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Post-fire effect of savannah vegetation on the establishment of new colonies of *Atta sexdens rubropilosa* (Hymenoptera: Formicidae)

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Abstract

Establishing their initial colony is probably the most critical moment in the life of leaf-cutting ants. The non-establishment is connected to abiotic and biotic factors, and the high mortality rates of initial colonies are possibly associated with entomopathogenic or antagonistic microorganisms to the symbiotic fungus present in the soil, hosted by these ants. Fire in the vegetation, depending on the intensity, is known to cause significant changes to the soil physical, chemical, and microbiological properties. The impact of a fire in savannah vegetation (Cerrado) on the establishment of early colonies of *Atta sexdens rubropilosa* Forel (Hymenoptera: Formicidae) was evaluated. For this end, two areas were selected, one where there had been an accidental fire, and a contiguous one with the same size and vegetation characteristics without burning. In these areas and in soil collected in the same areas and stored in the laboratory, females recently fertilized in the nuptial flight were placed to excavate the soil and establish their colonies. Post-fire changes in the soil chemical and microbiological properties were quantified and correlated successfully in the establishment of new colonies of this leaf-cutting ant. Under field conditions, the females of *A. sexdens rubropilosa* did not show preference for which areas to excavate: the ones that had been burned or the ones that were unburned; under this condition, no colony survived according to the evaluation performed 120 d after the nuptial flight. Under laboratory conditions, the majority of the females excavated the soil, whether it had been burned or not. However, the establishment of initial colonies was significantly higher in soils collected far from the surface and in areas that had not directly been affected by the fire, showing a negative effect of fire on colony establishment under laboratory conditions.

Key Words: female; initial nest; nuptial flight; leaf-cutting ant; fire

Resumo

Estabelecer sua colônia inicial provavelmente seja o momento mais crítico da vida das formigas-cortadeiras. O não-estabelecimento está ligado a fatores abióticos e bióticos e, possivelmente, as altas taxas de mortalidade de colônias iniciais estejam associadas aos microrganismos entomopatogênicos ou antagônicos ao fungo simbionte cultivado por essas formigas presentes no solo. Sabe-se que o fogo na vegetação, dependendo de sua intensidade, provoca alterações significativas nas propriedades físicas, químicas e microbiológicas do solo. Assim, neste trabalho avaliou-se o impacto de um incêndio em vegetação de savana (cerradão) no estabelecimento de colônias iniciais de *Atta sexdens rubropilosa* Forel (Hymenoptera: Formicidae). Para isso, foram selecionadas áreas onde ocorreu uma queima acidental e outra, de mesmo tamanho, com mesmas características vegetacionais e contígua sem queima. Nessas áreas e em solo coletados nessas mesmas áreas e acondicionados em laboratório foram colocadas fêmeas recém copuladas no dia da revoada para perfurarem o solo e fundarem suas colônias. Alterações nas propriedades químicas e microbiológicas do solo pós-fogo foram quantificadas e correlacionadas com sucesso no estabelecimento de novas colônias dessa formiga-cortadeira. Em condições de campo, as fêmeas de *A. sexdens rubropilosa* não apresentaram preferência para perfurar o solo de áreas queimadas ou não e, nessa condição, nenhuma colônia sobreviveu, em avaliação realizada 120 dias depois da revoada. Em condições de laboratório, a maioria das fêmeas perfurou o solo, independentemente de o solo onde elas foram acondicionadas ter sido queimado ou não. Entretanto, o estabelecimento inicial de colônias foi significativamente maior em solos coletados distantes da superfície e em areas que não sofreram ação direta do fogo, o que mostrou efeito negativo do fogo no estabelecimento de colônias no laboratório.

Palavras Chave: fêmeas; ninhos iniciais; voo nupcial; formigas-cortadeiras; fogo

Unlike other disturbing physical agents, fire is influenced by the type of plant community, and its occurrence plays a key role in the ecosystem dynamics of many world biomes (Whelan 1995). The direct effects and subsequent implications depend on the fire severity, which in turn depends on the availability and arrangement of the combustible material, soil moisture and temperature, topography, wind, and frequency and duration of heat (Frizzo et al. 2011). The negative effects of fire are in general less evident in ants than in other arthropods because most of them build nests in places that protect them against the intense heat. Furthermore, due to their social organization, ants are adapted for fast recovery (Naves 1996).

