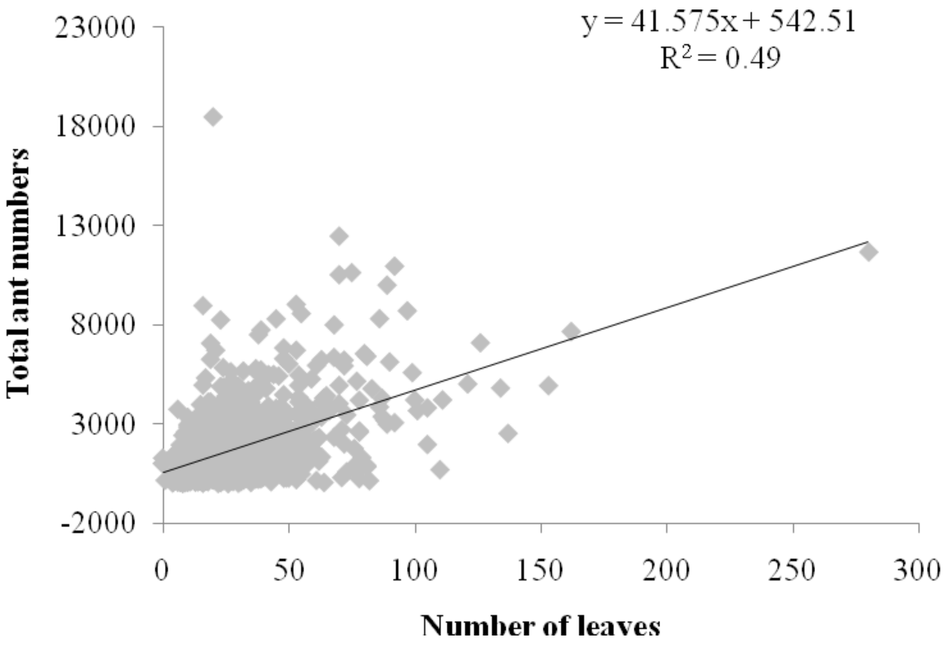

Figure 5.7: **Correlation between total number of ants and numbers of leaves used to construct single nest in citrus field Tanga.**

5.4 Discussions

The studied *O. longinoda* were naturally established in the sampled citrus fields. Colonies sizes varied during the sampling period. Variation in colony sizes were previously reported [Peng *et al*. 1998; Way 1954]. According to Way [1954] one colony of *O. longinoda* had 151 nests, covered 8 coconut trees and 4 clove trees. In a mango orchard, one colony of *O. smaragdina* occupied 2 to 30 trees with 25 to 153 nests [Peng *et al*. 1998]. A number of previous studies associated colony sizes with antagonistic colonies fights among ants from antagonistic colonies, in that case stronger colonies prevailed and extended to territories of weaker ones. A study by Paul and Nguyen [2007] showed that stronger colonies accessed more food than weaker colonies because they occupied a big area which increased the foraging area, especially at times of food scarcity. Colony size also depends on time since establishment. New colonies established shortly after nuptial flights are normally young and small [Vanderplank 1960]. One colony of weaver ants in citrus fields occupied a small area with many nests per colony, and the largest colony covered an area of 270 m^2, which is different from a coconut field where by one colony occupied an area between 800 m^2 and 1000 m^2 [Way 1954; Paul and Nguyen 2007].

The numbers of minor workers and major workers fluctuate with season. Decline of workers which were observed during the dry season in January, previous studied associated with lack of enough water. Abdullah *et al*. [2015] reported that lack of enough water, sugar and proteins especially during the dry season caused a decline in the number of workers, and consequently reduced predatory efficiency. In another observation, declines in the number of workers coincide with the increase in the numbers of sexuals [Rwegasira *et al.*, 2014]. According to Hölldobler and Wilson [1990]; Hasegwa [2013] reported that colonies invested more on production of new queens than males, consequently the numbers of workers declined. Reduced number of workers has an impact on fresh weight of ants since workers dominated the content of the nest.

