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Daily Foraging Activity of *Acromyrmex* (Hymenoptera: Formicidae) Leaf-cutting Ants

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Abstract

Leaf-cutting ants are well-known insects due to their remarkable activity as herbivores and the considerable economic damage they cause to many crops. The identification of season and time of day when leaf-cutting ants are most active is an important tool, not just to understand the foraging ecology of these ants, but also to optimize their control in plantation areas where they are pests. Thus, the aims of this study are to evaluate the daily foraging activity of leaf-cutting ant species of the genus *Acromyrmex*, which occur in forest plantations in Southern Brazil. Foraging activity of *Acromyrmex crassispinus* (Forel) and *Acromyrmex subterraneus subterraneus* (Forel) were correlated with weather conditions, and it was more intense during spring and summer. Workers that forage at night are significantly heavier than workers that forage during the day. This study showed that *A. crassispinus* and *A. subterraneus subterraneus* did not forage at temperatures below 10-11°C. Then, the use of granulated baits to control these leaf-cutting ants species where they are pests should be done just under favorable conditions of temperature for *Acromyrmex* foraging activity (over 12°C), to ensure maximum collection of baits by ants and the least left-over baits.

Introduction

Leaf-cutting ants (*Atta* and *Acromyrmex*, Formicidae) are possibly the most important herbivores of terrestrial environments throughout the Neotropics, leading to considerable economic losses to a variety of crops (Hölldobler & Wilson, 2011). Foragers of these ants cut fragments of leaves and other plant parts and carry them to their nests to be used as substrate for the cultivation of a mutualistic fungus (Weber, 1972; Hölldobler & Wilson, 1990). Traditionally, attempts to control leaf-cutting ants involve the use of insecticides from diverse chemical groups and different formulations (liquid concentrates such as nebulizers, fumigants, but mainly granulated baits) (Araújo et al., 2003).

Temperature and relative humidity have marked effects on the activities of all animal species (Begon et al., 2006) and the behavior of leaf-cutting ants is strongly influenced by those parameters (Roces & Kleineidam, 2000; Bollazzi & Roces, 2002). Temperature primarily affects both worker activities by inhibiting or reducing foraging, as well as the speed of brood development, conditions that directly determine the growth rate ofa colony (Gamboa, 1976; Porter, 1988; Porter & Tschinkel, 1993). Also, the fungus gardens have strict demands of high humidity and temperatures between 20 and 30ºC for proper growth (Powell & Stradling, 1986).

Foraging activity of *Atta sexdens* (L.) and *Atta cephalotes* (L.) is mainly daytime during the winter and nighttime during the summer in Paraguay (Fowler & Robinson, 1979) and in Costa Rica (Wetterer, 1990). Foraging activity of *Atta colombica* Guérin-Méneville is more frequent during the day in the Republic of Panama (Wirth et al., 1997). *Atta capiguara* Gonçalves shows two daily foraging journeys, one in daytime and another one at night, a variation that is a function of dry and wet seasons (Caldato, 2014). For *Acromyrmex* species, studies showed that *Acromyrmex versicolor versicolor* (Pergande) is primarily nocturnal during the hot summer months, but shifted toward diurnal activity as fall progressed in Tempe, Arizona (Gamboa, 1976). Foraging

activity of *Acromyrmex crassispinus* (Forel) occurred throughout the day during April in Paraguay (Fowler, 1979). The annual foraging activity of *A. subterraneus subterraneus* (Forel) was exclusively nocturnal in Viçosa, MG, Brazil (Maciel et al., 1995), while in *Acromyrmex balzani* (Emery) (Mendes et al., 1992) and *Acromyrmex laticeps nigrosetosus* Forel (Araújo et al., 1998) is mainly nocturnal in Minas Gerais, Brazil.

There are suggestions that the micro-weather near each nest, or even peculiar to each trail or nest-entrance may affect their foraging rhythms. Nocturnal foraging may be encouraged by warm nights, by cool or very hot dry days, and by full clear moonlight; conversely, diurnal foraging is supposedly encouraged by cool nights, warm days and moonless or dull nights (Lewis et al., 1974a).

