TIAGO VINÍCIUS FERNANDES

TESTING THE INFLUENCE OF THE MYRMECHOCORY ON SEED FATE AND PLANT ESTABLISHMENT

Dissertação apresentada à Universidade Federal de Viçosa, como parte das exigências do Programa de Pós-Graduação em Ecologia, para obtenção do título de *Magister Scientiae*.

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APROVADA: 28 de fevereiro de 2018.

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(Orientador)

Dedico aos meus pais, Vitor e Simone, pelo amor aos filhos

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SUMMARY

ABSTRACT

TIAGO, Vinícius Fernandes, M.Sc., Universidade Federal de Viçosa, February, 2018. **Testing the influence of the myrmechocory on seed fate and plant establishment**. Adviser: Ricardo Ildefonso de Campos.

Ants are considered one of the most remarkable invertebrates to disperse seeds. Seed dispersion by ants (myrmecochory) can be divided in three main phases: i) Removal; ii) Manipulation and iii) Deposition. However, all three phases have never been tested simultaneously and the contribution of each one on seed fate and seedling establishment remains unknown. In this purpose, our main aim was to determine experimentally and in field conditions the separate effect of each myrmechocory's phase on the most critical plant stages: seed germination and seedling establishment. We choose as model organisms, the myrmecochous tree *Mabea fistulifera* and the leaf-cutting ant *Atta sexdens*. To simulate the effect of removal we used 30 distances of from the closest conspecific adult tree. To test the manipulation, in each distance we set three seed treatments levels manipulated by *A. sexdens* in laboratory: unmanipulated, non-scarified/without elaiosome, and scarified/without elaiosome. Finally, to test the deposition effect, we placed seed treatments over and away from ant nests. We evaluate seed germination for 90 days and seedling growth and survival for a year. We found that the increase in distance from the closest *M. fistulifera* adult tree decreases seed germination and increases seedling growth but have no effect on seedling survival. Moreover, neither the nest environment nor ant manipulation treatments affect seed germination, seedling growth, and survival. We experimentally showed in field conditions the role of myrmecochorous seed distancing from conspecific plants. Moreover, we suggest a life history conflict on this plant, associate to seed distancing that impairs seed germination and beneficiate seedling growth.

RESUMO

TIAGO, Vinícius Fernandes, M.Sc., Universidade Federal de Viçosa, fevereiro de 2018. **Testando a influência da mirmecocoria na germinação de sementes e no estabelecimento das plântulas**. Orientador: Ricardo Ildefonso de Campos.

As formigas são consideradas um dos mais importantes invertebrados dispersores de sementes. A dispersão de sementes por formigas (mirmecocoria) pode ser dividida em três fases principais: i) Remoção; ii) Manipulação; e iii) Deposição. Contudo, essas três fases nunca foram testadas simultaneamente e a importância relativa de cada uma delas na germinação de sementes e estabelecimento das plantas não é conhecida. Dessa maneira, nosso objeto foi determinar experimentalmente e em condições de campo os efeitos separados de cada fase da mirmecocoria nos estágios mais críticos da vida das plantas: a germinação e o estabelecimento das plântulas. Nós escolhemos como modelos biológicos, a árvore mirmecocorica *Mabea fistulifera* e a formiga cortadeira *Atta sexdens*. Para simular o efeito da distância de remoção nos usamos 30 distancias diferentes das árvores de *M. fistulifera* mais próximas. Para testar a manipulação colocamos em cada uma dessas distancias três tratamentos de sementes manipuladas por *A. sexdens* no laboratório: não manipulada, não escarificada/sem elaiossoma e escarificada/sem elaiossoma. Finalmente, para testar o efeito da deposição colocamos esses mesmo grupos de sementes perto e longe do ninho. Nós acompanhamos a germinação por 90 dias e a sobrevivência e crescimento das plântulas por um ano. Nós encontramos que com o aumento da distância para a árvore de *M. fistulifera* mais próxima, há um decréscimo na germinação e um aumento no crescimento das plântulas mas, a mortalidade não foi afetada. Além disso, não houve nem efeito do ninho nem da manipulação das sementes pelas formigas na germinação, crescimento e sobrevivências das plântulas. Dessa forma, nós mostramos que em condições de campo o papel do distanciamento de sementes mirmecocoricas das proximidades de plantas co-especificas. Além disso, nós sugerimos a existência de um conflito na historia dessa planta, associado ao distanciamento das sementes que prejudica a germinação e beneficia o crescimento das plântulas.

INTRODUCTION

Seed dispersal is considered one of the most important ecological interactions influencing plant communities and species distribution (Levin *et al.*, 2003). A great number of plants species rely on mutualistic animals that act as seed dispersal agents (van der Pijl, 1982). Animals, usually attracted by a food reward (e.g. fruit pulp and aril), remove seeds away from the parental plant (van der Pijl, 1982) and those seeds have great odds to reach better sites for germination and establishment (Howe & Smallwood, 1982; Christianini & Oliveira, 2010; Schupp *et al.*, 2010; Farji-Brener & Werenkraut, 2017). Almost all animal groups (e.g. mammals, birds, reptile, and insects) are frequently involved in seed dispersion (Farwig & Berens, 2012; Padilla *et al.*, 2012; Bello *et al.*, 2017). Recently, more attention has been paid to the role invertebrates as seed dispersers (Christianini *et al.*, 2014), due to their high abundance, wide distribution and also due the increasing extinction rates of large animals worldwide (Christianini *et al.*, 2014). Among invertebrates, ants stand out as one of the most important seed dispersers, being present in more than 11 thousand species of angiosperms (Giladi, 2006; Lengyel *et al.*, 2009). Those plants have a special lipid-rich appendix (known as elaiosome) that attracts ants, which characterize this as a dispersal syndrome called myrmechocory (Lengyel *et al.*, 2009). Ants detached this appendix from seeds and use it as food source (Servigne & Detrain, 2010).

