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Observations about Founding Queens (*Atta sexdens*) and their Unusual Behavior

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Abstract **– Nest foundation in *Atta* leaf-cutting ants is claustral, and the single founding queen completely relies on its body reserves throughout several weeks until the first workers emerge and initiate foraging. However, little is known about the founding queens under laboratory conditions. In this study, we determine the survival of mated females of *Atta sexdens*. In addition, we observed an unusual behavior of these queens. Newly-deposited eggs were already present in the first week after nuptial flight. Larvae were observed from the fourth week onwards, and pupae from the sixth week. The workers emerged in the eighth and ninth week, when the weekly observed. Survival decreased markedly in the first week after nest founding, averaging 65,47%, and continued decreasing over the 11-week period of observations until a minimal value of 42,26%. Another interesting result was in the 10th week, when plants were offered, some queens exhibited unusual behavior, in which it cut and chewed small leaf pieces. Our study contributes to knowledge of the founding queens under laboratory conditions.**

Keywords **– Leaf-Cutting Ants, Nest Founding, Nuptial Flight.**

I. INTRODUCTION

Nuptial flights of leaf cutting ants queens occur in the afternoon, during the months of October – November in southeastern Brazil. They are triggered by the first rains breaking a period of drought. During these flights, males form "clusters" with a diameter of 200 m, which can be found 150 m above ground level [1]. The distances traveled during nuptial flights vary from species to species, and also depend on the air speeds achieved. *Atta texana* Buckley, 1960 can fly at speeds of 5.33 ms^{-1}, suggesting that a distance 10.4 km can be covered, and to *Atta sexdens*, speeds of 1.57 ms^{-1} suggest a travel distance of 11.1 km [1].

Both sexes need energy to engage in nuptial flights. Before leaving the nest of origin, males and females are well fed and ready for mating. Jutsum and Quinlan [1] found that 21% of the dry weight of winged forms is composed of carbohydrates, which are completely consumed during the nuptial flight. Similarly, carbohydrates (stored as glycogen) have been shown to represent the main source of energy for the nuptial flight in a study involving *Formica lugubris* Zetterstedt, 1838 [2]. In male *Cataglyphis cursor* Fonscolombe, 1846 and *Linepithema humile* (Mayr) (formerly *Iridomyrmex humilis*) which fly, exhibit a much higher carbohydrate content than do the non-flying females of these species [3]. From these studies it can be safely assumed that the first energy resource to be exhausted in the nuptial flight is the carbohydrate reserve in the body. Lipids and proteins are additionally more difficult to break down than carbohydrates, and spared for colony-founding activities which follow the nuptial flight.

After the nuptial flight, the queens land on the ground, shed their wings, and start excavating the soil. After 6 to 10 hours [4] of digging, a tunnel and a chamber result. During the excavation, the queens perform 300 trips on average for piling excavated soil outside, each trip lasting from 1 to 2 minutes, resulting in an average speed

of 3 cm/hour [5]. Because this activity is very intense, it is assumed that the energy costs of the excavation are high. However, there are no data available in the literature of the amount of energy required for nest excavation, and of the substrate used to fuel it. It has been postulated that the required energy is drawn from lipid reserves, because the carbohydrates are mostly depleted during the nuptial flight [1], [2]. In order to make claustral colony founding possible, the queen must have a large reserve of fats (lipids), which is consumed at this stage of the colony life. However, *A. sexdens* queens feed on the fungus garden when founding new colonies, indicating that, although not exogenous, fungal staphylae, together with trophic eggs, supply the founding queens with a ready energy source they need during the founding stage [6].

The founding queen loses about 40% of its weight during this phase, achieving her lightest body mass four months after the nuptial flight (*A. sexdens* and *A. laevigata* F. Smith, 1858) [7]. However, there are no studies on survival of *A. sexdens* species under laboratory conditions. In this study, we determine the survival of mated females of *A. sexdens*. In addition, we observed an unusual behavior of these queens.