The direct impact of fire on *Atta* and *Acromyrmex* (Hymenoptera: Formicidae) nests in the field has already been studied by some au-

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thors (Anjos et al. 1998; Araújo et al. 2004a,b; Oliveira et al. 2011). High rates of colony mortality have been reported in reforestation areas in Brazil during the fires (Anjos et al. 1998). In this sense, the impact on the colonies could be direct, as mentioned earlier, and indirect because after the fires there are scarce resources to be foraged by worker ants.

The mortality of initial leaf-cutting ant colonies is associated with the physical, chemical, and microbiological conditions of the soil where the ants try to establish them after the nuptial flight (Bento et al. 1991). Accidental fires and controlled burns not only keep the soil bare until the beginning of rainfall but also cause changes to the soil chemical and microbiological properties. They prevent the establishment of colonies possibly due to microorganisms that are entomopathogenic or antagonistic to the ant's symbiotic fungus (Araújo et al. 2003).

The leaf-cutting ants (*Atta*) have a peculiar behavior: After the nuptial flight, the newly fertilized females fall to the ground, lose their wings, quickly start moving around looking for a place to excavate their new nest, and then cloister to form a colony (Araújo et al. 2011). Female preference for places cleared of vegetation, such as road sides, open paths, or even areas with little vegetation created by fires, has been observed in *Atta cephalotes* (L.) and in *Acromyrmex octospinosus* (Reich) (Cherrett 1968; Vasconcelos 1990), in *Atta sexdens* (L.) (Diehl-Fleig 1995), in *Acromyrmex striatus* (Roger) (Diehl-Fleig & Rocha 1998), and in *Atta bisphaerica* Forel (Araújo et al. 2003).

The nuptial flights usually occur after rains that moisten the soil, which not only facilitates excavation but also provides a favorable microenvironment for initial cultivation of the ants' symbiotic fungus (Bento et al. 1991; Araújo et al. 2003). The nest building process is the most crucial period in the life of an ant colony (Wilson 1971), and failure of *Atta* ants to establish new colonies is directly associated with high female mortality due to predation (Autuori 1950; Mintzer & Vinson 1985; Diehl-Fleig 1995), to rainfall (Mariconi 1970), and to microorganisms naturally existing in soils (Bento et al. 1991; Araújo et al. 2003).

Other factors may also affect the establishment of new leaf-cutting ant nests, though they are not well known, and one of these factors may be fire. The long-term effects of fires are known to bring about changes to environmental conditions and shelters, as well as to food quality (Majer 1984; Collett 1998; Araújo et al. 2004a,b).

Given the apparent economic unimportance and complex procedures required to study the impact caused by fire on small soil fauna, little has been researched in this area when compared with plants and large vertebrates. Consequently, the post-fire ecology of such organisms is not well understood (McCullough et al. 1998).

The knowledge generally accepted about ants is that they are less affected by fires than many other insects. However, the effect of climate conditions and disorders caused by human interference, such as controlled burns or fires, on the establishment of new colonies of these eusocial insects is poorly studied (Araújo et al. 2003). Thus, this study was aimed at assessing the impact of a fire in a savannah vegetation area (Cerrado) in the Ipameri Region, Goiás State, Brazil, on the early establishment of *Atta sexdens rubropilosa* Forel colonies.

Materials and Methods

STUDY AREA

This study was conducted at the Experimental Farm of Goiás State University, Ipameri Câmpus, in the municipality of Ipameri, GO, Brazil. This site is located at 17.721023°S, 48.159606°W, and 764 m altitude. In this area, the savannah vegetation (in regeneration) type is dominant and is characterized by trees, bushes, and subshrubs, with herbaceous plants composed predominantly of exotic grasses *Urochloa decumbens* Stapf and *Hyparrhenia rufa* (Nees) Stapf (Poaceae), with no history of burns in the last 6 yr. According to Köppen, the climate type is Aw. The average temperature of the region is 21.9 °C, with average air humidity ranging from 58 to 81% and annual rainfall of 1,448 mm, with around 80% of rain in Dec, Jan, and Mar, and the rest distributed mainly throughout Oct, Nov, and Feb (Rodrigues et al. 2009). The predominant soil type is dystrophic red-yellow latosol (Embrapa 2006).

