Sociobiology
An international journal on social insects

RESEARCH ARTICLE - ANTS

Is the initial nest depth adapted to favorable conditions for the incipient colony in leaf-cutting ants?

RS Camargo[1], LC Forti[1], CAO Matos[2], NCaldato[1], OS Fonseca[1]

1 - Faculdade de Ciências Agronômicas/UNESP, Botucatu-SP, Brazil

2 - Campus Experimental de Itapeva/UNESP, Itapeva-SP, Brazil

Article History

Edited by
Inge Armbrecht, University del Valle, Colombia
Received 05 January 2016
Initial acceptance 07 April 2016
Final acceptance 06 June 2016
Publication date 15 July 2016

Keywords
Atta sexdens, nest, leaf-cutting ants, social insects, claustral foundation.

Corresponding author
Roberto da Silva Camargo
Laboratório de Insetos Sociais-Praga
Departamento de Produção Vegetal
Faculdade de Ciências Agronômicas/UNESP
Caixa Postal 237, 18603-970, Botucatu-SP, Brazil
Email: camargobotucatu@yahoo.com.br

Abstract

The nest dug by the leaf-cutter ant queen of the genus *Atta* is a vertical tunnel interconnected to a small chamber which holds its offspring and the symbiotic fungus. The depth of the initial chamber of the *Atta sexdens rubropilosa* ranges from 10 to 30 cm below the soil surface. With this information, we asked whether the ideal initial nest depth is adapted to favorable conditions for the initial colony. We hypothesized this depth can provide a minimum temperature range with almost constant temperature, leading to the development of symbiotic fungus and brood yet to emerge. To test this hypothesis, laboratory experiments were carried out and the soil temperature was measured at different depths. The colony development at different temperatures was studied in the laboratory and the brood production (number of eggs, larvae, pupae and adults) was measured until the first workers emergency. Additionally, lipid content and the survival of queens at different temperatures were determined. Our results show a suitable temperature range (ranging from 24.82±3.14°C to 24.11±1.30°C) at a depth of 5 to 25 cm from the ground, an ideal brood development at temperatures of 24 and 28 °C, and consequently a reduction in lipid content of the queens at high temperatures, without affecting their survival in the trial period. These results indicate that the depth of the initial chamber excavated by the queen is suitable for the success of the incipient colony.

Introduction

Nest foundation in *Atta sexdens* is claustral, where the queen uses body reserves for self-preservation and to cultivate the symbiotic fungus and its brood until the emergence of the first workers (Autuori, 1940). Moreover, the nest excavation by the queen requires significant energy investment due to the excavation hours and excavated soil volume (Camargo et al., 2011). However, the ideal depth of the initial chamber and how natural selection acts on the queens are still unknown issues. One can hypothesize that a shallow initial chamber requires the queen's less time and energy, but the queen, the symbiotic fungus and her brood would be exposed to environmental variables such as temperature and humidity (Bollazzi et al., 2008; Lapointe et al., 1998). In contrast, a deep initial chamber could provide a more stable microclimate, but would require more time and energy spent with additional exposure to predators during its construction (Autuori, 1940). In addition, the survival queen and colony development can be affected in several ways: first, predation is intense at the nuptial flight and nest excavation, the main agents is birds, frogs, lizards , armadillos and insects, such as *Canthon* spp. (Coleoptera, Scarabaeidae) and ants (*Solenopsis*, *Paratrechina* and *Nonamyrmex*) (Autuori, 1950; Erthal & Tonhasca, 2001; Forti et al., 2012); second, excessive nest digging and greater depths require the queen's substantial digging effort, which directly affects her survival at this initial stage of the colony (Camargo et al., 2011); third, entomopathogenic or parasitic

fungi of symbiotic fungus may infect the founding colony (Bento et al., 1991; Rodrigues et al., 2010; Marti et al., 2015; Currie et al., 1999).

Probably, there is an adequate depth for the initial chamber, as there are various nest depths among the *Atta* species. For example, initial chamber depths range from 7.5 to 12 cm in *A. colombica* (Weber, 1972), 6.5 to 13 cm in *A. cephalotes* (Weber, 1937), 15 to 25 cm in *A. texana* (Moser, 1967), 10 to 30 cm in *A. sexdens rubropilosa* (Camargo & Forti, 2013), 10 to 15 cm in *A bisphaerica* (Cardoso et al., 2014), 11 to 34 cm in *A capiguara* (Mariconi, 1974) and 9 to 15 cm in *A. insulares* (Bruner & Valdes Barry, 1949). It is known that digging behavior is affected by temperature and soil moisture, reflecting the nesting habits of species. The initial chamber depth significantly differed between the ant species, with *A. bisphaerica* showing deeper chambers than *A. sexdens rubropilosa* (Cardoso et al., 2014). A*tta bisphaerica* presents nests in full sun and forages predominantly grasses, while *Atta sexdens rubropilosa* presents nests in shaded places and forages dicots (Mariconi, 1970; Fowler et al., 1986; Nagamoto et al., 2009), therefore both species differ in nesting habits and foraging strategies. Probably, the differences in nest depth between species are correlated to soil temperature, because shading alters soil temperature regimes by locally diminishing soil temperature (Rosenberg et al., 1983). In this perspective, it is reasonable that nest exposed in grassland should have deeper fungus chamber than nest under shade of trees or inside the woods, given that soil temperature is negatively correlated with soil depth (Rosenberg et al., 1983). For leaf cutting ants, soil moisture and temperature act together: (i) Bollazzi et al. (2008) verified that workers' thermopreferences lead to the construction of superficial nests in cold soils, and subterranean ones in hot soils; and (ii) Pielström and Roces (2014) verified that soil moisture also varies according to soil depth, and demonstrably affects the digging behavior of leaf cutting ants.