The advantages of seed dispersion by ants to plants could be summarized in three main phases (Prior *et al.*, 2014): i) Removal (i.e., when ants take the seeds away from where they fell) – with the increasing in distance from a conspecific plant, it is expected an increase of the probability to be in a promising site with better abiotic condition to establish (Andersen, 1988), a decrease in seed predation and intraspecific competition (e.g., sunlight; Giladi, 2006); ii) Manipulation (i.e., the process of elaiosome detachment and eventually seed scarification made by ants inside the nest) – however the effects of this phase are controversial (Fernandes *et al.*, 2018 submitted) some studies showed a increase in seed germination and the dormancy break of seeds after being manipulated by ants (Horvitz, 1981; Ohkawara, 2005; Leal *et al.*, 2007); iii) Deposition (i.e., after manipulation ants discard seeds in waste piles, generally close to their nest entrance) - It has been reported an increase in performance for seeds deposited over ant nests, mainly due to improved abiotic conditions (e.g., temperature, moisture and light availability) and higher soil nutrient concentration caused by the nest (Leal *et al.*, 2014a, Wenny & Levey, 1998; Wenny, 2001; Farji-Brener & Werenkraut, 2017). Despite the clear importance of those plant advantages promoted by myrmecochory, little information is available about the simultaneous effects of its phases and the interactions among them on plant development (Christianini & Oliveira, 2010).

Even though myrmechocory is a well-studied seed dispersal syndrome (Giladi, 2006; Gómez & Espadaler, 2013), its three phases have never been simultaneously tested following the chronological order in the same habitat and for the same ant-plant pair of species and, particularly in field conditions. Especially, it is hard to find researches that empirically tests myrmecochory effect on seedling establishment (but see Giladi, 2006; Leal *et al.*, 2014a). Furthermore, the distancing of seeds by ants although largely related to advantages of myrmecochory to plants (Goméz & Espadaler 2013), its effects on seed germination and plant establishment was barely tested for myrmecochouos seeds (but see Handel, 1976).

Here we aim to determine – experimentally and in field conditions – the effect of the three myrmechocory phases (seed removal distance, manipulation, and deposition) on the two most critical plant stages: seed germination and seedling establishment. Specifically, we aim test the hypothesis that each one of the three myrmecochory phases has an individual and positive contribution to seed fate and plant establishment. Furthermore, we want to understand the mechanisms involved in each phase measuring abiotic conditions (light incidence).

For this purpose, we used as biological study models the myrmecochorous tree *Mabea fistulifera* Mart. (Euphorbiaceae) and the leaf-cutting ants *Atta sexdens rubropilosa* Forel, 1908 (Hymenoptera: Formicidae). This pair of species share the same kind of habitat (Wirth *et al.*, 2007; Lorenzi, 2000), have a widespread and joint distribution (Lorenzi, 2000; Antweb, 2018), and are known to present the three myrmecochory phases (Peternelli *et al.*, 2009).

METHODS

Study site

We conducted this study in a fragment of a secondary semi-deciduous Atlantic Forest (11 ha) in regeneration since the 1970s. This area is managed by the Engineering Department, from Federal University of Viçosa (UFV) and it is located inside UFV campus in Viçosa, Minas Gerais State, Brazil (20°46'40"S-42°52'27"W). The climate in this area has a cold and dry season (April to September) followed by warm and humid summer (October to March), classified as Cwb according to Köopen’s classification (Peel *et al.*, 2007). It has an average temperature of 19°C and the annual rainfall varies from 1,300 to 1,400 mm per year (Silva *et al.*, 2010). The soil predominant is Distrofic Red Yellow Latosol (Filomeno *et al.*, 2016).

Studied species

Mabea fistulifera is a pioneer Euphorbiaceae tree with broad distribution in all Brazil, being commonly found in forest borders and early secondary growth forest (Lorenzi, 2000). Moreover, the Euphorbiaceae family is one of the most representative plant families in Brazil and in the and Neotropical region (Carvalho & Ribeiro, 2018). It is also among the most important family where the myrmecochory evolved (Lengyel *et al.*, 2009). As others myrmecochorous plants, *M. fistulifera* has globular seeds with an oleaginous appendix (elaiosome) attached to its upper part. The elaiosome is a nutritious lipid-rich appendage that is attractive to many ant species, including leaf-cutting ants (Peternelli *et al.*, 2004, 2008).

Atta sexdens rubropilosa is a leaf-cutting ant with large colony size, with millions of ants and several nest entrances (Hölldobler & Wilson, 1990). They are one of the most widespread leaf-cutting ants, being found from Mexico down to Paraguay, and it is present almost all Brazil (Antweb, 2018). This ant species inhabits open areas, forest borders, secondary growth forests and other disturbed habitats. They use fresh leaves to cultivate their symbiotic fungus that is used as the main food source. However, they can also use other plant parts as seeds, stems, and flowers as fungus subtract (Hölldobler & Wilson, 1990). It is well recorded that ants from the genus *Atta* are able to carry a large number of myrmecochorous seeds to their nests (Leal *et al.*, 2014a; Farji-Brener & Werenkraut, 2015). In the nests, those ants are able to detach the elaiosomes from *M. fistulifera* and even scarify seed coat of some seeds (Fernandes, unpublished data, Leal *et al.*, 2009).