Leaf-cutting ants' workers are highly polymorphic (Wilson, 1980; Endringer et al., 2012). In some leaf-cutting ant species, there are dial changes in worker weight. Workers that foraged at night were heavier than workers that foraged during the day. Heavier leaf cutters do not forage during the day in order to avoid diurnal parasitoids that preferentially attack larger ants or they might avoid activity outside the nest during the day due to greater risks with desiccation or heat stress (Wetterer, 1990).

Acromyrmex crassispinus and *A. subterraneus subterraneus* (Forel) were the most commonleaf-cutting ant species in pine plantations in Southern Brazil (Nickele et al., 2009). The identification of season and time of day when leaf-cutting ants are most active is an important tool, not just to understand the foraging ecology of these ants, but also to optimize their control in plantation areas where they were pests. Thus, the aims of this study are to evaluate the daily foraging activity of leaf-cutting ant species of the genus *Acromyrmex*, which occur in forest plantations in Southern Brazil.

Material and methods

Observations were conducted on one adult colony of *A. crassispinus* (nest size = 1 m²) and one adult colonyof *A. subterraneus subterraneus* (nest size = 6.25 m²) at the same time, during all four seasons of 2010 (February, 10, May, 10, July, 10, and October, 10). Colonies were located in a *Pinus taeda* (L.) plantationin Rio Negrinho city (26º15'16" S; 49º31'06" W; alt. 790 m), state of Santa Catarina, Brazil. The colonies were monitored during 24 hours in all seasons. Surveys began at 18:00h and ended at 17:00h the following day. Foraging rates were measured at one-hour intervals by counting all ants passing a fixed point in the active foraging trail close to the nest entrance. Workers leaving the nest and workers returning (either laden or unladen) were counted for 3 minutes at each sampling period (24 samples per colony per season).

In order to investigate the correlation between ant activity and climate parameters, temperature and relative humidity of theair were measured usinga thermohygrometer. Measurements were made near the monitored nests during the first minute of each sampling interval.

In order to study the ant size variation during the activity period (diurnal or nocturnal workers), 20 workers were collected at each interval (10 ants per colony per hour) following the measurement of foraging rates. Workers were stored in individual Eppendorf® vials and they were weighed to the nearest 0.1 mg on a Mettler balance. Differences in body weight of ants that foraged during the day (ants collected in the period from 7:00h until 18:00h) or during the night (ants collected in the period from 19:00h until 6:00h) were tested using Student's t tests.

Results

Foraging activity of *A. crassispinus* started at 4:00h and stopped at 14:00h, on summer. There was significant correlation between foraging activity and humidity, in this season. On fall, *A. crassispinus* workers did not foraged from 2:00h until 08:00h. In this case, there was significant correlation in foraging activity and both climatic parameters (temperature and humidity). On winter, *A. crassispinus* workers did not foraged from 3:00h until 07:00h, and there was significant correlation in foraging activity and temperature. On spring, *A. crassispinus* workers showed foraging activity all day long and in this season there was not significant correlation in foraging activity and bothclimatic parameters. *Acromyrmex crassispinus* workers did not forage at temperatures below 10°C (Table 1, Fig 1).

Table 1: Correlation between ant activity and climate parameters.

Season	Climate parameters	Species	*r*	p
Summer	temperature	*A. subterraneus subterraneus*	-0.44	0.020
		A. crassispinus	-0.22	0.300
	humidity	*A. subterraneus subterraneus*	0.32	0.124
		A. crassispinus	0.61	0.001
Fall	temperature	*A. subterraneus subterraneus*	0.52	0.007
		A. crassispinus	0.87	0.000
	humidity	*A. subterraneus subterraneus*	-0.42	0.038
		A. crassispinus	-0.90	<0.001
Winter	temperature	*A. subterraneus subterraneus*	-0.08	0.695
		A. crassispinus	0.40	0.050
	humidity	*A. subterraneus subterraneus*	0.31	0.134
		A. crassispinus	-0.14	0.503
Spring	temperature	*A. subterraneus subterraneus*	0.17	0.430
		A. crassispinus	0.23	0.269
	humidity	*A. subterraneus subterraneus*	-0.02	0.939
		A. crassispinus	-0.30	0.151

