BROOD PRODUCTION AND MORTALITY RATE OF YOUNG SAÚVA QUEENS (*Atta sexdens* LINNAEUS, 1758) (HYMENOPTERA: FORMICIDAE) REARED IN ARTIFICIAL COLONIES WITH MIXED SUBSTRATUM

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ABSTRACT – The use of plaster as a substratum for colonies of leaf-cutting ants in laboratory is widespread. Because *Atta* Fabricius, 1804 species excavate the soil in order to found new colonies in nature, we wanted to test whether mixing plaster with soil (Latossolo Vermelho) would influence the number of eggs, larvae, pupae and workers in *A. sexdens* Linnaeus, 1758 colonies. Here we report results that possibly indicate the plaster substratum as the most suitable for rearing young *A. sexdens* colonies in laboratory.

Palavras-chave: Atta, claustral founding, leaf-cutting ants, saúva.

*PRODUÇÃO E TAXA DE MORTALIDADE DE RAINHAS JOVENS DE SAÚVA (*Atta sexdens *LINNAEUS, 1758) (HYMENOPTERA: FORMICIDAE) CRIADAS EM COLÔNIAS ARTIFICIAIS COM SUBSTRATO MISTO*

RESUMO – É comum o uso de gesso estuque como substrato em colônias artificiais de formigas cortadeiras (Forti et al., 1994). Devido ao fato de espécies de Atta *Fabricius, 1804 escavarem o solo para fundar novas colônias na natureza, deseja-se testar se o uso de solo de terra de barranco (Latossolo Vermelho) misturado ao pó de gesso como substrato teria influência sobre o número de ovos, larvas, pupas e operárias em colônias de* A. sexdens *Linnaeus, 1758. Reportamos aqui os resultados preliminares que possivelmente indicam o substrato feito unicamente de pó de gesso como o mais adequado para criar colônias de* A. sexdens *em laboratório.*

Keywords: Atta, fundação claustral, formigas cortadeiras, saúva.

1. INTRODUCTION

The leaf-cutter ant *Atta* (Hymenoptera: Formicidae) is phylogenetically the most derived taxon among the fungus-growing ants (Attini) (Schultz & Meier, 1995). Mature colonies may hold 5 million polymorphic workers (Weber, 1972) and perform a complex fungiculture repertoire (Weber, 1972; Currie, 2001; Wirth et al., 2003; Augustin et al., 2011).

Atta colonies are generally initiated by a single claustral founding queen (Weber, 1972; Fernandéz-Marín et al., 2004). Following the nuptial flight, each *Atta* gyne sheds her wings, digs a primary chamber between 5 and 25 cm deep, and closes the entrance and tunnel of the nest using soil from the primary chamber (Autouri, 1942; Mariconi, 1970; Mintzer, 1987; Weber, 1972, 1982). One to three days after nuptial flight (Autouri, 1942; Augustin, 2007) an *Atta* foundress expels a fungal pellet that has been previously collected from her natal nest and transported in the infrabuccal pocket.

Among the leaf-cutters, *Atta* queens are the one that place the fungal pellet directly on the chamber floor, where the incipient fungus garden grow. By contrast,

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Acromyrmex queens employ roots as a platform where they attach the fungal garden as a prophylactic behavior against fungal contamination by pathogens from the soil (Fernandéz-Marín, et al., 2004, 2007). The use of plaster as a substratum for colonies of leaf-cutting ants in laboratory is widespread (Forti et al., 1994) because it keeps humidity inside the chambers where the colonies are reared, thus favoring the fungus garden to grow and preventing insect desiccation. In the case of *A. sexdens rubropilosa* colonies, it has been found that after an average of three days since the fungus garden has been expelled the foundress then lays the first batch of eggs. It takes an average of 26 days for the first *A. sexdens rubropilosa* eggs to develop into larvae, which the queens nourish with trophic eggs (Augustin, 2007). *A. sexdens rubropilosa* larvae take an average of 23 days to develop into pupae, which in turn take an average of 21 days to grow into workers (Augustin, 2007). Upon emergence, the first generations of workers gradually start to forage for leaf material to use as substrate to support the continued growth of the fungus garden (Augustin & Lopes, 2008).

Here we describe the preliminary results on brood production and mortality rate of laboratory *A. sexdens rubropilosa* colonies reared in different substratum, showing that dampened plaster is probably the best substratum for rearing this species under laboratory conditions.

2. MATERIAL AND METHODS

Following the nuptial flight on November 6, 2005 in pasture fields within the city of Juiz de Fora (21°46'S–43°21'W, 678m average altitude), southeastern Brazil, *A. sexdens* gynes were collected when they were digging the nest tunnel. The gynes were transported to the laboratory into individual transparent plastic boxes (250 mL).