Nests and trees mapping

Inside our study site and during *M. fistulifera* fruit maturation period (September 2016), we actively searched for *A. sexdens* nest entrances, which had at least one *M. fistulifera* seed without elaiosome (manipulated) nearby (less than 30 cm). We considered that ants carry those seeds to their nests. Just after, we counted the number of manipulated seeds around each nest entrance, removed all seeds, and measured the entrance distance from the closest *M. fistulifera* tree we found. Finally, we tagged the nest using a numbered flag and recorded their geographical coordinates to help in the posterior location and nest identification. After 45 days of mapping, we marked 125 nest entrances.

Experimental seed preparation

In order to prepare the seeds to be used in the field experiment, we hand-collected unripe fruits of *M. fistulifera* from 20 trees from a single population in the same fragment of secondary semi-deciduous Atlantic Forest (September 2016). After that, we placed the collected fruits under direct sunlight for maturation and seed release during seven days.

Since one of our aims is to measure the effect of ant manipulation on seed fate, we offered 500 seeds of *M. fistulifera* to five mature colonies of *A. sexdens* kept in laboratory conditions for more than five years (100 seeds per colony). After 48 hours we collected all seeds that were discarded by ants outside the fungus chamber and classified them according to their physical condition: i) seeds without elaiosome and non-scarified or ii) seeds without elaiosome and scarified. Seeds that still with their elaiosome attached were discarded. We set up a third seed group (control) by taking unmanipulated seeds from the same seed sampling (Figure 1). Then, we maintained the seeds from those three groups refrigerated at 5°C for one month to preserve seed viability (Lorenzi, 2000), until we set up the field experiment.

Unmanipulated
(Control)

Non-scarifed
Elaiosome detached

Scarified
Elaiosome detached

Figure 1- Seed manipulation treatments of *Mabea fistulifera* used in our field experiment. Excepting the unmanipulated control group, all seed were manipulated by the leaf-cutting ant *Atta sexdens* in laboratory conditions. The arrow shows the elaiosome that still attached to unmanipulated seeds.

Field experimental design

We set 30 sampling stations, each one composed by one *M. fistulifera* tree, one nest entrance and six seed depots with one pair of seeds each, where three seed depots were "over" ant nest entrance and three grouped "away" from ant nest entrance (Figure 2).

To determine the place of each sampling stations we used 30 ant nest entrances with different distances from the closest *M. fistulifera* tree raffled from all entrances previously marked (N=125). The different distances allowed us to analyze the effect of distance from the closest conspecific tree on seed germination and seedling establishment. Around each of these 30 nest entrances (15 cm distant), we placed on the ground three pairs of seeds (seed depots) also distant 15 cm one each other (Figure 2).

To quantify the effect of seed manipulation on seed germination and seedling establishment, each seed depot belonged to one ant manipulation treatment: i) unmanipulated seeds with elaiosome attached and non-scarified (control); ii) seeds without elaiosome and non-scarified (N), and iii) seeds without elaiosome and scarified (S) (Figure 2). This number of six seeds around each nest aims to match the natural average number of seeds we found during the nest mapping stage of this work. We took all seed treatments described above (control, N, S) and put them in pairs inside an isolation, consisting of a PVC tube (15 cm height x 15 cm diameter) buried 5 cm deep in soil and surrounded by *tanglefoot* ® to allow seed germination, avoiding removal by ants.

Finally, to measure the influence of the nest environment on seed germination and seedling establishment, we placed another three pairs of seeds (in exactly the same manner) on the ground, two meters from the nest entrance but at the same distance from the closest *M. fistulifera* tree (Figure 2).