STUDY SITES AND CHARACTERIZATIONS

The area of the savannah fragment studied was 13 ha. Four hectares of this area were burned. The rest of the fragment, which had not directly been affected by the fire, had vegetation and soil characteristics similar to the burned areas. In order to infer the burning intensity, combustible material was collected in five 1.0 m^2 samples randomly selected ahead of the firing line. This shallow combustible material, like dried leaves and shrub branches and grass, was weighed.

On the nuptial flight day of *A. sexdens rubropilosa* in the studied region, which happened 67 d after vegetation burning, homogenized soil from burned (B) and unburned (NB) areas, collected randomly at multiple locations (n = 15) and 2 depths (0.0 to 10.0 cm and 10.1 to 20.0 cm), was placed in 250 mL plastic cups. From this material, only 1 sample of the soil was taken, and a routine chemical analysis was performed. Also, polyvinyl chloride (PVC) tubes (10 cm in diameter) were used to collect a whole section of soil (B and NB) in the field, without disruption. For this purpose, 25 cm pieces of PVC tubes were pressure inserted into the soil with the use of a mallet. After that, PVC tubes containing the soil were pulled up and taken to the laboratory.

COLONY FOUNDATION IN THE FIELD

In each treatment fragment (B and NB), 10 *A. sexdens rubropilosa* females fertilized on the nuptial flight day were placed along a 60 m transect separated by 6.0 m. These queens were surrounded by a plastic barrier to prevent them from escaping their site and prevent them from excavating the ground. All females when captured where without wings and in search of a site to excavate their nest outside the experimental area. Excavating behavior in regard to soil to build the nest was evaluated for a period of 24 h, and the number of surviving colonies was counted 120 d after the females had excavated the same soil. Under field conditions, the duration of survival of leaf-cutting ant colonies on average is between 71 and 118 d when the workers make the first scouting opening (Autuori 1941). Surviving colonies were considered those that had a living queen with workers and symbiotic fungus forms. In the surviving colonies, the presence of a living queen was sought, the numbers of workers, pupae, and larvae counted, and the dry weight of the symbiotic fungus colony measured.

COLONY FOUNDATION IN THE LABORATORY

A newly fertilized female was placed into each cup or PVC tube. Twenty-five females were placed in plastic cups with B and NB substrate treatments, and 45 females were placed in PVC tubes to excavate the soil. The method to observe leaf-cutting ant colony establishment under laboratory conditions was the same as that adopted by Araújo et al. (2003). The containers were kept at room temperature, with a daily water supply in cotton pads attached to their covers, which ensured a favorable environment for the development of the entire colony, as recommended by Bento et al. (1991).

The parts of the soil (0.0 to 10.0 and 10.1 to 20.0 cm deep) collected from the B and NB areas to fill the 250 mL plastic cups were subjected to chemical and microbiological analyses. The chemical analysis was the standard usually recommended for mineral fertilization of agricultural crops. The microbiological analysis was the same as that adopted by Araújo et al. (2003), where the amount of microorganisms present in the soil was inferred by measuring the CO_2 production.

STATISTICAL ANALYSES

A Chi-squared (χ^2) test and Mann–Whitney U tests were used to compare the females' choices for nest place and their successful establishment of initial colonies. CO_2 production in the soils from B and NB areas collected at 2 depths was compared using the Mann–Whitney U test. An analysis of variance (ANOVA) and an HSD Tukey test were performed to compare the morphometric characteristics and composition of surviving colonies between nests excavated in B and NB areas. The Levene test was used to check for homogeneity of variance between treatments, and the Shapiro–Wilk test was used to verify whether the data were normally distributed. These analyses were processed with the software SISVAR 5.3 (Ferreira 2008).