Each box was covered with a layer of approximately 1 cm of dampened plaster at their base to keep humidity inside the chambers. The plaster layers were prepared mixing up plaster, water and soil in three different manners: 1- plaster and water, in proportions of 1:0,5 (v:v), respectively, resulting in what was called plaster substratum (P); 2- plaster, soil (Latossolo Vermelho) and water, in proportions of 3:1:2 (v:v:v), respectively, resulting in what was called soil substratum (S), and finally 3- plaster, sterilized soil (Latossolo Vermelho) and water, in proportions of 3:1:2 (v:v:v), respectively, resulting in what was called sterilized substratum (St); in this case, soil was sterilized following the methodology of Bessa & Araújo (1995). With the aid of a needle tip, small holes on each chamber cover were made in order to allow air exchange. Only colonies that held the fungus garden were studied, giving a total of 51 queens in (P), 47 queens in (S) and 52 queens in (St). The number of eggs (E), larvae (L), pupae (P) and workers (W) were counted weekly in each colony under a stereomicroscope at magnifications of up to 30x. The nests were maintained from November 6, 2005 to March 25, 2006, at 25 ± 5 °C, 70 to 85% of relative humidity in the dark, and were exposed to light only when observations were made. In order to evaluate differences in the absolute frequencies of both the mortality and number of offspring in colonies with the three different substratum, all data were submitted to *Kruskal-Wallis* (5%) test.

3. RESULTS

Queens in (St) survived to care for their larvae but died just after the first pupae emerged. Similarly, queens in (S) survived to care for their pupae but not their workers. After an observational period of twenty weeks, all queens in (S) and in (St) had died. Only queens in (P) produced workers, even though 79.8% of them died before that. By the end of the observation period, 95.7% of all the queens perished. The mean number of eggs were significantly higher in (P) ($H = 128.8289$; $p < 0.001$) comparatively to both (S) and (St), which did not differ significantly from each other ($p = 0.0856$). Similarly, the mean number of larvae were significantly higher in (P) ($H = 48.0787$; $p < 0.001$) comparatively to both (S) and (St), which did not differ significantly from each other ($p = 0.7791$) (Fig. 1). The number of

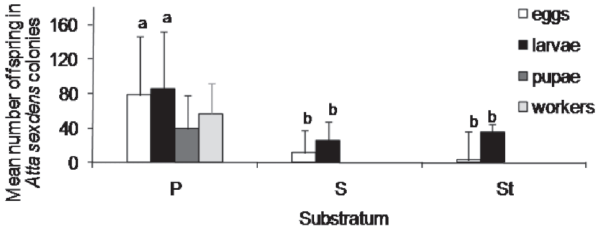

Figure 1 - Mean number and standard error of offspring in *Atta sexdens* colonies in laboratory, according to substratum type. P = Plaster substratum; S = Soil substratum; St = Sterilized substratum. Different letters indicate significant difference according to *Kruskal-Wallis* (5%) test.

pupae and workers could not be compared among the substratum because queens had died just after the first pupae emerged in (S).

4. DISCUSSION

These results possibly indicate potentially different environmental conditions that each substratum provides to the ant colonies in the laboratory. Gypsium, or dihidratade calcium sulfate ($CaSO_4.2H_2O$) is commonly found in sedimentary rocks and is formed by a chemical reaction between both sulfuric acid and carbon molecules (Assis et al., 1992). Dampened gypsum-based plaster is commonly used as substratum for leaf-cutting ants in laboratory, as it helps keeping humidity inside the chambers where these insects are maintained, thus favoring the fungus garden to grow and preventing insect desiccation (Forti et al., 1994). Possibly due to its chemo-physical characteristics, (P) allowed queens to survive and successfully raise their offspring, comparatively to (S) and (St), both of which contained soil (Latossolo Vermelho) in their structure.

The high mortality of the queens may be due to insufficient body reserves and/or possible non-insemination of the virgin queens upon their nuptial flights. Although we seek data to confirm this, high mortality has also been found for other *Atta* species in nature and even under laboratory conditions, where predators, competitors or desiccation do not contribute to mortality (Hölldobler & Wilson, 1990).

It seems likely to conclude that, when struggling to founding new colonies claustrally and independently, *A. sexdens* gynes face with high rates of mortality, even under controlled conditions of relative humidity and temperature in laboratory. In reference to the types of substratum, further work is needed to evaluate the chemo-physical structure of the different substratum used here. However, our preliminary results show that (P), in relation to (S) and (St), is possibly the one that better keeps humidity inside the chambers, being the most appropriate to rear *A. sexdens* colonies in laboratory.

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