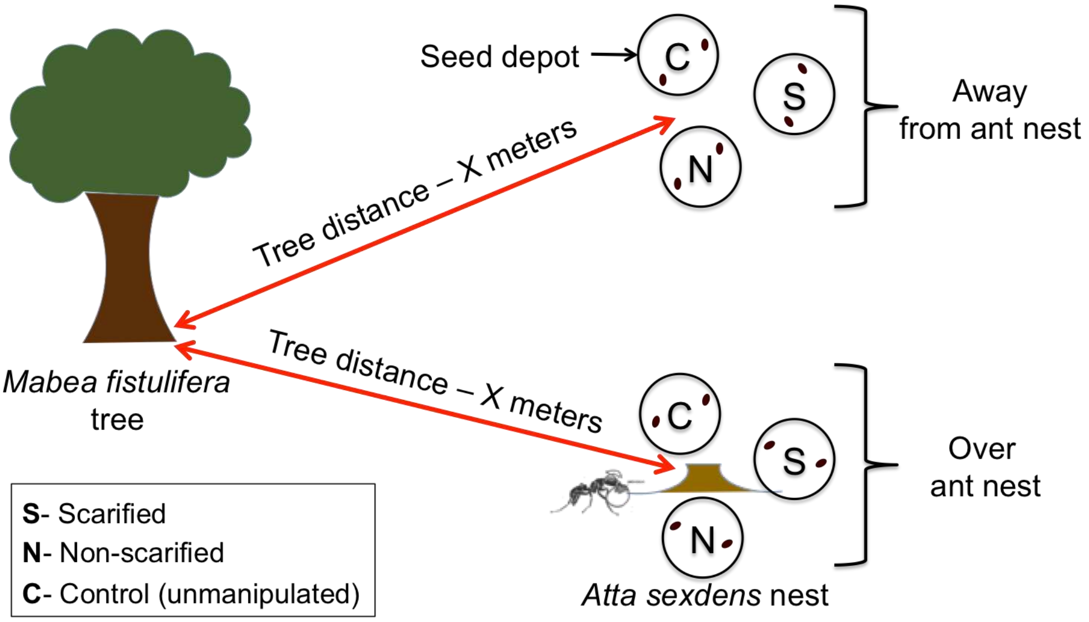

Figure 2: Schematic representation of a sampling station design used to test the effect of conspecific tree distance, ant manipulation and nest environment on seed fate and seedling establishment as follow: *tree distance*: X represent the distance of ant nest entrance to the closest *Mabea fistulifera* tree; *ant manipulation*: each seed depot (open circles) was composed by one of the following seed treatments: **S-** two scarified seeds without elaiosome, **N-** two non-scarified seeds without elaiosome and **C-** unmanipulated seeds, non-scarifed and with elaiosome (Control). Each pair of seeds was protected from ant removal by a PVC tube (open circles, 15 cm height x 15 cm diameter). Finally, for the *nest environment:* we placed six pair of seeds on the ground: three placed “over” (15 cm) and three “away” (2 m) from the nest entrance, but both groups at the same distance from the closest tree.

We count the number of seed that germinated (sprouting of radicle) in each seed depot every two days for 90 days. In other to measure the number of seedling that germinated and remains alive on the end of germination trial, we count the number of live seedlings on the 90th day from the beginning of the experiment. This group of seedlings will be hereafter called "young seedlings". After finished the germination trial, we removed the PVC protection to permit a complete access to the seedlings (e.g., herbivores). We followed those seedlings during other 12 months, every 15 days, counting the number of live seedlings in each depot and measure their heights (distance from base to its higher apical apex). The seedling after the removal of PVC protections will be hereafter called "old seedlings".

To calculate the old seedlings growth rate (cm/day) we used the formula: GR = (Hfinal - Hinitial)/t, where Hfinal is height measured on the last day before plants died or the end of the experiment, Hinitial is height measured on the first day, and t is time in days of the last measurement. We did not consider seedlings that die before the second height measurement (i.e. 15 days after removal of PVC protections).

Light incidence

In order to measure the influence of an abiotic factor that could affect seed fate and seedling establishment, we indirectly measure the light incidence by the proportion of canopy openness over the seed depots. In this purpose, we took hemispherical photographs under each group of three seed depots (30 "near" and 30 "far" from nest entrance, n = 60), at a standardized height of 1m from the ground. We took these pictures around sunrise when the sky was overcast. We used an Fc-E9 fisheye adapter lens mounted on a Nikon Coolpix 5700 camera to take the pictures. Then, we analyze the images in Gap Light Analyzer software (Frazer *et al.*, 1999), and we used the percentage canopy openness to estimate light-availability.

Statistical Analyses

To test whether seed germination, young seedling survival and old seedling survival differs according to i) distance from closest *M. fistulifera* tree (meters), ii) influence of the nest (over ant nest and away from ant nest), and iii) ant manipulation treatments (scarified, non-scarified and unmanipulated), we used three Generalized Linear Mixed Models (GLMM) with a binomial distribution. We set distance from the closest tree, ant manipulation treatments, and influence of the nest as explanatory variables and the proportion of germinated seeds, young seedling survival and old seedling survival on each seed depot as the response variables. We considered as the random effect the group of three seed depots that were placed together (see Figure 2; Crawley, 2013).

To test the effect of distance from the closest tree, categorical distance from ant nest entrance, and ant manipulation treatments on the seedling growth rate, we conducted a Linear Mixed-Effect Models (LME). We set those three variables as explanatory variables, seedling growth rates as the response variable, and the group of three seed depots placed over and away from nest entrances as the random effect (Crawley, 2013). To correct for heteroscedasticity and non-normality, we applied square-root transformations to the growth rate.

Finally, to evaluate if the light availability is different according to nest distance and distance from the closest *M. fistulifera* tree, we performed an Analysis of covariance (ANCOVA). We set the percentage of canopy openness in each group of tree seed depots as the response variable, the distance from the nest as predictor, and distance from the closest *M. fistulifera* tree as covariate.

We conducted all analyzes in the software R (R Core Team 2015). We also analyzed the residuals and checked for model suitability in all models. We graphically examined model assumptions, including normality of errors and homogeneity of variances. We used the *lme4* package v 1.1-12 (Bates *et al.*, 2016) to build the models. We access the global model values of GLMM's using a type III Wald Chi tests, and for LME model we used a type III Wald F tests with Kenward–Roger degrees of freedom approximation. In both cases, we use the *mixlm* package v 1.2.3 (Liland , 2018). We also used a backward model selection approach using likelihood ratio to determine the simplest fitted models.

RESULTS

Nests, seeds and trees distribution in the field

We found 125 *Atta sexdens* nest entrances with manipulated seeds. The average distance between the nest and the closest *Mabea fistlifera* tree was 3.89 meters and ranged from 0.40 to 12.45, being 84.4% located within the first 6 meters (Figure 3). *A. sexdens* is able to carry similar amount of seeds independent of distance ($F_{1,121}$=0.384, p=0.54; 5.98±0.59 seeds; mean±SE: Figure 3). We found in a unique ant nest entrance a maximum of 898 manipulated seeds. We removed this and another ant nest entrance (with 126 seeds) because their massive amount of seeds that affect drastically the average. Furthermore, the removing of this two ant nest entrances did not affect the significance of the number of carried seeds according to the distance from the closest tree ($F_{1,123}$ = 0.49, p=0.48).