So, is the ideal depth of the initial nest adapted to favorable conditions for the incipient colony? We hypothesized that this depth would provide a minimum temperature range with almost constant temperatures, conducive to the development of the symbiotic fungus and the brood yet to emerge. To test this hypothesis, we used brood production as a measure of the colony development at different artificially controlled temperatures in the laboratory.

Material and Methods

*Collection of queens of **Atta sexdens rubropilosa***

The collection was carried out during the nuptial flight that occurred on October 5, 2014, by capturing queens that were founding their colonies at the Lageado Experimental Farm, Faculdade de Ciências Agronômicas – Universidade Estadual Paulista–Botucatu, São Paulo state, Brazil. The newly collected queens were stored in plastic containers, 11 cm in diameter and 8 cm in height, containing 1 cm of plaster at the bottom to maintain humidity. The queens were transported to the Laboratory of Social-Pest Insects FCA/UNESP – Botucatu, where all experiments were performed. . The queens were allocated in laboratory (BOD Incubators) at different artificially controlled temperatures.

Field experiment - Soil temperature and initial nest depths

The temperature was measured at different soil depths (Dark red latosol): 0 cm (ground surface) 5 cm, 25 cm, 45 cm, 150 cm. The temperatures were measured using a thermal sensor with data logger TESTO (model 175-T2), with daily readings every five minutes using the thermal sensor in October (N=7801), November (N=8001), December (N=8001), January (N=6311), February (N=2000) and March (N=2000). The sensors were buried at different soil depths, as mentioned before. The readings were discontinued due to some days of excessive rain which could compromise the measurement accuracy.

At the end of March, 89 early nests were excavated in order to measure the depth (cm) of the initial chamber (Fig 2). Additionally, for better view of its structure some were cement molded (two nest), according to the methodology used by Moreira et al. 2004. For molding, a mixture of cement and water at a proportion of 2:10 (kg/L) was pumped into the holes of each nest. The nests were excavated one week after they had been filled with cement using small and manual tools to avoid destruction of the nests. After excavation, the nests were photographed (Fig 2).

Laboratory experiment:
Temperature effect on the establishment of initial colonies

We tested the following hypotheses: the observed depth would provide a small temperature range with almost constant temperature which leads to successful development of brood. The rationale of the experimental approach was to compare the influence of temperature in colony development, measured by offspring production over time (numbers of eggs, larvae, pupae and adults raised until the first workers emerge) of queens at different temperatures.

To test this hypothesis, we used brood production as a measure of the colony development at different artificially controlled temperatures in the laboratory (BOD Incubators, Eletrolab). The brood production (number of eggs, larvae, pupae and adult) was carefully examined and recorded weekly using a stereoscopic microscope (Nikon, SMZ 1000) according to Camargo et al. (2011). The experimental groups were divided as follows:

1- Development at 15°C: 25 queens remained under the effect of low temperature (15°C) until the emergence of the first workers.

2- Development at 20°C: 25 queens remained under the effect of medium temperature (20°C) until the emergence of the first workers.

3- Development at 24°C: queens remained under the effect optimal temperature (24°C) until the emergence of the first workers.

4- Development at 28°C: 25 queens remained under the effect of high temperature (28°C) until the emergence of the first workers.

Temperatures of 15°C, 20°C, 24°C and 28°C were chosen due to temperature preference for the allocation of brood and fungus by worker, according to Bollazzi and Roces (2002), Powell and Stradling (1986). The mortality of queens was also measured weekly.

Lipid determination

The queens were immersed in organic solvent (pentane) until they reached a constant weight (Cook et al., 2010). The procedure was as follows: Fresh weight was individually determined; queens were dried for 24 hours at 50°C and their dry weight was determined; lipids were extracted with pentane for 24 hours and then dried and weighed on an analytical balance. The procedure was repeated for 72 hours of extraction. Values are expressed as a percentage (%) of the dry mass of the queen.