Foraging activity of *A. subterraneus subterraneus* started at 2:00h and continued all day long, on summer. There was significant correlation between foraging activity and humidity, in this season. On fall, workers did not foraged from 22:00h until 09:00h. In this case, there was significant correlation in foraging activity and bothclimatic parameters. On winter, workers did not foraged from 2:00h until 09:00h, and there was significant correlation in foraging activity and temperature. On spring, workers did not foraged from 2:00h until 07:00h, and there was significant correlation in foraging activity and both climatic parameters. *Acromyrmex subterraneus subterraneus* workers did not forage at temperatures below11°C (Table 1, Fig 2).

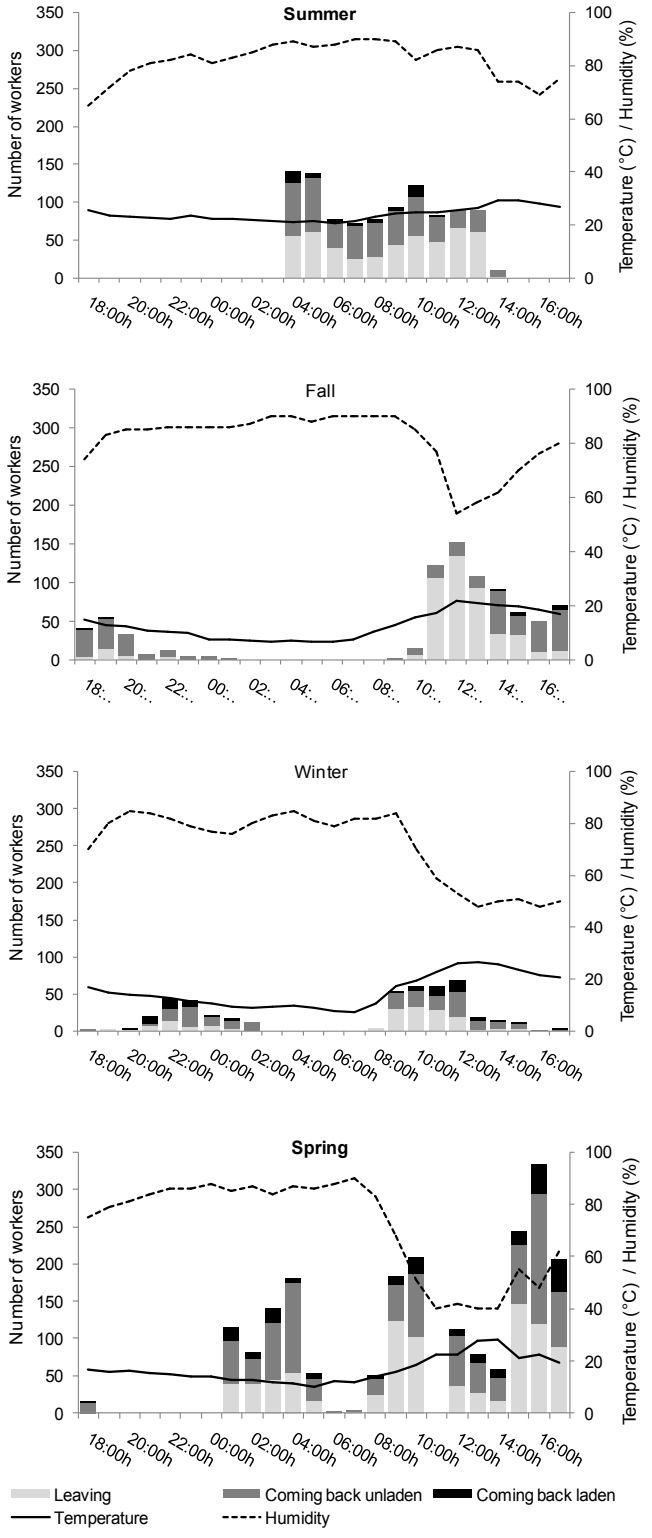

Fig 1. Daily foraging activity of the leaf-cutting ant *Acromyrmex crassispinus* in Rio Negrinho, SC, Brazil, 2010.