II. Material and Methods

A. *Studied Colonies*

Forty the collection was accomplished during then nuptial flight that occurred on October 25th 2021 at the Lageado Experimental Farm, FCA - UNESP – Botucatu, Brazil, by capturing 672 queens immediately after mating, before they started to dig their nests. Queens were individually maintained in small plastic containers (11 cm in diameter and 8 cm in height) with a 1-cm floor of plaster to maintain humidity. All experiments were performed at the Laboratory de Insetos Sociais (of Social Insects) -Pragas – FCA/UNESP – Botucatu.

Queens were immediately placed in small plastic containers as described above for observation of offspring production over a period of 11 weeks (Fig. 1). Queen mortality, offspring production were quantified weekly throughout the 9 weeks after nuptial flight, using a stereoscopic microscope (Nikon, SMZ 1000). Data on queen mortality was submitted to a Survival Analysis (Kaplan Meier). Statistical analyses and graphs were processed by BioEstat 5.0.

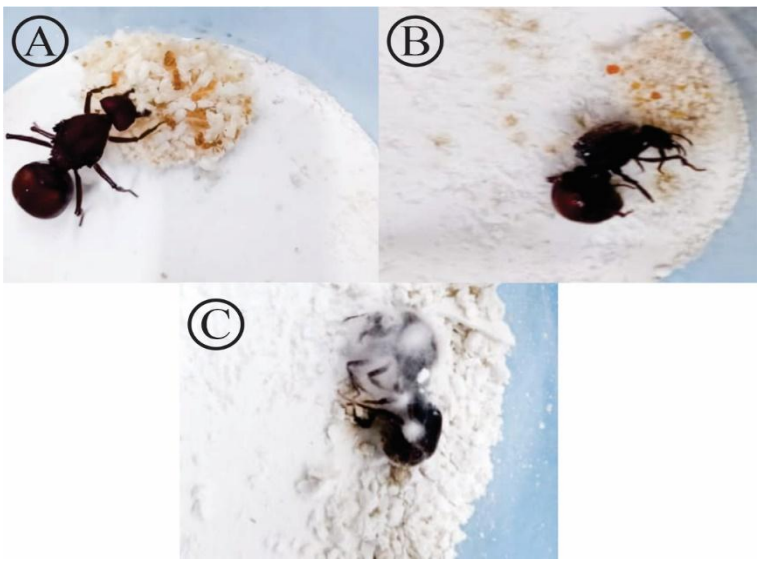

Fig. 1. (A) Healthy queen (*Atta sexdens*); B) Queen dead without the presence of contaminating fungus; and (C) Queen dead with the presence of contaminating fungus.

III. RESULTS

Newly-deposited eggs were already present in the first week after nuptial flight. Larvae were observed from the fourth week (21st day) onwards, and pupae from the sixth week (42nd day). The workers emerged in the eighth and ninth week (56th and 63rd day), when the weekly observed.

Survival rates of queens are presented in Fig. 2. Survival decreased markedly in the first week after nest founding, averaging 65,47%, and continued decreasing over the 11-week period of observations until a minimal value of 42,26%.

Queens mortality occurred due to several factors, from unknown causes to entomopathogenic fungi, as can be observed in Fig. 1. Another interesting result was in the 10th week, when plants were offered, some queens exhibited unusual behavior, in which it cut and chewed small leaf pieces. This behavior can be observed in the video (https: //youtu.be/ee-bNtYHhzU).