Statistical Analysis

The external and internal temperature (soil) was compared by the F test at each depth, assuming there is an equal temperature variation between the internal (soil inside) and external temperature (soil surface) (H0), with 5% significance. The total production (egg, larva, pupa and adult) was subjected to multiple comparison test of variances (Ryan, 1960) between the temperatures 15°C, 20°C, 24°C and 28°C. After that, a generalized estimating equations model (Generalized linear model for dependent data) was used in the calculation using an iterative process proposed by Liang and Zeger (1986) that was fitted using a Poison probability model that has the same mean-variance relationship. A correlation structure that minimized Pan's QIC was chosen. QIC is a statistic that generalizes AIC to generalized estimating equations, according to Pan (2001), and comes in two versions. One version is used for selecting a correlation structure and the second version is used for choosing models, all of which were fitted with the same correlation structure. A correlation structure AR-1 that includes time dependence and reflects the manner in which the data were collected was selected for eggs and larvae. An independent correlation structure was selected for pupae. The model considering the variables time and temperature as discrete was selected from three models.

The Cox proportional hazards regression model was fitted to the queens' survival at different temperatures. In addition, a linear regression was fitted between variations in lipid content of queens and temperature. Statistical analyses and graphs were processed by R 2.9.0 for Windows.

Results

The temperature variance of the measured temperatures at different soil depths did not change (Table 1). This result corroborates the hypothesis that the temperature is relatively constant at different soil depths when compared to the external temperature (Table 1). The depth of the initial chamber was 17.5±5.5 (Fig 2), in a temperature range varying from 24.82±3.14°C (5 cm) to 24.11±1.30°C (25 cm) considering the averages of every month (Fig 1). As for the months, October had the lowest temperature at all depths, but over the following months it tended to increase and remain constant (Fig 1).

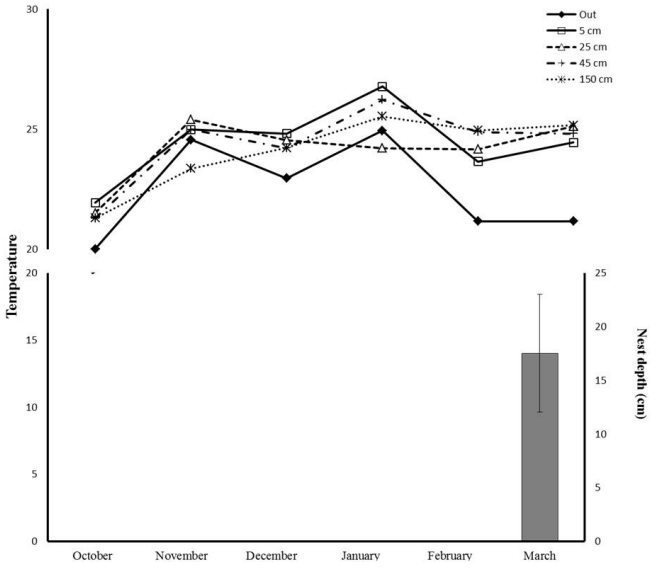

Fig 1. Mean temperature (°C) of soil and nest depth (cm± SD) of initial nest of *Atta sexdens* for six months after nuptial flight.

In the laboratory, the queens showed differences in offspring production under different temperatures. At 15°C, no queen produced offspring, there was only underdeveloped symbiotic fungus cultivation. At 20°C, the queens produced eggs (396±98.73) and larvae (15.85±17.51), adults during the six weeks of observation. At 24°C the queens produced eggs (309±67.07), larvae (173.12±78.38), pupae (51.44±31.88) and adults (1.40±0.55). And at 28°C the queens produced eggs (238.40±80.47), larvae (184.00±79.30), pupae (81.24±51.67) and adults (36.92±29.92).

The variance of the total production (egg, larva, pupa and adult) was 0.00029for 15°C, 0.00096 for 20°C, 0.00313 for 24°C and 0.00892 for 28°C. The multiple variance comparisons showed no differences between the 15°C and 20°C groups (p-value $F_{7,23} = 0.1117$), but there were differences ranging between 20°C and 24°C (p-value $F_{23,21} = 0.006973$), 20°C and 28°C (p-value $F_{23,19} = 1.863\ 10^{-6}$) and finally between 24°C and 28°C (p-value $F_{21,19} = 0.02244$) (Table 2). The GLM showed significant effects regarding the number of eggs produced during the weeks, in the 2nd week the average was 0.41 times higher than the average of the 1st week ($P>|Z| = 4.767\ 10^{-18}$). In larvae, in the 6th week the average was 0.48 times lower than the average of the 3rd week ($P>|Z| = 2{,}361\ 10^{-6}$). There was no significant effect for pupae and was not estimated for adults.

Table 1. Summary statistics for soil temperature (mean ±sd) during 6 months.

