



RESEARCH ARTICLE

UNDERSTANDING THE PHENOLOGY OF NEW FOUNDING COLONY OF WEAVER ANT: A  
PRELIMINARY SCREEN HOUSE OBSERVATIONS

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ABSTRACT

Weaver ant, *Oecophylla longinoda* is eusocial insect used as a bio-agent against more than fifty insect pest species in several crops. Colony rearing in the screen house involves the collection of mated winged females after their nuptial flight, rearing to allow broods development. The time taken for forming different broods is not well known for the new founding colony of *O. longinoda*. We conducted a screen house experiment to study the phenology of new founding *O. longinoda* colonies. We found that are eggs hatched 9 days after they are laid; furthermore, it takes 9 days from larvae to develop to pupae. However it requires at least 10 days for pupae to develop into worker ants. It takes 30 days from nuptial flight day to the first emergence of worker ants. The study found that, it can take at least 11 days for workers in a colony freely exposed in seedlings to initiate nests buildings. These are useful pieces of biological information which can be used in colony managements during rearing. This information can also be used in estimating time taken by a colony in the screen house before it is introduced in the field to control insect pests.

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INTRODUCTION

Weaver ants (*Oecophylla smaragdina* and *O. longinoda*) are predatory insects that are widely used as bio-agents for the control of insect pests in multiple tree crops including cashew, citrus and mango (Way and Khoo, 1992; Peng and Christian, 2007; Abdulla et al., 2015). The two species of weaver ants (*O. smaragdina* and *O. longinoda*) are effective bio-agent against more than 50 different pests in multiple tree crops and forest trees (Way and Khoo, 1992; Peng et al., 1995). These species can be as effective as or even more effective than synthetic chemicals (Peng and Christian, 2005a; Dwomoh et al., 2009; Offenberget al., 2013); result into an increase of crop yields and or higher fruit quality (Barzman et al., 1996; Peng and Christian, 2007). The use of weaver ant as a bio-agent is sustainable and successful when a colony consists of maternal queen (Peng et al., 1997; Peng et al., 1998; Peng et al., 2008). Two ways can be used to obtain maternal queen containing colonies namely; (i) relocation of mature colony consisting of maternal queen nest into new plantation (Nene et al., 2017) and (ii) trapping newly mated queens with the use of artificial nests (Nene et al., 2016a; Nene et al., 2017a), reared in nurseries and be introduced in a new orchard particularly during rainy season (Peng et al., 2013; Rwegasira et al., 2014; Nene et al., 2015). The new founding mated queen lays eggs

which can be hatched into other developmental stages such as larvae, pupae and adult workers. The timing of the emergence of these developmental stages can be considered as insect phenology (Haggerty and Mazer, 2008). However, these development stages particularly for African Weaver Antare not well known (AWA), *O. longinoda*. In the case of *O. smaragdina*, it can takes 3 to 4 weeks for an egg to develop to a worker and 5 to 6 weeks for ant workers to start making new leaf nests (Peng et al., 2008). The knowledge on insect phenology is important and can contribute to multiple advantages including conservation of beneficial insect species (Ruml and Vulić, 2005). Species are very variable in their ecological and life history strategies (Ruml and Vulić, 2005). Phenology varies among species, seasons and locations (Hodgson et al., 2010; Primack et al., 2009). Several studies have indicated variability in insect phenology across species. For instance, the timing of the emergence peaks of butterfly species (Hodgson et al., 2011; Navarro-Cano et al., 2015; Williams et al., 2014), hatching date of avioan predator, the breeding of insectivorous birds and peak biomas of caterpillars (Both et al., 2009) are highly variable across species. The rate of offspring development from the first egg to the hatching of the first larva, the first larva to the first pupa and the first pupa to the first adult in *O. smaragdina* is well documented (Lockkers, 1990). The timing of performing these activities by each of the weaver ant form (morph) in the early colony of *O. smaragdina* also is clearly described (Lokkers, 1990; Peng et al., 2008). However, this is not the case for the early colony of

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*O. longinoda*, where there is no clear information on the time taken for brood development (egg laying and hatching to larvae, larvae to pupae and pupae to adults). Thus, the aim of this study was to explore information on the phenology of new founding queen of *O. longinoda*. The information obtained can help timely planning on the requirements including feeding of broods during colony rearing.