The brood to adult ratio increased from April which was shortly after a decline in the weaver ant weight. Increases in the brood to adult ratio meant more broods were produced to compensate for the decline in the numbers of workers. This occurred during the rainy season, when water and proteins were not scarcity.

Weather parameters affected the numbers of major workers, minor workers and winged queens of *O. longinoda.* Numbers of major workers decrease when Temperature and rainfall increased. On the contrary the numbers of winged queens increased with increased in temperature. According to Rwegasira *et al.* [2014] the numbers of winged queens increased during the dry season and mating flights occurred during rainy season. According to Nene et al [2016], Relative humidity, sunshine and wind speed trigger nuptial flights in a unimodal rainfall region in southern Tanzania. Increase in the numbers of winged queens corresponded with decreased on the numbers of major workers and minor workers.

Numbers of leaves used to construct a single nest of *O. longinoda* did not highly correlate with numbers of ants. In that case the numbers of leaves cannot be used to estimate ants abundance. Results from the present studies have several implications on biological pest control using *O. longinoda.* Colony should be boosted during the decline on the numbers of workers for effective control. According to Peng and Christian [2004] mango trees with abundant weaver ants were less damaged by insect pests than trees with fewer, or without, weaver ants. In the present studies farmers reported less or no citrus fruits drop on orchard occupied by weaver ants. Major workers do most of the activities in the colony, and their distribution through the tree canopy is very important. They virtually do all the foraging, care for the queen, defend the colony and assist in care of the broods and minor workers which remain inside the nests to nurse the family broods [Hölldobler and Wilson 1978].

In conclusion, *O. longinoda* colonies varied in sizes, one colony could occupy several trees depending on the strength of colony. Production of minor workers and major workers were consistent throughout the season. Weather parameters affected on the numbers of major workers, minor workers and winged queens. Nest size could not correspond with ant abundance.

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5.6 Acknowledgements

This study was funded by DANIDA through project DFC No. 10-25 at Sokoine University of Agriculture. We are grateful to leaders and farmers at Semngano village in Muheza, Tanga for allowing us to conduct experiments in their citrus farms. Thanks to the agricultural extension officer Mr. Jimmy Mhina for assisting on day to day supervision of the experiments. Mr. John Kusolwa assisted in field data collection and MS Saverina Tiba had a hand on the Laboratory work. The technical guidance was provided by Dr. Renkang Peng of the University of Darwin, Australia to whom we owe much our findings.

CHAPTER SIX

6.0 GENERAL CONCLUSION AND RECOMMENDATIONS

6.1 General Conclusion

The colony behaviour of weaver ants (*Oecophylla longinoda)* was studied in citrus fields where they existed naturally.

a) This study has revealed that production of winged queens and males began during the first rainfall in November after long dry season.

b) Sexuals of *O. longinoda* were observed in the nests almost throughout the year with a peak between January and April. This was the longest period compared to what was reported before on related species *O. smaragdina*.

c) Prolonged period occurrences of exuals in the nests implied mated queens can be collected for a prolonged period with more concentration during the peak season.

d) There was a high variation between the numbers of males and the number of new queens per nest. The number of males was higher than number of queens throughout the occurrence of sexuals.

e) Also numbers of workers varied with seasons. Numbers decreased from January to mid – March, which is the similar period with peak production of sexuals.

f) Rainfall played a major role in production and dispersal of the sexuals.

g) Knowing the period of sexuals appearance guided the time for setting traps for collecting mated queens of *O. longinoda*. Among the different traps tested in Tanga experiment, leaf traps were the best for trapping mated queens compared to paper traps and search and catch methods.

h) Light trap was the best method for trapping mated Queens

i) Trapped queens were reared in the screen house using different methods. Mated queens reared in continuous indirect access to water and continuous direct access to water produced high numbers of first workers than limited access to water method. Also the number of workers on continuous indirect and direct access to waster was very high by the time when last queen produced first workers (60 days).