Depth	thermal sensor	October	November	December	January	February	March
5 cm	in	21.95±3.11 σ^2=9.67	24.98±0.95 σ^2=9.04	24.80±2.15 σ^2=4.63	26.77±2.19 σ^2=4.80	23.65±2.29 σ^2=5.90	24.44±1.47 σ^2=2.15
	out	20.01±3.90 σ^2=15.21	24.55±1.29 σ^2=16.61	22.95±2.95 σ^2=16.61	24.91±3.40 σ^2=11.59	21.15±3.91 σ^2=15.26	23.29±4.26 σ^2=4.26
	F test	F=1.572 P≥0.05	F=1.8386 P≥0.05	F=1.88 P≥0.05	F=2.42 P≥0.05	F=2.88 P≥0.05	F=8,4323 P≥0.05
25 cm	in	21.51±1.37 σ^2=18.88	25.38±1.19 σ^2=14.13	24.54±1.06 σ^2=11.22	24.21±1.56 σ^2=2.43	24.16±0.82 σ^2=0.67	25.11±0.65 σ^2=0.42
	out	20.74±1.50 σ^2=22.40	24.68±1.25 σ^2=15.59	23.70±1.20 σ^2=14.47	22.04±3.64 σ^2=13.26	24.45±3.18 σ^2=10.12	23.91±3.22 σ^2=10.33
	F test	F=1.1867 P≥0.05	F=1.1031 P≥0.05	F=1.29 P≥0.05	F=5.46 P≥0.05	F=15.08 P≥0.05	F=24.67 P≥0.05
45 cm	in	21.29±0.81 σ^2=6.70	24.98±0.95 σ^2=9.03	24.25±0.76 σ^2=5.81	26.22±0.72 σ^2=5.14	24.88±1.21 σ^2=1.47	24.81±1.06 σ^2=1.13
	out	20.64±1.55 σ^2=24.0	24.55±1.28 σ^2=16.61	23.75±1.14 σ^2=12.92	25.37±2.08 σ^2=43.57	20.96±3.98 σ^2=15.87	24.47±3.72 σ^2=13.80
	F test	F=3.58 P≥0.05	F=1.8386 P≥0.05	F=2.22 P≥0.05	F=8.48 P≥0.05	F=10.77 P≥0.05	F=12.19 P≥0.05
150 cm	in	21.29±0.21 σ^2=0.05	23.36±0.77 σ^2=0.59	24.21±0.24 σ^2=0.05	25.53±0.10 σ^2=0.01	24.93±0.14 σ^2=0.018	25.16±0.05 σ^2=0.002
	out	20.61±1.67 σ^2=2.80	24.59±1.30 σ^2=1.71	23.84±1.11 σ^2=1.23	21.65±3.74 σ^2=14.01	23.02±3.16 σ^2=10.01	23.78±3.13 σ^2=9,85
	F test	F=6.179 P≥0.05	F=2.8692 P≥0.05	F=2.11 P≥0.05	F=1389.8 P≥0.05	F=548.19 P≥0.05	F=4311.59 P≥0.05

In – thermal sensor in the soil
Out – thermal sensor at soil surface

Fig 2. Initial nest of *Atta sexdens*: A) Excavated at 4 months after nest foundation; B) Initial nest molded with cement at four months after nest foundation.

The average lipid content was 32.58±2.98% for queens at 15°C, 30.90%±2.94 at 20°C, 28.84±3.41% at 24°C and 26.78±5.70% at 28°C. Linear regression analysis of the lipid content according to temperature was significant (p-value $F_{1,74}$ < 0.0001), but due to the high variation, the quality of the model fit is low (R^2 adjusted = 0.1836) (Fig 3).

Table 2. Summary of multiple comparisons of variances at 5% level experimentwise.

Group	K number of groups	n	Nominal level	Nominal level/2	Significance
15-28	4	4	0,00833	0,00417	**
15-24	3	4	0,0125	0,00625	**
20-28	3	4	0,0125	0,00625	**
15-20	2	4	0,025	0,0125	n.s.
20-24	2	4	0,025	0,0125	**
24-28	2	4	0,025	0,0125	**

n.s. no significance

In relation to queen survival, 32% of queens survived (17 deaths) at 15°C, 92% of queens survived (2 deaths) at 20°C, 88% of queens survived (3 deaths) at 24°C and 80% of queens survived (5 deaths) at 28°C. The Cox proportional hazards regression model fitted to the data shows no difference for queens' survival in relation to the different temperatures (P>|Z|=0.729).

