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Notas Científicas / Scientific Notes

Testing an artificial aril as a new ant-attractant

Testando um arilo artificial como um novo atrativo de formigas

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Abstract
Ants are attracted by structures called arils, which have different biochemical compositions. In this study, we tested seed removal by ants by offering a new ant-attractant and we analyzed its efficiency to induce seed removal. We installed six observational points observed for 6 h thereafter. We collected 12 ant species removing the ant-attractants. There was a significant difference in the removal of the different types of resources. The new ant-attractant used as artificial aril was significantly more removed than the other types of resources. Therefore this new ant-attractant used as artificial aril will be a useful tool in studies concerned about ant-plant interactions and evaluating ecosystem functions.
Keywords: Formicidae, seed removal, *Copaifera langsdorffii.*

Resumo
Formigas são atraídas por estruturas chamadas arilos, os quais apresentam diferentes composições bioquímicas. Neste estudo, nós testamos a remoção de semente por formigas oferecendo um novo atrativo para elas e analisamos sua eficiência em induzir a remoção de sementes. Nós instalamos seis pontos amostrais observados por 6h. Nós coletamos 12 espécies de formigas removendo os atrativos para as formigas. Existiu diferença significativa na remoção entre os diferentes tipos de recurso. O novo atrativo para as formigas usado como arilo artificial foi significativamente mais removido que os outros tipos de recurso. Portanto esse novo atrativo usado como arilo artificial será uma ferramenta útil em estudos interessados nas interações formiga-planta e avaliação das funções ecossistêmica.
Palavras-chave: Formicidae, remoção de semente, *Copaifera langsdorffii.*

INTRODUCTION

Seeds contain a nutritive appendage attractive to ants known as elaiosome with different biochemical compositions (Rico-Gray & Oliveira, 2007), including lipid, carbohydrates, proteins and vitamins (Fischer et al., 2008). Non myrmecochorous plants (e.g. *Copaifera langsdorffii* Delf.) have lipids as main component of the fleshy portion of their seeds (aril) (Pizo & Oliveira 2001).

Hence, produce ant-attractant, which could be used as an artificial aril, provide an excellent opportunity to study the ecological function of seed dispersal. An ant-attractant designed for mimicking an aril should present some attributes: attractiveness, easily transportable, for not being detached from the seed *in situ*. Characteristics such as being resistant to de-composition, similar chemical composition to natural arils and low production cost are also important requirements (Henao-Gallego et al., 2011).

Some studies have already used artificial arils for evaluating environmental conditions. Raimundo et al. (2004) used artificial fruits with the same composition of non-myrmecochorous plants. Henao-Gallego et al. (2011) used artificial aril with ingredients based on the chemical composition of natural arils. The use of artificial arils, based on the approach of Raimundo et al. (2004), was also used by Bieber et al. (2012). However, to our knowledge, no study used natural arils for manufacturing an artificial aril used as ant-attractant for attracting seed removing ants.

Thus, we sought to induce removal by ants by offering two types of ant-attractants and seeds without ant-attractants. We hypothesized that ant-attractants prepared with 'copaíba' aril should be more attractive and more removed by ants than the others types of resource, independent of their size.

MATERIAL AND METHODS

The study was carried out at the 'Parque Estadual do Ibitipoca'(S 21˚42'32,3" and W 43˚53'45,3") at Minas Gerais state, Brazil, during October 2011. We installed six observational points, 10 m distant from each other. Henao-Gallego et al. (2011) and Raimundo et al. (2004), both using artificial arils, showed that 10m distance is sufficient to ensure independent discoveries by different ant colonies. We prepared two types of ant-attractant:

1 – A 'Copaíba ant-attractant': natural arils of *Copaifera langsdorfii* Delf. are found widely in Cerrado biome (Ribeiro et al., 2001) with intercalated years of intensive fructification. This plant produces a seed partially covered by a lipid-rich aril (Pedroni et al. 2002) highly consumed by ants (Georg et al. 2010). We triturated and mixed *C. langsdorfii* natural arils (~212 g) with 250 ml of water, 350Tamb g of wheat flour and 100 ml of soya oil. We used two circular plastic molds (big: 8mm and small: 4mm) to make tablets that were subsequently oven-dried for 3 h at 100˚C. We got one set of tablets weighing 49.3 mg, named SC (small 'copaíba ant-attractant') and another weighing 244.43 mg, named BC (big 'copaíba ant-attractant').

2 – An artificial flour ant-attractant: 200 g of wheat flour mixed with 100 ml of water. Following the same procedure before, the tablets produced weighed 50.5 mg, named SF (small artificial flour aril), and 223.1 mg, named BF (big artificial flour aril). We also used sunflower seeds (SS; individual weigh ~ 72.5 mg), which were observed to be removed by ants (personal observation), to evaluate which structure, free seeds or ant-attractants was considered more attractive to ants.

In each observational point at 09:00 h, we placed five units of each type of resource on white filter paper (10 x 10 cm) to facilitate ant visualization. The papers were placed within wired cages (15 x 15 x 10 cm) to avoid vertebrates' actions.

We observed the points for 30 min and checked at 10-min intervals for 6 h. After 24 h, we checked again and the remaining resources were counted. The resources were considered removed when carried more than 30 cm from their original location (Christianini & Oliveira, 2010). The ants collected were identified to the lowest taxonomic level possible in the Laboratory of Ant Ecology at Federal University of Lavras, according to Palacio & Fernandéz (2003).

We performed a non-parametric Kruskal-Wallis test for determining whether there are significant differences on the removal rates among the five types of resource. After, we performed posteriori Test Z for pairwise comparisons. We used the program Statistica 7.0. types of resource.