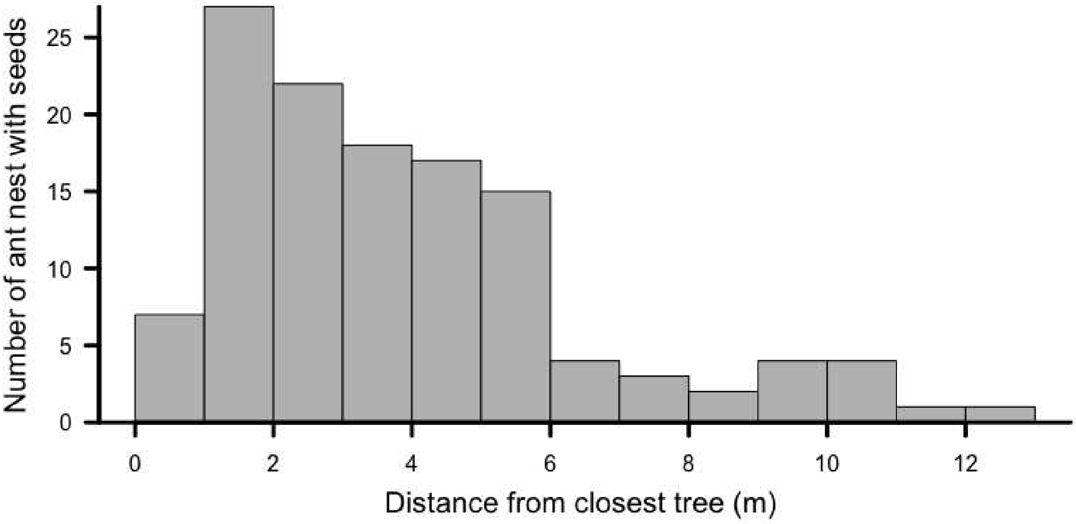

Figure 3. The frequency distribution of *Atta sexdens* nest entrances with at least one manipulated *Mabea fistulifera* according to their distance from the closest *M. fistulifera* tree.

Seed germination

From the total of 360 seeds (deposited in the 180 seed depots) in the beginning of the experiment, 107 germinated (29.72%) in 97 seed depots. We found a negative relationship between distance from *M. fistulifera* tree and the Proportion of germinated seeds (*Wald* χ^2 (1, N = 180) = 9.54, p = 0.02; figure 4). However, there was no difference between seeds deposited over or away from the nest (*Wald* χ^2 (1, N = 180) = 0.45, p = 0.49; mean proportion of germinated seeds: over nest = 29.44±3.43%; away from nest = 28.89±3.53%), as well as no difference on seed germination among the three ant manipulation treatments (*Wald* χ^2 (2, N= 180) = 2.29, p = 0.31; mean proportion of germinated seeds: *unmanipulated* = 27.5±4.01%; *scarified* = 29.16±3.99%; *non-scarified* = 30.83±4.76). Finally, the interaction among the three variables or between pairs of variables was not significant (χ^2 (2, N = 180) < 2.21, p > 0.33).

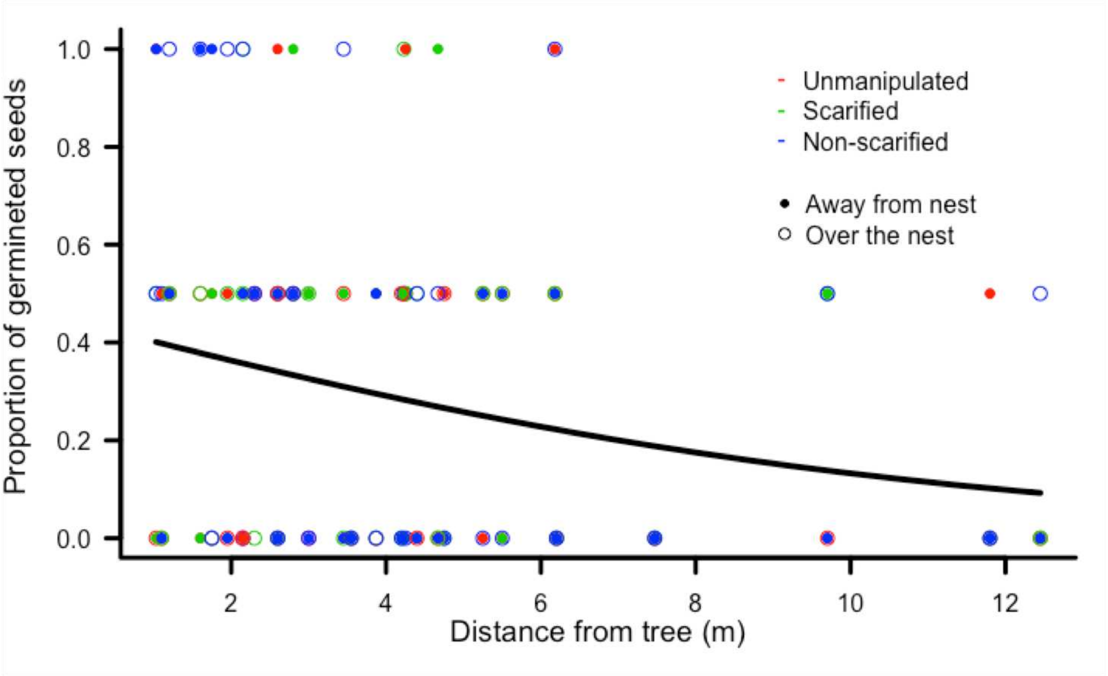

Figure 4: Proportion of germinated seeds in each seed depot according to its distance from the closest *Mabea fistulifera* tree. The colors represent different *Atta sexdens* manipulation treatments on seeds and the circle filling the distance of seeds from *A. sexdens* nest. The black curve shows the germination tendency.