Results

SITE CHARACTERIZATIONS

The dry weight (average ± standard deviation) of the combustible plant material by 1.0 m^2 portion was 0.196 ± 0.028 kg. The burning of this material caused chemical changes in the soil, especially an increase in pH and in K, P, Ca^{2+}, and Mg^{2+} (Table 1). The organic matter content of the soil was 2.1% in the B areas and 1.8% in the NB areas. The clay contents in the analyzed samples were 459.8 g/kg in B and 493.2 g/kg in NB, meaning that the occurrence of a fire did not affect this soil characteristic. The amount of CO_2 released in the soil (value accumulated during 10 d) did not differ between the B and NB areas for the 2 depths studied (Mann–Whitney U test, $P > 0.05$) (Fig. 1).

COLONY FOUNDATION IN THE FIELD AND THE LABORATORY

Under field conditions, *A. sexdens rubropilosa* females excavated B and NB soils similarly ($\chi^2 = 1.82$; $P = 0.178$), and none of these excavating queens managed to establish their own colony (Table 2). However, in the laboratory, the collection of soil samples with PVC tubes was aimed at obtaining undisturbed samples to observe females naturally excavate them as under field conditions. The soils from B and NB areas were equally excavated by the *A. sexdens rubropilosa* females ($\chi^2 = 1.91$; $P = 0.167$) (Table 3). No depth difference in initial chambers was observed in the soils from B and NB areas (ANOVA, $F_{(1, 28)} = 4.196$). The average (± standard error) depths of constructed chambers were 8.0 ± 0.12 cm and 8.5 ± 0.14 cm in the B and NB areas, respectively. Likewise, no difference was observed in the dimensions of the initial chamber built between B and NB soils (ANOVA, $F_{(1, 28)} = 4.194$). Chamber dimensions were 34.03 ± 6.02 cm^3 in B and 33.82 ± 4.64 cm^3 in NB. In PVC pipes, successful establishment of initial ant colonies (11.1%) occurred in NB soils but not in B soils (Table 3).

The number of *A. sexdens rubropilosa* females that excavated the soil from B and NB areas collected at 2 depths is shown in Table 4. Soils from B or NB surface areas were equally excavated by the females ($\chi^2 = 0.80$; $P = 0.371$), with similar rates of initial colony survival ($\chi^2 = 0.60$; $P = 0.440$). Non-surface soils collected at 10.1 to 20.0 cm depth from B or NB areas were also equally excavated by the females ($\chi^2 = 7.71$; $P = 0.057$). However, in this situation, the fire negatively affected the initial colony establishment: The survival rate of initial ant colonies was significantly greater in colonies that were established in NB soils compared with B soils ($\chi^2 = 8.01$; $P = 0.004$).

The characteristics of leaf-cutting ant colonies that survived in the 250 mL plastic cups are shown in Figs. 2 and 3. There was no difference in the numbers of larvae, pupae, workers, and symbiont fungal dry weight of initial colonies established in B or NB areas (ANOVA, $P > 0.05$).

Discussion

The study site was predominantly composed of *U. decumbens* and readily enabled fire spreading. According to Miranda et al. (2002), fire in Brazilian savannahs is usually superficial and mostly consumes herbaceous plants. The fire's unpredictability made it impossible to reliably measure the speed of the firing line, and therefore the burning intensity could not be estimated in this study. In a previous study during a controlled burning of dry sugar cane straw, which was monitored with a higher material volume than that used in this study, the fire intensity (615 KJ/m/s) was low (Araújo et al. 2003). Based on this previous assessment, the fire in our study could be considered of low intensity.

The increase in nutrient content was probably due to the ashes resulting from the fire and incorporated into the soil by the rains, as also reported by Redin et al. (2011). According to Nuernberg et al. (1984) and Cattelan & Vidor (1990), these nutrients directly foster soil microorganisms. In our study, 67 d after direct fire action, the amount of CO_2 released in the soil (value accumulated during 10 d) did not differ significantly between B and NB areas at the 2 depths studied. In the present work, the microorganism community in the soil was not identified, but it probably interfered with the new colony setting of *A. sexdens rubropilosa* (Rodrigues 2007). Up to the date when we observed no survival of the initial colonies (120 d after nuptial flight) in the field, the microorganism community was most likely influenced by climate fluctuation such as temperature and rainfall.