Discussion

The depth that the queen of *Atta sexdens rubrupilosa* digs her initial chamber promotes a minimum temperature range, with a temperature that is favorable to the development

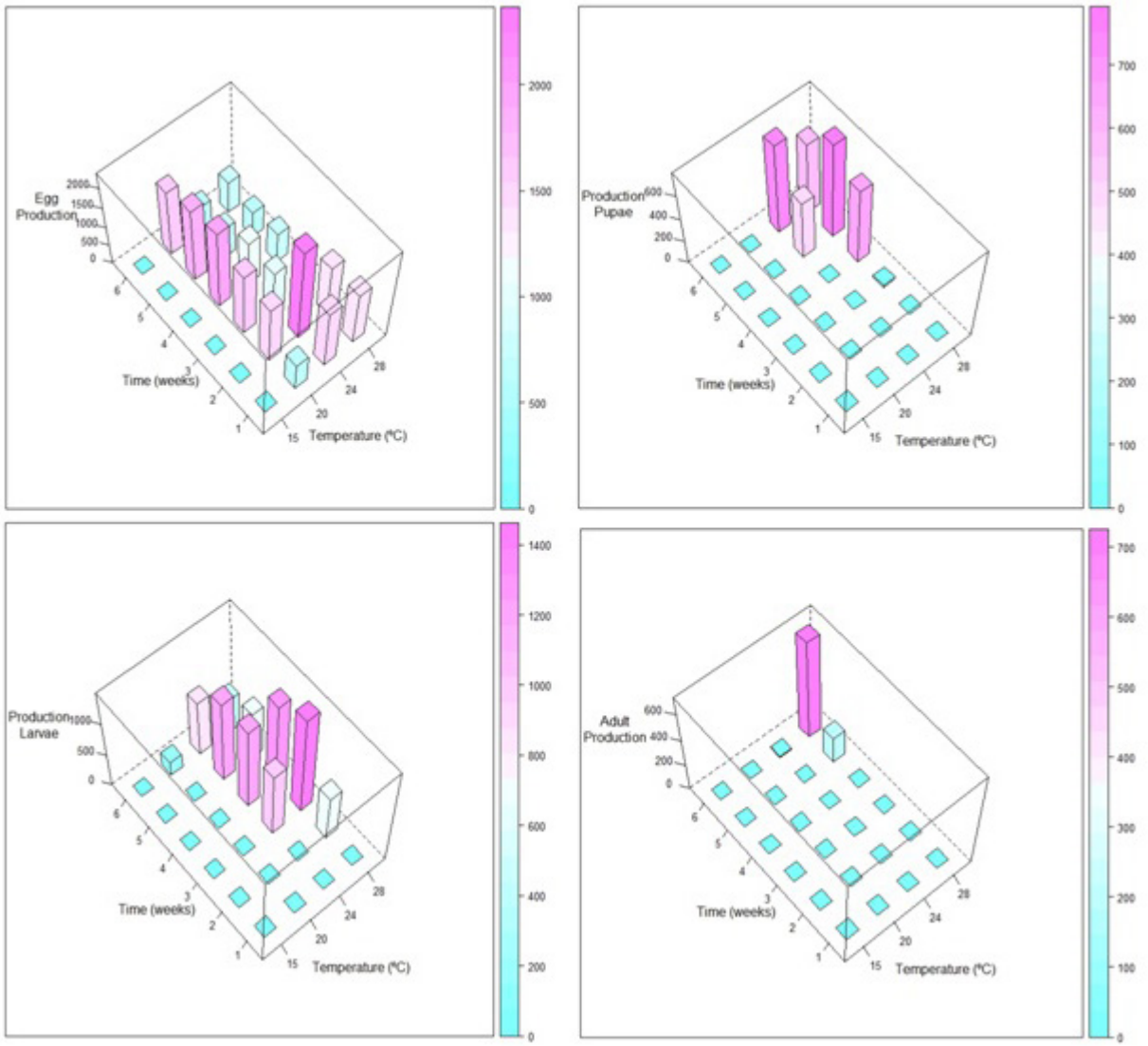

Fig 3. Eggs, larvae, pupae and adults produced by the queens in different temperatures (15°C, 20°C, 24°C e 28°C) during six weeks**.**

of symbiotic fungus and her brood. This hypothesis was confirmed by the results obtained which showed that the depth of the initial chamber was 17.5±5.5 in a temperature range varying from 24.82±3.14°C (5 cm) to 24.11±1.30°C (25 cm) (Table 1, Fig 1). Combined with the laboratory results, the temperatures ranging from 24 to 28°C allowed the queens to produce in large numbers and rapidly in the 6th and in the 4th week there were adults (Fig 3).