Young seedlings

From all 107 germinated seeds, 54 seedlings (15% of sowing seeds) were alive after 3 months (the moment when we removed the PVC protection). We did not find any effect of distance from the closest *M. fistulifera* tree (*Wald* χ^2 (1, N = 97) = 0.51, p = 0.47), ant nest environment (*Wald* χ^2 (1, N = 97) = 1.42, p = 0.23), and ant manipulation treatments (*Wald* χ^2 (2, N = 97) = 4.82, p = 0.08) on the proportion of young seedling survival. Also, the interaction among the three variables or between pairs of variables was not significant (*Wald* χ^2 (2, N = 97) < 3.31, p > 0.19).

Old seedlings

From the 54 young seedlings, 23 remained alive (old seedlings) after one year since we removed the PVC protection (which represents 6.38 % of the initial sowing seeds). We did not find any significant effect of the three variables we analyzed for seedling mortality on the 360 days of experiment: Distance from the closest *M. fistulifera* tree (*Wald* χ^2 (1, N= 49)= 0.39, p=0.52), distance from ant nest (*Wald* χ^2

(1, N= 49)= 0.25, p=0.61), and ant manipulation treatments (*Wald* χ^2 (2, N= 49)= 1.31, p=0.52). Furthermore, the interaction among the three variables or between pairs of variables was not significant (*Wald* χ^2 (2, N= 49)< 1.75, p>0.37).

Seedling growth

The seedlings height ranged from 1.5 cm to 14.5 cm on the beginning of the growth measurement and from 1.5 cm to 56.5 cm when we last measured them (i.e. either on the 360th day or just before they died). We found a positive relationship between daily growth rate and the distance from the closest *M. fistulifera* tree ($F_{1,37}$ = 5.83, p=0.02, $R^2_{(c)}$= 0.3; figure 5). However, we found no difference on seedlings growth rate between from the treatments over and away from ant nests ($F_{1,35}$ = 0.0004, p= 0.94; over nest = 0.02±0.005 cm/day; away from nest = 0.03±0.004 cm/day) neither among ant manipulation treatments ($F_{1,38}$=2.26, p= 0.11; unmanipulated=0.03±0.006 cm/day; scarified= 0.03±0.007 cm/day; non-scarified= 0.02±0.002 cm/day).

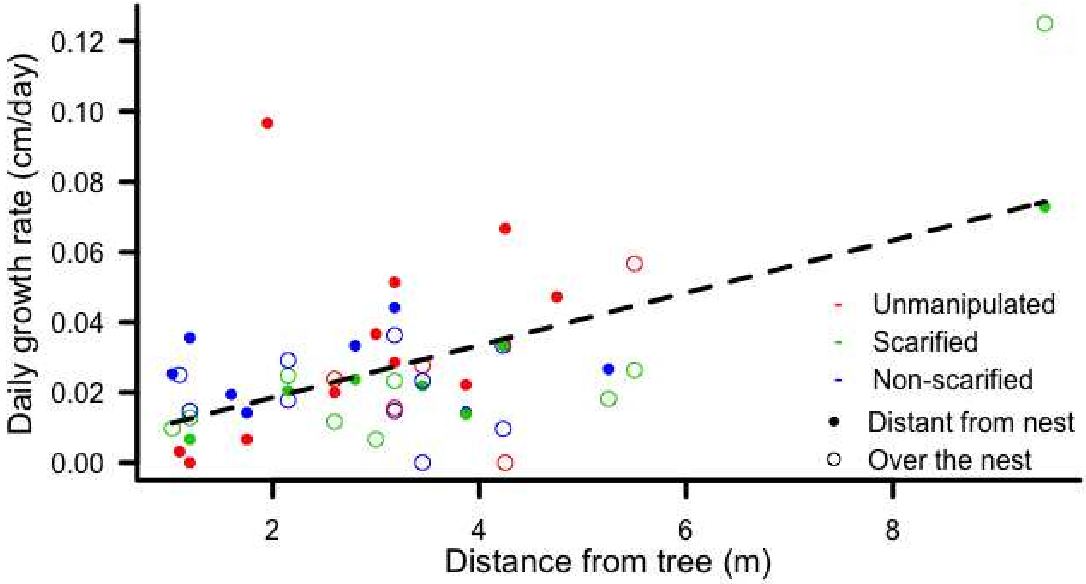

Figure 5: Daily growth rate (cm/day) of *Mabea fistulifera* seedling according to its distance from the closest co-specific tree. The colors represent different *Atta sexdens* manipulation treatments on seeds and the circle filling the distance of seeds from *A. sexdens* nest. The dashed slope shows the Growth rate tendency.

Canopy openness

The canopy openness above each group of three seed depots had a positive relationship with distance from *M. fistulifera* tree ($F_{1,56} = 44.27$, $p<0.001$, $R^2 = 0.43$; figure 6). There was no difference on light incidence according to the distance from ant nest ($F_{1,56} = 0.39$, $p=0.53$; over nest = 31.91±1.54% ; away from nest = 32.92±1.46%).