## MATERIALS AND METHODS

### The study area

A screen house experiment was conducted between February and April, 2017 at Naliendele Agricultural Research Institute (NARI), (10° 21' 22.49" S, 40° 09' 57.05" E and 140 Meters above sea level), which is about 10 km from Mtwara town in the southern part of Tanzania. The experiment examined the phenology of new founding queen colony of *O. longinoda*. Thus, the study involved the collection of winged queens after the nuptial flight which takes place during rainy season (Nene et al., 2016) and rearing.

### Collection of winged queens after their nuptial flights and rearing

Winged queens were collected by hand after a random search on the ground under the light sources (electric light) placed on NARI's residential houses. The collections were carried out between 6:30 and 8:00 p.m. This is because the nuptial flights occur in the evening hours (Nene et al., 2016a). The first stage rearing was done by keeping each collected winged queen in a test tube placed on tables in a screen house. Some water was sealed behind the cotton plug inside the test tube. The test tubes were kept horizontally so that the queen and brood cannot fall into the wet cotton plug. A dried cotton plug was sealed at the opening of the test tube.

plug was removed to enable the emerged workers to move in and outside the test tube. The colonies were provided with; a 20% sucrose solution, some water and ground anchovy or other sources of protein after the emergence of the first workers.

### Assessing for colony phenology

We recorded the time taken (in terms of number of days) by each winged queen to shed wings, to start laying eggs, eggs getting hatched to larva, larva developing to pupa and pupa to adult workers from the nuptial flight. We recorded the total number of broods (eggs, larva, pupa and workers) in each queen. We also recorded the dates for the construction of the first nest and the queens' mortality.

## RESULTS

### Collection of winged queens and assessing for their reproductive success

A total of 21 winged queens were collected at different dates in February 2017 after the nuptial flight as follows; 5 queens were collected on the 17<sup>th</sup>, 8 were collected on the 18<sup>th</sup> and other 8 were collected on the 27<sup>th</sup>. The results in Table 1 indicated that over 85 % of the collected queens laid eggs and 76.2% queens shed their wings. Eggs from 9 queens were hatched to larva and developed to pupa. This makes larvae and pupae formation successful by 42.9 %. Pupae from 5 queens (23.8 %) developed to workers within 30 days of rearing.

### Queens' mortality

The queen mortality of 28.6 % was recorded within 30 days of rearing. For all the died queens (i.e. 6 in total), 2 queens died within 3-6 days after the nuptial flight, 3 died within 6-9

**Table 1. Reproductive success of *O. longinoda* queens within 30 days rearing after nuptial flight**

	Queen numbers	% Queen number	Total number of queens under rearing
Number of queens laid eggs	18	85.7	21
Number of queens shed wings	16	76.2	21
Number of queens their eggs hatched to larvae	9	42.9	21
Number of queens their larva developed to pupa	9	42.9	21
Number of queens their pupa developed to workers	5	23.8	21
Mortality			
Number of queens died before laying eggs	3	-	-
Number of queens died after laying eggs	3	-	-
Total number of queens died within 30 days rearing	6	28.6	21

**Table 2. Time taken for initiation of wing shedding, egg laying, and forming of; larva, pupa and workers in new founding *O. longinoda* colonies**

Event	Emergence day following nuptial flight	Queen numbers	% queen number	Queen total
Shedding wings	2	7	33.3	21
Laying eggs	2	7	33.3	21
Forming larva	11	5	31.1	16 (5 died)
Forming pupa	20	5	33.3	15 (1 more died)
Forming workers	30	5	33.3	15

This kind of setting enables the queens and the broods to stay in the middle inside the test tube, to access water through wet cotton plug and to be protected against other ants. The second stage rearing involved the transfer of colony to the seedlings in the screen house. In this stage, after the emergence of first workers, each test tube with queens and broods was tied on a mango or a cashew seedling using a rubber. The dried cotton

days and 1 queen died in the 15<sup>th</sup> day of rearing. Half of the queens in this group laid eggs before death. However, their eggs were not hatched. Other 6 queens died before they were introduced in the second rearing (i.e. the use of seedlings), making a total of 12 queens. The remaining 9 colonies were introduced in the seedlings; however, 4 more queens died before building their nests.