The queen determines the initial chamber depth, this is a regulated process involving the use of internal references: queens excavated their tunnels either until a particular depth was reached or for some predetermined length of time according to Fröhle and Roces 2012. For example, *Atta sexdens* queens dug around 11 to 30 cm in the soil to build their chamber (Camargo & Forti, 2013) to a depth for suitable temperature for fungus garden and brood. Some studies have recorded fungus temperatures in field colonies: 25–28°C for *Atta sexdens* (Stahel and Geijskes1940; Eidmann 1935), 27.5 °C for *Atta vollenweideri* (Kleineidam & Roces, 2000), and around 27°C for *A. heyeri* (Bollazzi & Roces, 2002). For brood, Bollazzi and Roces (2002) found that *A. lundi* workers avoid, while digging, soil temperatures that are unsuitable for brood development, and prefer those temperatures that are known to maximize fungal growth (Bollazzi & Roces, 2002). Thus, our results show that soil temperature at depths of 5 to 25 cm in the months of October to March provide ideal conditions for the fungus garden and brood (Fig 1).

The number of eggs produced during the 2nd week was 0.41 times greater than the average of the 1st week, probably due to the cumulative effect on the number of eggs (incubation period greater than seven days) and the absence of larval oophagy (Autuori, 1942, Camargo et al., 2007). In larvae, in the 6th week the average was 0.48 times lower than the average of the 3rd week. This result is related to the larvae becoming pupae during the observation period, thus the number decreased. Furthermore, there was no significant effect on pupae and adults, probably due to the low number found in the treatments. In general, comparing the development of initial colonies of *Atta* with data from the literature (Autuori, 1942; Pereira-da-Silva, 1979), differences were found in the

incubation, larval and pupal periods. For *Atta sexdens*, the incubation period lasted 25 days, the larval period 22 days and pupal period 10 days (Autuori, 1942). Camargo et al. (2011) obtained similar results from the same leaf-cutting ant species, incubation period lasted 30 days, larval period 25 days and pupal period 15 days. In *Atta capiguara*, in the sampled periods, the eggs were most prevalent at 1 to 18 days, larvae at 21–38 days, pupae at 39–55 and adults at 58–67 days (Pereira-da-Silva, 1979). When comparing the quantity of brood produced with the findings of Autuori (1942) and Pereira-da-Silva (1979), a pattern is verified in the production of eggs, larvae, pupae and adults, in other words, generally showing high variability (Fig 3).

The lipid content decreased in relation to increasing temperature (Fig 4), or high temperatures (24 °C and 28°C) producing more offspring than at low temperatures (15°C and 20°C), thereby consuming more lipids. The high lipid content is extremely important to the queens of *Atta sexdens*, responsible for maintaining a high brood production rate for several weeks (Camargo et al., 2011). According to Fowler et al. (1986), egg production by the queen during the 3 or 4 months of the colony lifespan is correlated with the activity cycle of the corpora allata. The corpora allata are responsible

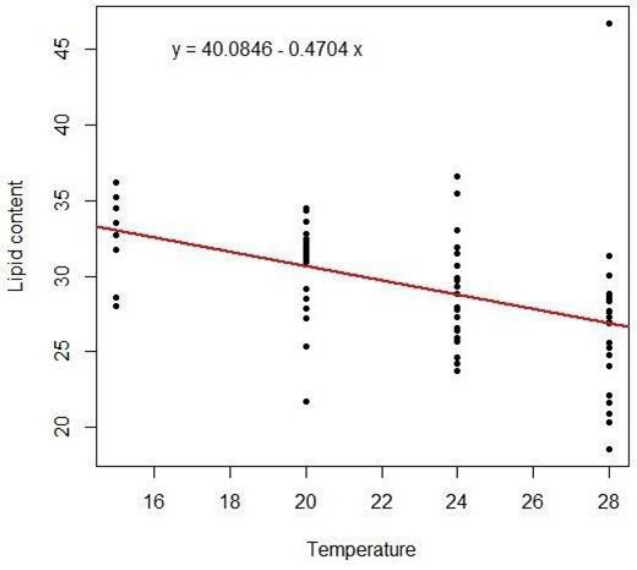

Fig 4. Linear regression between variations in lipid content of queens and temperature.

for the synthesis of juvenile hormone, which acts during oviposition of founder queens, as verified in females that suffer allatectomy (Barker, 1978). The juvenile hormone acts on the fatty body, initiating the synthesis of vitellogenins. Thus, the production of brood depends on body reserves (lipids from the fatty body and protein from the wing muscles), since the queen does not feed during the claustral foundation.

For queen survival at different temperatures, there was a marked mortality increase at low temperature (15°C), however with no significant difference among them. Although temperature does not affect the survival of queens, the first 3 months of *Atta* colony life have many obstacles for queens and their incipient colonies (Fowler et al., 1986; Marti et al., 2015). The survival of the queen can be reduced in several ways: predation (Erthal and Tonhasca, 2001; Forti et al., 2012), nest excavation effort (Camargo et al., 2011) and entomopathogenic or parasitic fungi of symbiotic fungus (Bento et al., 1991; Rodrigues et al., 2010; Marti et al., 2015; Currie et al., 1999).