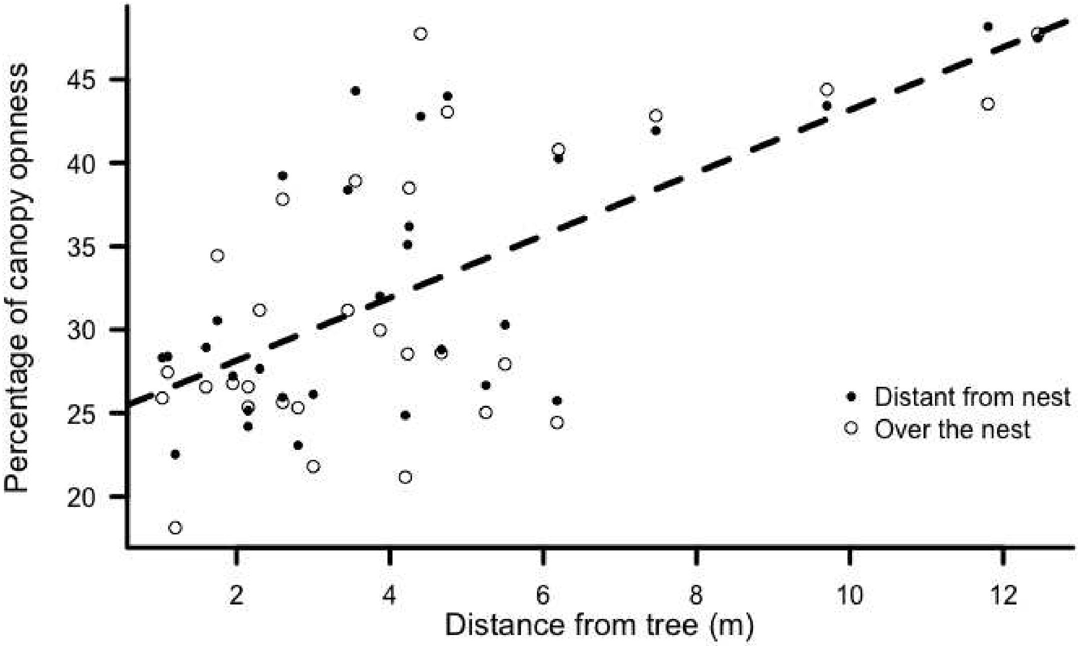

Figure 6: Percentage of canopy openness in each of the 60 experimental points according to their distance from the closest *Mabea fistulifera* tree. The circle filling shows the distance from the *Atta sexdens* nest entrance. The dashed slope shows the percentage of canopy openness in each point.

DISCUSSION

We experimentally demonstrate here that distance from the closest conspecific tree is an important driver influencing seed germination and plant growth while seed manipulation by ants (elaiosome removal and scarification) and nest environment (over and away the nest) were not important. We showed that as the distance from the nearest adult tree increases, there is a decrease in seed germination but an increase in seedling daily growth rate. Thereby, we suggest that myrmecochory might be promoting the so-called seed-seedling conflict (Schupp 1995), where the site of seed deposition impairs seed germination but increase seedling establishment. Furthermore according to our data, these contrasting effects seem to be mainly driven by light availability.

Seed removal distance is one of the most common mechanisms considered in studies of myrmechocory (revision in Gómez & Espadaler, 2013). Almost all studies consider the increase in distance from parental plant positive to plants (Gómez and Espadaler, 2013). However, those studies based their arguments on the great diversification of myrmecochorous plants lineages, which is likely to be promoted by a combination of factors such as decrease of parental-seedling competition, predation avoidance and increase in site suitability (Andersen, 1988; Boyd, 2001; Lengyel *et al.*, 2009; Leal *et al.*, 2015). In fact, empirical data about the effect of distance from parental plant on seed germination and seedling growth of myrmecochrous species is almost absent. From our knowledge, it was only empirically tested once in a greenhouse condition by Handel (1976), that showed an increase in growt of *Carex pedunculata* (cyperaceae) seedlings when growing away from the parental plant. What we demonstrated is that the effects of distance from adult trees, are conflicting between less germination and faster growth, at least for a pioneer tree dispersed by leaf-cutting ants. This is a typical example of seed-seedling conflict, when different life history stages could have agonistic development requirements, ending in a life history conflict of phases (Schupp 1995).

We propose that the seed-seedling conflict found here is mainly influenced by sunlight incidence. First, high sunlight incidence is responsible to increase in temperature and decrease in humidity (Tymen *et al.*, 2017) that can affect directly the capacity of seeds to germinate (Leal *et al.*, 2014a). Second, *M. fistulifera* is a heliophilous species, so it is expected that an increase in sunlight favors its growth rate. In this way, parental plants can have a nursing effect on seed germination because trees crown shadow decreases sunlight incidence, which could increase seed germination suitability. Otherwise, the tree crown shadow decreases the light availability to seedling, impairing its growth. Consequentially, it might affect the future plant productivity and even the probability of survival to maturity (Schupp, 1995). However, especially for our studied species, this last argument must be taken carefully since we found no effect of distance to parental-plant on seed and seedling survival after a year.