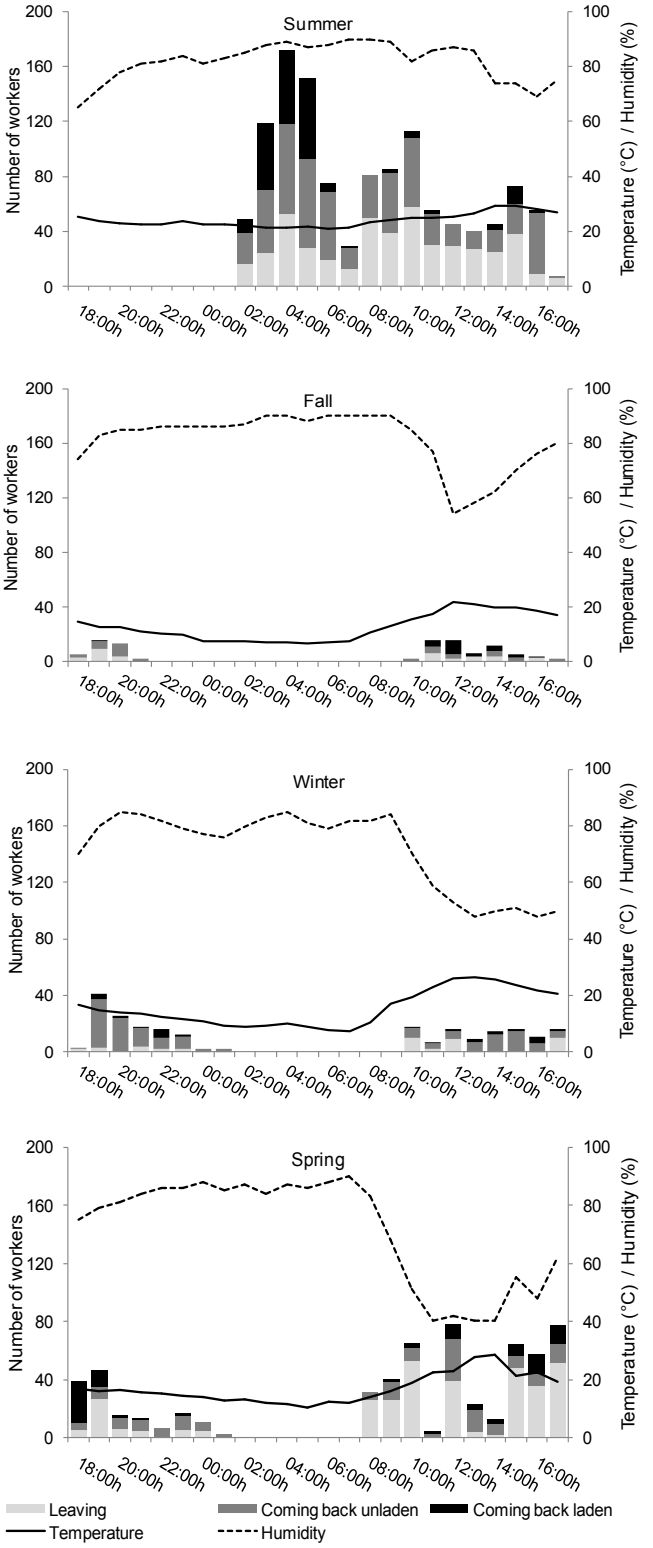

Fig 2. Daily foraging activity of the leaf-cutting ant *Acromyrmex subterraneus subterraneus* in Rio Negrinho, SC, Brazil, 2010.

Foraging activity of *A. crassispinus* was more intense during the spring and foraging activity of *A. subterraneus subterraneus* was more intense during the summer. A considerable number of workers were observed returning unladen to the nest, for both *Acromyrmex* species in all seasons (Fig 1 and 2).