Under field conditions, none of the colonies survived in B or NB areas (Table 2). It is worth mentioning that under such field conditions, the beginning of soil excavation by the females took place under intense solar radiation, with the females being completely exposed to direct radiation, a fact that probably hindered their activity.

Under laboratory conditions, the behavior of excavating the soil to establish a colony was similar to that observed by Araújo et al. (2003) with another leaf-cutting ant species, *A. bisphaerica*, which also excavated the soil offered regardless of its being from burned or unburned areas. The higher excavation rates in the laboratory (91.1 and 97.8%

Table 1. Chemical analysis of soils from burned areas (B) and surrounding unburned areas (NB). Ipameri, GO, Brazil, Nov 2013.

Area	Depth (cm)	pH[a]	P (mg/dm³)	K (mg/dm³)	Ca^{2+} (cmol$_c$/dm³)	Mg^{2+} (cmol$_c$/dm³)	Al^{3+} (cmol$_c$/dm³)	$H^+ + Al^{3+}$ (cmol$_c$/dm³)	OM[b] (%)	Texture (g/kg)
B	10.1–20.0	5.20	2.25	128	4.2	1.4	0.10	2.0	2.1	459.8
NB	10.1–20.0	4.80	0.36	68	3.2	1.1	0.30	3.0	1.8	492.2

[a]pH determined in water; [b]OM = Organic matter.

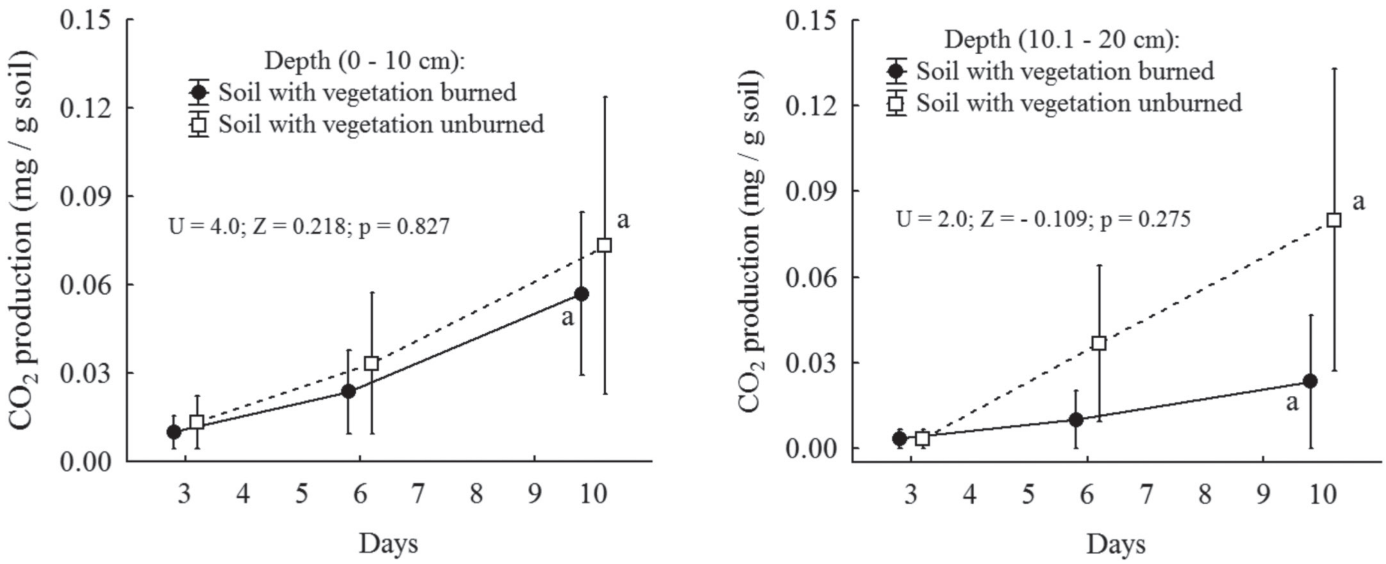

Fig. 1. Microbial activity (mean and standard error) in soils collected at 2 depths (0.0 to 10.0 cm and 10.1 to 20.0 cm) in areas with burned and unburned vegetation in Ipameri, GO, Brazil. Means followed by the same letter (accumulated CO_2 production at 10 d), for each depth where the soil was collected, did not differ from each other (Mann–Whitney U test, $P > 0.05$).