j) Mated queens reared on limited access took long time to produce first workers than continuous indirect access to water and continuous direct access to water.

k) A*d libitum* supply of water to young colonies in ant nursery also increased the number of workers found outside the nest. *O. longinoda* colonies responded with the availability food such as water, sugar solution and different source of protein.

l) Colonies sizes of *O. Longinoda* varied during the sampling period, one colony of weaver ants in citrus fields occupied a small area with many nests per colony, and the largest colony covered an area of 270 m^2.

m) The numbers of minor workers and major workers fluctuated with season. Decline of workers which were observed during the dry season in January could be caused by lack of enough food and water.

n) The brood to adult ratio increased from April which is shortly after a decline in the weaver ant weight. Increases in the brood to adult ratio meant more brood were produced to compensate for the decline in the numbers of workers, and it occurred during the rainy season, the time when there is no scarcity of water and proteins.

6.2 Recommendations

a) More studies should be done to observe the mating behaviour on *O. longinoda*, for example to determine how many males mate with single queen.

b) Develop a light trap for mated queens of *O. longinoda,* which will contain the queens without escaping.

c) There is a need to develop method for transferring young colonies to nurseries in open field and later into orchards.

d) To develop a method for estimating *O. longinoda* population using number of nests per tree.

e) To establish population size of *O. longinoda* required to give an effective control of pests in different crops.

f) Farmers perception on the naturally existence of weaver ants on their field should also be determined.

g) There is a need to find out the proper utilization of weaver ants on smallholder farmers who grow mix cropping

PUBLISHED PAPERS (SUMMARY)

Paper 1: Occurrence of Sexuals of African Weaver ant (*Oecophylla* longinoda Latreille) (Hymenoptera: Formicidae) under a bimodal Rainfall Pattern in Eastern Tanzania

Bulletin of Entomological Research, Page 1 of 5 doi: 10.1017/S0007485314000868

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(Accepted 11 November 2014, Published December, 2014)

Abstract

The African weaver ant, *Oecophylla longinoda,* is being utilized as a biocontrol agent and may also be targeted for future protein production. Rearing of mated queens in nurseries for colony production is needed to cater for such demands. Thus, newly mated queens must be collected for use as seed stocks in the nurseries. To collect mated queens efficiently it is important to identify when sexuals occur in mature colonies. We studied the occurrence of sexuals in *O. longinoda* colonies for two years in Tanga, Tanzania, a region characterized by a bimodal rainfall pattern. We found that *O. longinoda* sexuals occurred almost throughout the year with abundance peaks from January to April. Production of sexuals appeared to be triggered by rainfall, suggesting that populations in areas with long rainy periods may show prolonged mating periods compared to populations experiencing extended dry periods. The bimodal rain pattern may thus cause a low production over a long period. The average yearly production of queens per tree and per colony was estimated to be 449 and 2753 respectively. The average number of queens per nest was 17. Worker abundance declined from January to March with minimum by the end of this period, being inversely proportional to the production of sexuals. In conclusion, mated queens may be collected almost throughout the year, but most efficiently by the onset of the long rainy season when the majority disperse.

Key word: *Oecophylla longinoda*, sexuals, bimodal rainfall, dispersal flights, Tanzania.

Paper 2: Comparing different methods for trapping mated queens of weaver ants (*Oecophylla longinoda* Latreille) (Hymenoptera: Formicidae)

Biocontrol Science and Technology, 2015

Vol. 25, No. 5, 503–512, http://dx.doi.org/10.1080/09583157.2014.992861

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Accepted 2nd December, 2014; Published 19 January, 2015