There was variation in body weight during the activity period. Workers that foraged at night were significantly heavier than workers that foraged during the day. The weight of *A. crassispinus* workers that foraged during the day was 5.32 ± 0.172 mg (n=219) $(\overline{x} \pm s_{\overline{x}})$ and 6.07 ± 0.264 mg (n=154) at night (t = -2.35, p = 0.0193). The weight of *A. subterraneus subterraneus* workers that foraged during the day was 7.73 ± 0.246 mg (n=247) and 9.72 ± 0.294 mg (n=178) at night (t = -5.46, p <0.001).

Discussion

Foraging activities of *A. crassispinus* and *A. subterraneus subterraneus* leaf-cutting ants occurred at night and during the day, however it was correlated with weather conditions, such as temperature and humidity. These *Acromyrmex* species were most active during spring and summer, but they have also foraged during fall and winter. However, foraging intensity was lower in these two last seasons. Similar results were observed for *Acromyrmex* species in the high lands of Santa Catarina state, Brazil, where ants were most active during the warmest months (Geisel et al., 2008).

Temperature and humidity fluctuations might cause changes in ant respiration and water loss, as well as affect the water balance of the plants being cut, which indirectly affect handling time and nutritional quality of the plants as fungal substrate (Fowler, 1979). *Acromyrmex crassispinus* and *A. subterraneus subterraneus* did not forage at temperatures below 10°C and 11°C, respectively. In Viçosa, MG, Brazil, it was observed that *A. subterraneus subterraneus* cease their foraging activity at temperatures below 14°C (Maciel et al., 1995). *Atta sexdens* (L.) and *Atta mexicana* (F. Smith) do not forage at temperatures below 10°C and 12°C, respectively (Fowler & Robinson, 1979; Mintzer, 1979). Foraging activities of the leaf-cutting ant *Atta sexdens piriventris* Santschi were concentrated during the night in the summer, in the afternoon and in the winter, allowing ants to forage under favorable temperature conditions (Geisel et al., 2013).

A considerable number of workers were observed returning unladen to the nest in both studied *Acromyrmex* species. A similar pattern was reported for *Acromyrmex heyeri* Forel, in which a large proportion of workers returned to the nest unladen at the initial foraging phase, which could represent a response to the high needs for information at the beginning of a daily foraging process (Bollazzi & Roces, 2011). However, in this study we observed a great number of unladen workers returning to the nest not only at the initial foraging phase. These workers could be scouts (Lewis et al., 1974b), workers involved in trail clearing (Lewis *et al.,* 1974b; Howard, 2001), transport of plant sap (Stradling, 1978), reinforcement of the chemical trail (Evison et al., 2008), or in a combination of recruiting and food transport, where these workers cut no fragments, but return to the nest displaying recruiting behavior (Jaffé & Howse, 1979).

Workers that foraged at night were significantly heavier than workers that foraged during the day. The same pattern was observed in *Atta cephalotes* (L.) (Wetterer, 1990; Feener & Brown, 1993). However, daytime *A. sexdens* foragers were not significantly smaller than nighttime foragers (Tonhasca, 1996; Tonhasca & Bragança, 2000). One explanation is that heavier leaf cutters do not forage during the day in order to avoid diurnal parasites that preferentially attack larger ants (Wetterer, 1990). *Atta cephalotes* colonies shifted the size distribution of workers on foraging trails as a second line of defense against parasitism by *Neodohrniphora curvinervis* (Malloch) (Feener & Brown, 1993). However, it seems not to be an effective defense against phorids in *Acromyrmex*, because a study reported that *Acromyrmex* phorids selected ants from all sizes available outside the nests. Phorids did not have preference to attack larger *Acromyrmex* workers as in *Atta* (Elizalde & Folgarait, 2011). Another possible explanation for the diel changes in forager size is that larger ants might avoid activity outside the nest during the day due to greater risks with desiccation or heat stress (Wetterer, 1990).

This study showed that *A. crassispinus* and *A. subterraneus subterraneus* were most active during spring and summer and they did not forage at temperatures below 10-11°C. This result is an important tool, not just to understand the foraging ecology of these ants, but also to infer in leaf cutting ants control. The use of granulated baits to control these leaf-cutting ants species where they were pests should be done just under favorable conditions of temperature for *Acromyrmex* foraging activity (over 12°C), to ensure maximum collection of baits by ants and the least left-over baits.

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