RESULTS

We collected 12 ant species from four genera: *Solenopsis*, *Camponotus*, *Pheidole*and Crematogaster), with *Solenopsis* (4) and *Pheidole* (6) being the most abundant, which were the same among the observational points. All these species removed both SC and BC, but only one *Pheidole* species removed the SF and only once. SS also were removed only once, but we did not observe which ant species realized the removal.

There was a significant difference in the removal of the different types of resources ($H_{4,30}$ = 25.21, $P < 0.001$). The SC and BC were four times more removed by ants than the other types of resources (Fig 1). This indicates that the type of resource influences reamoval by ants, and that the 'copaíba ant-attractant', irrespective of its size, was more attractive to the ants (Table 1).

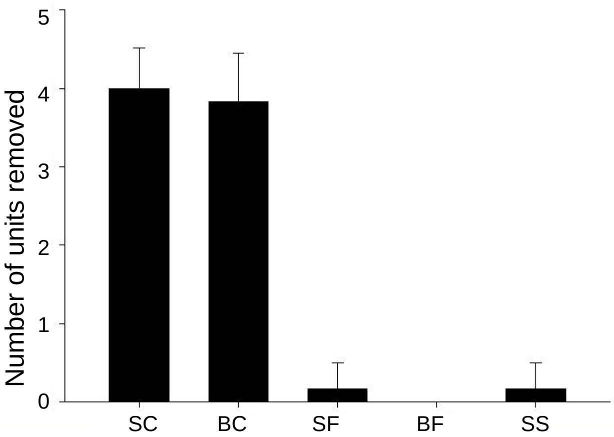

Figure 1. Ant-attractants and seed removal by ants in a trial experiment at 'Parque Estadual do Ibitipoca' in 2011. Vertical bars are standard errors (SE). SC - small 'artificial copaíba ant-attractant', BC - big 'artificial copaíba ant-attractant', SF - small artificial flour ant-attractant, BF - big artificial flour ant-attractant, SS - sunflower seed without ant-attractant.

Table 1. Ant-attractants and seed removal by ants in a trial experiment at 'Parque Estadual do Ibitipoca'. P and Z values, respectively, from Z posteriori test. SC - small 'artificial copaíba ant-attractant', BC - big 'artificial copaíba ant-attractant', SF - small artificial flour ant-attractant, BF - big artificial flour ant-attractant, SS - sunflower seed without ant-attractant.

	SC	BC	SF	BF	SS
SC		1.00; 0.16	0.03; 2.93	0.01; 3.22	0.03; 2.93
BC	1.00; 0.16		0.06; 2.77	0.02; 3.06	0.06; 2.77
SF	0.03; 2.93	0.06; 2.77		1.00; 0.29	1.00; 0,00
BF	0.01; 3.23	0.02; 3.07	1.00; 0.29		1.00; 0.29
SS	0.03; 2.93	0.06; 2.77	1.00; 0,00	1.00; 0.29	

The consistency of the 'copaíba ant-attractant' enabled ants of different sizes to carry them, and its largest weigh did not affect its removal. During the removal by ants, both 'copaíba ant-attractants' and 'flour ant-attractant' did not shatter.

Eight months after the preparation of these ant-attractants there was no sign of fungal infection, and neither type of ant-attractant had been damaged by desiccation. They were stored in a box in the Laboratory of Ant Ecology, with room temperature.

DISCUSSION

The ant species richness indicates the attractiveness of the new ant-attractant. Our results also suggest that there was no ant species behavioral dominance, as indicated by the number of ant species sampled and species similarity among sampling points. *Pheidole* and *Solenopsis* are generally recorded like the most abundant genera interacting with artificial and natural aril (Pizo & Oliveira, 2000; Bieber et al., 2012).

The attractiveness of arils to ants is controverse. Sheridan et al. (1996) and Escobar-Ramírez et al. (2012) concluded that ants found the seeds randomly. Ciccarelli et al. (2005) suggested that the ants found seeds by detecting the volatiles compounds present in structures such as arils. However, researchers agree that the aril is responsible for initiating ant foraging behavior (Byrne & Levey, 1993; Pizo & Oliveira, 1998).

The chemical content of our 'copaiba ant-attractant' was more attractive for ants than the other types of resources. Although it is not clear how the ants found these arils, in our field observations the ants were observed to inspect and manipulate the other types of resources, sometimes before inspecting our 'copaíba ant-attractant'. This fact suggests that the ants did not find the ant-attractants through volatile compound, otherwise, ants should be attracted directly to 'copaíba ant-attractant'.

The higher removal of 'copaíba ant-attractant' supports the hypothesis that the presence of an aril induces and increases the seed removal by ants independent of aril size. We hypothesized that the interaction between ants and 'copaiba that the interaction between ants and 'copaiba ant-attractant' in this study was determined by the presence of lipids, which is present in *C. langsdorffii* natural aril.

Our 'copaíba ant-attractant' met the criteria necessary to use them as artificial arils according to Henao-Gallego et al. (2011). Hence, 'copaíba ant-attractant' successfully attracted ants and could become a new handle for ants and to facilitate the choice of which part use for transporting the seeds. The use of artificial arils has advantages including the possibility of controlling the size, morphology and chemistry of the aril (Rowels & O'Dowd, 2009).

In conclusion, the new 'copaiba ant-attractant' could be used successfully in studies of ant-plant interactions, mainly in Cerrado areas where *Copaifera lansdorffii* is a common plant (Lorenzi, 2000). Myrmecochory studies are also important for understanding the dynamics of establishment of many plant communities and maintenance of ecological functions, with important implications for tropical habitat regeneration and conservation. Furthermore, these arils provide a basis for new studies to assess the presence and influence of volatiles compounds in seed dispersal.

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