In summary, our results show an appropriate temperature range (from 24.82 ±3.14°C to 24.11±1.30°C) at a depth of 5 to 25 cm from the ground, good brood development at temperatures of 24 and 28 °C, thus reducing the lipid content of the queens at high temperatures without affecting their survival in the trial period. These results indicate that the depth of the initial chamber excavated by the queen is suitable for the success of the incipient colony.

Acknowledgements

We are grateful to Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) for the financial support and stipends to the authors [grant numbers 2007/04010-0 and 2007/07091-0]. R.S. Camargo thanks Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) for the Postdoctoral fellowship. LCF thanks Conselho Nacional de Desenvolvimento Cientifico e Tecnológico for the research assistance (301917/2009-4).

References

Autuori M (1940). Algumas observações sobre formigas cultivadoras de fungo (Hymenoptera: Formicidae). Revista de Entomologia, 11 (1-2): 215-226.

Autuori M (1942). Contribuição para o conhecimento da saúva (Hymenoptera: Formicidae) II - o sauveiro inicial (*Atta sexdens rubropilosa* Forel, 1908). Arquivos do Instituto Biológico, 13: 67-86.

Autuori M (1950). Contribuição para o conhecimento da saúva (*Atta* spp.). V. Número de formas aladas e redução dos sauveiros iniciais. Arquivos do Instituto Biológico, 19: 325-331.

Barker JF (1978). Neuroendocrine regulation of oocyte maturation in the imported fire ant *Solenopsis invicta*. General and Comparative Endocrinology, 35: 234–237.

Bento, J.M.S., Della Lucia, T.M.C., Muchovej, R.M.C. & Vilela, E.F. (1991). Influência da composição química e da população microbiana de diferentes horizontes do solo no estabelecimento de sauveiros iniciais *de Atta laevigata* (Hymenoptera: Formicidae) em laboratório. Anais da Sociedade Entomológica do Brasil, 20: 307-316.

Bollazzi M, Kronenbitter J, Roces F (2008). Soil temperature, digging behaviour, and the adaptive value of nest depth in South American species of *Acromyrmex* leaf-cutting ants. Oecologia, 158: 165-175.

Bollazzi M, Roces F (2002). Thermal preference for fungus culturing and brood location by workers of the thatching grass-cutting ant *Acromyrmex heyeri*. Insectes Sociaux, 49: 153-157

Bollazzi, M., Kronenbitter, J. & Roces, F. (2008). Soil temperature, digging behaviour, and the adaptive value of nest depth in South American species of *Acromyrme*x leafcutting ants. Oecologia, 158: 165-175. Mariconi, F.A.M. (1970). As saúvas. São Paulo: Agronômica Ceres.

Bruner SC, Valdés Barry F (1949). Observaciones sobre la biología de la bibijagua (Hymenoptera: Formicidae). Memorias de la Sociedad Cubana de Historia Natural, 19: 135-154.

Camargo RS, Forti LC, Fujihara RT and Roces F (2011). Digging effort in leaf-cutting ant queens (*Atta sexdens rubropilosa*) and its effects on survival and colony growth during the claustral phase. Insectes Sociaux, 58: 17-22.

Camargo RS, Forti LC (2013). Queen lipid content and nest growth in the leaf cutting ant (*Atta sexdens rubropilosa*) (Hymenoptera: Formicidae). Journal of Natural History, 47: 65-73.

Camargo RS, Forti LC, Lopes JF, Noronha Junior NC, Ottati ALT (2007). Worker laying in leafcutter ant *Acromyrmex subterraneus brunneus* (Formicidae, Attini). Insectes Sociaux, 14: 65-75.

Cardoso SR, Forti LC, Nagamoto NS, Camargo RS (2014). First-year nest growth in the leaf-cutting ants *Atta bisphaerica* and *Atta sexdens rubropilosa*. Sociobiology, 61: 243-249.

Cook SC, Eubanks MD, Gold RE, Behmer ST (2010). Colony level macronutrient regulation in ants: mechanisms, hoarding and associated costs. Animal Behaviour, 79: 429-437.

Currie CR, Muller UG, Malloch D (1999). The agricultural pathology of ant fungus gardens. PNAS USA, 96: 7998-8002.

Eidmann H (1935). Zur Kenntnis der Blattschneiderameise *Atta sexdens* L., insbesondere ihrer Okologie. Zeitschrift für Angewandte Entomologie, 22: 185-436.

Erthal Jr M, Tonhasca AJ (2001). *Attacobius attarum* spiders (Corinnidae): myrmecophilous predators of immature forms of the leaf-cutting ant *Atta sexdens* (Formicidae). Biotropica 33: 374–376.

Forti LC, Rinaldi IMP, Camargo RS, Fujihara RT (20012). Predatory Behavior of *Canthon virens* (Coleoptera: Scarabaeidae): A Predator of Leafcutter Ants. Psyche, 1-5.