**Table 3. Maximum number of broods in each queen in the 30<sup>th</sup> day following each nuptial flight**

Queen number	Number of eggs	Number of larva	Number of pupa	Number of worker
1	19	19	16	4
2	16	1	1	0
3	16	15	10	2
4	21	19	13	8
5	25	23	12	0
6	21	19	15	0
7	14	13	10	5
8	13	12	8	7
9	22	19	8	0
10	8	0	0	0
11	10	0	0	0
12	1	0	0	0
13	7	0	0	0
14	6	0	0	0
15	5	0	0	0
Total	194	140	82	26
Average	12.93	9.33	5.46	1.73

**Table 4. Time taken by AWA workers to start building nests after they are freely released**

Queen labeled	Date of establishment in the seedlings	Date for construction of first nest	Time taken by the workers to build nest (days)	Status (dead or live)
1	09 April	20 April	11	Died on 29 May, 2017
3	09 April	20 May	41	Live
4	09 April	29 April	20	Live
7	09 April	01 May	22	Died on 11 May, 2017
8	09 April	16 May	37	Live

Table 2 shows the time taken for the initiation of the wing shedding, egg laying, and the development to larva, to pupa, and to workers. The shedding of wings and the laying of eggs were first observed in seven queens (33.3%) in the second day after the nuptial flight. The hatching of eggs into larva was first observed on the 11<sup>th</sup> day after the nuptial flight in 5 (31.1 %) queens. It took 20 days to form pupae and 30 days to form workers in 5 queens following each nuptial flight. The results in Table 3 indicate that 194 eggs (average 12.93) were laid by 15 queens within 30 days. A total of 140 eggs were hatched to larva, 82 larvae developed to pupae and 26 pupae developed to workers on the 30<sup>th</sup> day following the nuptial flight. The highest numbers of eggs and larvae were 25 and 23 respectively. The highest (16) number of pupae was recorded in queen 1; whereas, the highest number of workers (= 8 workers) was recorded in queen 4 on the 30<sup>th</sup> day. The results in Table 4 show that the time taken by the workers to start building nests ranges from 11 to 41 with an average of 26.2 days.

## DISCUSSION

In this study, not all collected queens laid eggs or shed their wings. The shedding wings and the laying of eggs started in the second day after the nuptial flight. The emergence of larvae started on the 11<sup>th</sup> day while pupae were observed on the 20<sup>th</sup> day following each nuptial flight. Not all eggs which were laid by queens hatched to larvae. Thus, mating for viable offspring may sometimes be unsuccessful. Workers were observed on the 30<sup>th</sup> day. This indicates that each phenology event takes at least 9 days (i.e. 9 days each from eggs to larvae and from larvae to pupae). However, it took at least 10 days from pupae to workers. Workers were recognized as they started stretching their legs and initiating movement. The laying eggs, the formation of larvae, pupae and workers infers complete metamorphosis. The study revealed that, not all the pupae from queens developed to workers within 30 days of rearing.

Workers were observed on the 30<sup>th</sup> day which is 4 weeks and 2 days. A previous study by Peng *et al.* (2008) observed the formation of workers for *O. smaragdina* in 3 to 4 weeks after rearing. A total of 26 workers were recorded in 15 colonies within 30 days of rearing. It takes 5 to 6 weeks for *O. smaragdina* colonies to establish their nests (Peng *et al.*, 2008). Mature colonies of *O. smaragdina* consist of thousands of worker ants (Crozier *et al.*, 2009). When new founding queens are reared, they remained unfed until the first worker ants are formed. Thus, understanding the time of the emergence of workers can help to plan for feeding of a colony. Workers started to build nests on the 11<sup>th</sup> day following their release in the seedlings. Before nests building, colonies used test tubes which were opened at one end as artificial nests. This implies that, artificial nests are useful during colony establishment. Bottles were used as artificial nests for mature *O. smaragdina* colonies in the pomelo and mango plantation in the northeast Thailand (Offenberg, 2014). Artificial nests help the ants against exposures to harsh conditions (Offenberg, 2014). Harsh conditions such as heavy rains of greater than 60 mm per event can even kill the queen (Peng *et al.*, 2008). High queens' mortality was observed. The reason was not clear, but this could be due to other predators/weaver ant antagonists, particularly big headed ant which were observed moving around the seedlings. It could also be caused by unknown flying predators, as in most cases, the whole queens were removed where by the test tubes were found empty. It is therefore suggested that, the control of weaver ants' antagonists and other predators is important during colony rearing.

## Conclusion

To the best of our knowledge this is the first study to show that, it takes 9 days for *O. longinoda* larvae to form pupae and it requires at least 10 days for the pupae to develop into worker ants. It takes 30 days from the nuptial flight day to the first emergence of worker ants. These are useful pieces of

biological information particularly for bio-agents such as *O. longinoda* which control insect pests in multiple crops. This knowledge can be used in the management of new colonies which are established in screen houses or nurseries. However, this is a preliminary study; more researches are needed and should involve an increase of the number of queens for more colonies, in order to clearly understand the causes of death so as to reduce queens' mortality.

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