Abstract

The predatory efficiency of African weaver ants *Oecophylla longinoda* and their utilization in protein production is a function of ant abundance. Reliable control of insect pests in tropical crops is achieved when ant populations are constantly high. Transplanted populations of weaver ant colonies containing egg-laying queens are more stable than those without. Achieving such stability through collection of colonies established in the wild is usually difficult because of uncertainty in locating the nest containing the egg-laying queen. In this paper, we investigated four methods that may be used to collect mated queens that subsequently can be used to stock ant nurseries. The catch efficiencies of (i) leaf traps, (ii) paper traps (both types providing a refuge for founding queens), (iii) random search for queens, and (iv) light trapping, were compared. Light trapping was the most efficient way to collect queens followed by leaf traps, random search and, lastly, paper traps. Light trapping and random search, though, required the presence of a person throughout the ant's mating season (several months), whereas this was not required when using leaf and paper traps.

Key words; *Oecophylla longinoda*, trapping, mated queen

Paper 3: Optimizing methods for rearing mated queens and establishing new colony of *Oecophylla longinoda,* Latreille (Hymenoptera: Formicidae)

Submitted to International Tropical Insect Science-ICIPE

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Abstract

Oecophylla spp are used as bio control agents for many types of insect pests. A large and stable population is essential for effective control of pests. Colonies of *Oecophylla* spp can be transplanted from wild habitats into orchards. Transplanted colonies can only survive in presence of mother queens. It is usually difficult to trace nests with mother queens in large colonies which may sometimes contain more than 100 nests. Therefore, the need to explore and develop methods for rearing newly mated queen in nurseries may not be overemphasized hence the current study. In the first experiment, we tested three rearing methods on queen survival and colony establishment. In the second experiment we compared different weaver ants feeding techniques on young colony growth. We observed that queens were best reared under continuous indirect access to water methods. First workers emerged earlier (32 days on average) in continuous indirect access to water and continuous direct access to water methods than on limited access to water (sprinkled) (38 days on average). Moreover, rearing mated queens under continuous indirect access and continuous direct access to water methods saved labour and time, because of limited attendance to the colonies. Availability of water, sugar solutions and different sources of protein throughout the time improved the growth of young colonies and the number of workers increased rapidly.

Key words; ant nursery; ant colonies; *Oecophylla longinoda;* queens; rearing methods

Paper 4: Seasonal population structure of African weaver ants *Oecophylla longinoda* (Latreille) (Hymenoptera: Formicidae) under bimodal rainfall pattern in Tanzania

Submitted to Journal of Entomologia Generalis

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Weaver ant colony structure under bimodal rainfall pattern

Abstract

The ant colony of *Oecophylla* spp is like an extended family with many nests and individuals who all know each other and work closely together in a certain area. For effective control of insect pests; weaver ant population should be high and relatively stable throughout the cropping seasons. Citrus fields with naturally established weaver ant colonies were evaluated to determine the colony structure. One colony covered 3 to 30 trees, with up to 166 nests in an area of 27 m^2 to 270 m^2. The number of major and minor workers fluctuated throughout the season. There were highly significant difference in the number of major workers within a season ($F_{11.429}$ = 8.82, P< 0.0001*). Similarly, significant differences were observed on the number of minor workers ($F_{11.429}$ = 4.00, P < 0.0001*). The average number of workers was 1690.69 ± 79.79 and 1003.43 ± 42.13in the first year (2011/2012) and second year (2012/2013) respectively. The highest number of minor and major workers per nest was 2,365 and 1,065 respectively for both years while the lowest was 277 and 139 respectively. The poison regression results showed significant associations (df = 3, P< 0.001) between relative humidity, temperature, rainfall and major and minor workers of *O. longinoda*. Positive correlations were observed among the numbers of leaves used to build single nest with numbers of total ants per nest (R^2 = 0.49, P<0.0001).

Keyword; *Oecophlla longinoda*, citrus, colony structure, nests, populations

APPENDICES

Appendix 1: *Oecophylla longinoda* nest

Appendix 2: New mated queen with first larvae caught using leaf trap

Appendix 3: Paper and leaf traps set in citrus field in Tanga region

Appendix 4: Queen reared into the vial

Appendix 5: Ant nursery