Fowler HG, Pereira-da-Silva V, Forti LC, Saes NB (1986). Population dynamics of leaf-cutting ants: a brief review. In: Lofgren CS, Vander Meer RK (eds) Fire ants and leaf-cutting ants: Biology and management. Westview Press, 123–145.

Fowler, H.G., Forti, L.C.; Pereira-da-Silva, V. & Saes, N.B. (1986). Economics of grass-cutting ants. In C.S. Lofgren & R.K. Vander Meer (Eds.), Fire ants and leaf-cutting ants: biology and management (pp.18-35). Boulder: Westview Press.

Fröhle K, Roces F (2012). The determination of nest depth in founding queens of leaf-cutting ants (*Atta vollenweideri*): idiothetic and temporal control. Journal of Experimental Biology, 215: 1642–1650.

Kleineidam C, Roces F (2000). Carbon dioxide concentrations and nest ventilation in nests of leaf cutting ants *Atta vollenweideri*. Insectes Sociaux, 47: 241-248.

Lapointe SL, Serrano MS, Jones PG (1998). Microgeographic and vertical distribution of *Acromyrmex landolti* (Hymenoptera: Formicidae) nests in a neotropical savanna. Environmental Entomology, 27: 636-641.

Liang YL, Zegger SL (1986). Longitudinal data analysis using generalized linear models. Biometrika, 73(1): 13-22.

Pan W (2001). Akaike's information criterion in generalized estimating equations. Biometrika, 83: 551–562.

Mariconi FAM (1974). Contribuição para o conhecimento do sauveiro inicial da "saúva parda" *Atta capiguara* Gonçalves, 1944 (Hymenoptera: Formicidae). Anais da Sociedade Entomológica do Brasil, 3: 5-13.

Marti HE, Carlson AL, Brown BV, Mueller UG (2015). Foundress queen mortality and early colony growth of the leafcutter ant, *Atta texana* (Formicidae, Hymenoptera). Insectes Sociaux, 62: 357-363.

Moreira AA, Forti LC, Andrade APP, Boaretto MAC, Lopes J FS (2004). Nest architecture of *Atta laevigata* (F. Smith, 1858) (Hymenoptera: Formicidae). Studies on Neotropical Fauna and Environment 39: 109-116.

Moser JC (1967). Mating activities of *Atta texana* (Hymenoptera, Formicidae). Insectes Sociaux 14: 295-312.

Nagamoto, N.S., Carlos, A.A., Moreira, S.M., Verza, S.S., Hirose, G.L. & Forti, L.C. (2009). Differentiation in selection of dicots and grasses by the leaf-cutter ants *Atta capiguara*, *Atta laevigata* and *Atta sexdens rubropilosa*. Sociobiology, 54: 127-138.

Pagnocca FC, Legaspe MC, Rodrigues A, Ruivo CC, Nagamoto NS, Bacci JR M, Forti LC (2010) . Yeasts isolated from a fungus-growing ant nest, including the description of *Trichosporon chiarellii* sp. nov., an anamorphic basidiomycetous yeast. International Journal of Systematic and Evolutionary Microbiology, 60: 1454-1459.

Pereira-da-Silva V (1979). Dinâmica populacional, biomassa e estrutura dos ninhos iniciais de *Atta capiguara* Gonçalves, 1944 (Hymenoptera: Formicidae) na região de Botucatu, SP; Tese de Doutorado, Universidade Estadual Paulista 77 p.

Pielström, S. & Roces, F. (2014). Soil moisture and excavation behaviour in the Chaco leaf-cutting ant (*Atta vollenweideri*): digging performance and prevention of water inflow into the nest. PLoS ONE 9(4): e95658. doi:10.1371/journal. pone.0095658

Powell RJ, Stradling DJ (1986). Factors influencing the growth of *Attamyces bromaticus*, a symbiont of attine ants. Transactions

of the British Mycological Society, 87: 205-213.

R Core Team (2015). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. http://www.R-project.org/

Rodrigues A, Silva A, Bacci Júnior M, Forti LC, Pagnocca FC (2010). Filamentous fungi found on foundress queens of leaf-cutting ants (Hymenoptera: Formicidae). Journal of Applied Entomology, 134: 342–345.

Rosenberg, N.J., Blad, B.L., & Verma, S.B. (1983). Micro-climate-the biological environment. New York: Wiley.

Ryan TA (1960). Significance tests for multiple comparison of proportion, variance, and other statistics. Psychological Bulletin, 57: 318-328

Stahel G, Geijskes DC (1940). Observations about temperature and moisture in *Atta* nests**.** Revista de Entomologia, 11: 766-775.

Weber NA (1937). The biology of the fungus-growing ants – Part II. Nesting habits of the bachac (*Atta cephalotes* L.). Australian Tropical, 14: 223-226.

Weber NA (1972). Gardening Ants – the Attines. PA: The American Philosophical Society, Philadelphia.