Table 2. Number of female leaf-cutting ants (*Atta sexdens rubropilosa*) that excavated the soil after the nuptial flight in burned (B) and unburned (NB) areas, and success in establishing colonies after 100 d in the field. Ipameri, GO, Brazil, Nov 2013.

Parameter	B	NB	χ^2 value	*P* value
Number of excavating females	7	4	1.82 n.s.	0.18
Successful establishment[a]	0	0	0	

[a]Success in initial colony establishment was measured through the presence of the queen, workers, larvae, and symbiotic fungi 120 d after female reclusion; n.s. = not significant.

for B and NB, respectively), when compared with those observed in the field (70 and 40% for B and NB, respectively), were probably due to favorable laboratory conditions such as temperature, relative air humidity, and soil moisture. Under these favorable laboratory conditions, no significant difference was observed in the depth of initial chamber construction or in chamber size in soils from B and NB areas. The colony depths were similar to those observed by Della Lucia & Araújo (1993) for the same leaf-cutting species in soil unaffected by fire.

In PVC tubes, females were able to establish colonies in NB soils (success rate 11.1%) but not in B soils. Araújo et al. (2003) reported that immediately after the fire, there was a reduction in the soil microbiological activity, and that after a few months, like in this study, nutrients originating from the burning may have benefited microorganisms, including those not beneficial to the colony, which may have occurred in our study because no colony survived in B soils.

When the females were allowed to excavate soil that had been collected at 2 soil depths (0.0 to 10.0 cm and 10.1 to 20.0 cm) from B and NB areas, we observed that, although females equally excavated soils from both areas, the highest survival rate of initial colonies was in non-surface soil from the NB area (Table 4). Even without a fire effect, there was lower colony survival in topsoils than non-surface soils, reinforcing the hypothesis of the action of entomopathogenic microorganisms, microparasites, or symbiotic fungi competitors of leaf-cutting ants. Bento et al. (1991), without considering the fire influence, also showed that females of leaf-cutting ants prefer to establish their colonies under laboratory conditions in soils from non-surface areas (A horizon) (Bento et al. 1991). Despite observing higher colony survival in soils from NB areas and in non-surface

Table 3. Establishment and survival of leaf-cutting ant (*Atta sexdens rubropilosa*) colonies in soil collected with PVC tubes and stored in the laboratory (n = 45 females). B: Soils from burned area; NB: soils from unburned area. Ipameri, GO, Brazil.

Parameter	B	NB	χ^2 value	*P* value
Number of excavating females (%)	91.1	97.8	1.91	n.s.
Successful establishment (%)[a]	0	11.1	5.29*	0.02

[a]Success in initial colony establishment was measured through the presence of the queen, workers, larvae, and symbiotic fungi 120 d after female reclusion.
* = Significant; n.s. = not significant.

Table 4. Percentage of female leaf-cutting ants (*Atta sexdens rubropilosa*) that excavated the soil under laboratory conditions, and their success in establishing initial colonies in homogenized soils coming from areas with burned (B) and unburned (NB) vegetation collected at depths of 0.0 to 10.0 cm and 10.1 to 20.0 cm (n = 25 females). Ipameri, GO, Brazil.

	Excavating females (%)			
Depth (cm)	B	NB	χ^2 value	*P* value
0.0–10.0	28 Aa	40 Aa	0.80	0.371
10.1–20.0	52 Aa	88 Ab	7.71	0.057
χ^2 value	3.00	12.50		
P value	0.083	0.0004		
	Rate of successful establishment[a] (%)			
	B	NB		
0.0–10.0	12 Aa	20 Aa	0.60	0.440
10.1–20.0	28 Aa	68 Bb	8.01	0.046
χ^2 value	2.00	11.69		
P value	0.157	0.001		

[a]Success in initial colony establishment was measured through the presence of the queen, workers, larvae, and symbiotic fungi 120 d after female reclusion. Percentage followed by different uppercase letters within rows or different lowercase letters within columns indicate significant differences between treatments (Chi-squared test, $P < 0.05$).