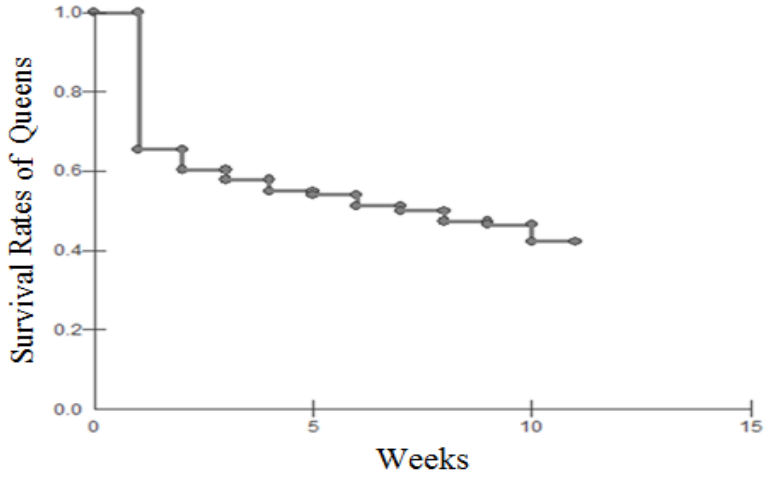

Fig 2. Survival rates of *Atta sexdens* queens over 11 weeks under laboratory conditions.

IV. DISCUSSION

The period of appearance of eggs, larvar, pupae and adults were comparable with those reported previously for the same species [4], [8], and also for *Atta capiguara* [9]. It can be concluded that *Atta* queens, with approximately 40% of their body mass comprised of fat [10], [11], possess enough energy reserves to maintain their rate of offspring production high over 9 weeks. Body reserves come primarily from the fat bodies localized in the queen gaster. Reserves are stored inside trophocytes and oenocytes [12], [13], which attach themselves firmly to the ovaries and other organs through tracheal branches [14]. It has been reported that in the fat body of *Atta sexdens rubropilosa* queens, trophocytes are abundant from the first to the 18th day, and decrease in cellular size starting on the 28th day until 60 days after the nuptial flight. By the 60th day (around 9 weeks) all reserves are consumed, probably utilized in vitellogenesis due to the ovarian development in this period, and also to support the metabolism of the fasting queen [14]. When foraging by workers is initiated, queen feeding is expected to increase, thus leading to a marked increase in egg-laying rates [15].

Survival rates of queens are presented in Fig. 2. Survival decreased markedly in the first week after nest founding, averaging 65,47%, and continued decreasing over the 11-week period of observations until a minimal value of 42,26%. Interestingly, the increased mortality in the queens occurred in the first week after digging,

while the mortality rates thereafter remained constant, with curve decreasing with a similar pattern (Fig. 2). Mortality rates due to several factors, from unknown causes to entomopathogenic fungi, as can be observed in Figure 1. Generalist entomopathogenic fungi, such as *Metarhizium* and *Beauveria*, potential biological-control agents, and can attack many insects at different stages [16]. In the case of leaf-cutter ants, the occurrence of *Metarhizium* and *Beauveria* has already been reported, as well as of other fungi such as *Purpureo cillium, Fusarium* and *Aspergillus*, in queens of *Atta sexdens rubropilosa, Atta laevigata* and *Atta capiguara* [16].

On the other hand, it can hardly be argued that queen mortality was based on the exhaustion of body reserves. More likely, the accumulation of oxidative damage associated with the intense flight activity, as known for flying insects [17], [18], may have been the main proximate cause of the mortality observed shortly after nuptial flight.

Some queens exhibited unusual behavior, in which it cut and chewed small leaf pieces (https://youtu.be/ee-bNtYHhzU). This behavior is very similar to found by workers of leaf-cutting ants, that the act of cutting and chewing the plant substrate is a primary behavior for the later incorporation into the fungus garden [19]-[25]. Why do *Atta* queens cut and chew the plant leaves? We suggest that there is ingestion of liquid substances during the preparation of the substrate to be incorporated, as it occurs in workers [26], and the queens can ingest plant sap. But it is unlikely to occur under natural conditions, however, it is an intriguing fact to be studied.

V. Conclusion

In short, it is concluded that the survival decreased markedly in the first week after nest founding, and continued decreasing over the 11-week during period of observations. In addition, we recorded the unusual behavior of queens of leaf-cutting ants.

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