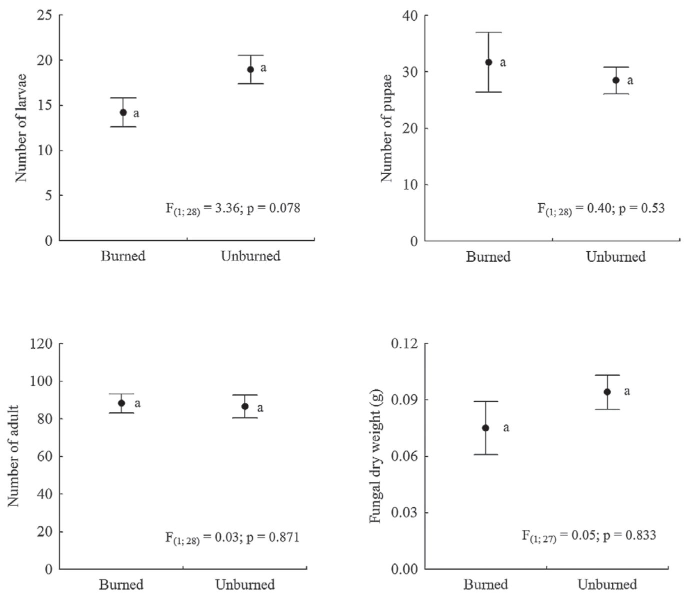

Fig. 2. Characterization of *Atta sexdens rubropilosa* surviving colonies in burned and unburned areas, after 120 d of queen reclusion (female). Means (and standard errors) followed by the same letter did not differ from each other (ANOVA, $P > 0.05$).

soils, no significant difference was seen in the composition of surviving colonies (fungal dry weight, number of larvae, pupae, and adults) (Figs. 2 and 3).

In summary, *A. sexdens rubropilosa* females did not show a preference in excavating soils from areas with burned or unburned vegetation, and initial colony survival was higher in non-surface soils, from areas that did not have vegetation burning. However, the composition of initial colonies created in the laboratory (numbers of larvae, pupae, and adults, and dry weight of symbiont fungus) was similar in soils collected from burned and unburned areas.

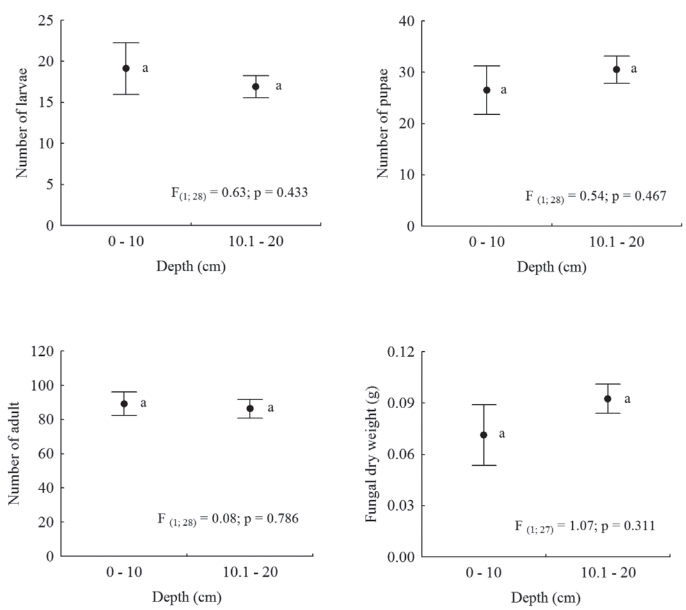

Fig. 3. Characterization of *Atta sexdens rubropilosa* surviving colonies in burned and unburned areas, after 120 d of queen reclusion (female), in soils from 2 depths (0.0–10.0 cm and 10.1–20.0 cm). Means (and standard errors) followed by the same letter did not differ from each other (ANOVA, $P > 0.05$)

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