Elaiosome detachment or seed scarification did not affect seed germination, seedling growth, and survival of seedlings in field conditions. In fact, the effects of ant manipulation on seeds remain very controversial and seem to depend on the pair of ant-plant species interacting considered (Fernandes *et al.* 2018 submitted, Giladi, 2006; Imbert, 2006; Leal *et al.*, 2007; Prior *et al.*, 2014). The effects of seed manipulation by ants on seed fate have been overlooked, especially when considering natural conditions where other factors might be more important than the ant manipulation per se (e.g., such as water availability, temperature, luminosity and soil quality). In addition, the scarification is usually associated to either embryo damage which decreases seed germination (Zettler *et al.*, 2001). Based on our results we show that *A. sexdens* ants do not compromise seed viability by damage the embryo during manipulation. Thus, this ant specie cannot be considered granivorous.

Regarding ant deposition effects, our results contrast with the most recent meta-analyses that found a higher plant growth over ant nests when compared to seed deposited far from nests (Farji-Brener & Werenkraut, 2015, 2017). We propose here that *A. sexdens* nest environment does not exercise any influence on seed and seedling performance for two main reasons. Firstly, the soil nutrient content comparing the sites over and away nest entrance could be similar (Madureira *et al.*, 2013; Farji-Brener & Werenkraut, 2015) but unfortunately we did not measure it in the present paper. Secondly, it is known that some plant species have different responses to soil nutrients availability (Farji-Brener *et al.*, 2010). *M. fistulifera* is adapted to poor nutrient soil and high sunlight availability (Lorenzi, 2000) and so far might be less influenced by soil nutrient content. Moreover, we also showed that *A.* sexdens does not seem to act as herbivore of *M. fistulifera* seedlings because the seedling mortality on ant nest-surroundings and away did not differ. However, we record five seedlings (1 over ant nest and 4 away from ant nest) that seemed to be consumed by defoliator herbivores but we were not able to infer the herbivores identity.

This is the first study to test in field condition the effect of myrmecochorous seed distancing from a conspecific adult plant on seed fate and plant establishment. Our results helps on the understand of the effects of seed removal distances by ants, a well study part of myrmecochory but with a lack of empirical support to their effects on seed fate and plant development. Furthermore, we showed that the increases of distance from a conspecific plant characterize a seed-seedling conflict, being negative for seed germination and positive for seedling growth. However, since we are not able to determine here the overall effect of *A. sexdens* on *M. fistulifera* fitness or population growth we suggest this subject for future studies. Finally, we showed that for our system *A. sexdens* do not act nor as a granivor neither as seedling herbivore. This associate to its large colonies and broad occurrence make this ant species an important agent of seed dispersion.

REFERENCES

Andersen, A.N. (1988) Dispersal distance as a benefit of myrmecochory. *Oecologia*, **75**, 507–511.

AntWeb. (2018) Available from http://www.antweb.org. Accessed 18 February

Bates, D., Maechler, M., Bolker, B. & Walker, S. (2016) lme4: Linear Mixed-Effects Models Using “Eigen” and S4. R Package Version 1.1-12. *R*.

Bello, C., Galetti, M., Montan, D., Pizo, M.A., Mariguela, T.C., Culot, L., *et al.* (2017) Atlantic frugivory: a plant–frugivore interaction data set for the Atlantic Forest. *Ecology*, **98**, 1729.

Boyd, R.S. (2001) Ecological benefits of myrmecochory for the endangered chaparral shrub *Fremontodendron decumbens*. *American Journal of Botany*, **88**, 234–241.

Camargo, P.H.S.A., Martins, M.M., Feitosa, R.M. & Christianini, A. V. (2016) Bird and ant synergy increases the seed dispersal effectiveness of an ornithochoric shrub. *Oecologia*, **181**, 507–518.

Carvalho, B. & Ribeiro, S.P. (2018) Architecture of Mabea fistulifera Mart. (Euphorbiaceae), a Neotropical semideciduous tree: development and variations in crown allometry between environments. *Flora*, **239**, 104–110.

Castro, S., Ferrero, V., Loureiro, J., Espadaler, X., Silveira, P. & Navarro, L. (2010) Dispersal mechanisms of the narrow endemic Polygala vayredae: Dispersal syndromes and spatio-temporal variations in ant dispersal assemblages. *Plant Ecology*, **207**, 359–372.

Christianini, A. V. & Oliveira, P.S. (2010) Birds and ants provide complementary seed dispersal in a neotropical savanna. *Journal of Ecology*, **98**, 573–582.

Christianini, A. V, Oliveira, P.S., Bruna, E.M. & Vasconcelos, H.L. (2014) Fauna in decline: Meek shall inherit. *Science*, **345**, 1129.

Comita, L.S., Queenborough, S.A., Murphy, S.J., Eck, J.L., Xu, K., Krishnadas, M., *et al.* (2014) Testing predictions of the Janzen-Connell hypothesis: A meta-analysis of experimental evidence for distance- and density-dependent seed and seedling survival. *Journal of Ecology*, **102**, 845–856.

Connell, J.H. (1971) On the role of natural enemies in preventing competitive exclusion in some marine animals and in rain forest trees. In *Dynamics of populations*. 298–312.

Crawley, J.M. (2013) *The R Book. Cran*. second. John Wiley & Sons Ltd, Chichester, United Kingdom.

Farji-Brener, A.G., Lescano, N. & Ghermandi, L. (2010) Ecological engineering by a native leaf-cutting ant increases the performance of exotic plant species. *Oecologia*, **163**, 163–169.

Farji-Brener, A.G. & Werenkraut, V. (2015) A meta-analysis of leaf-cutting ant nest effects on soil fertility and plant performance. *Ecological Entomology*, **40**, 150–158.

Farji-Brener, A.G. & Werenkraut, V. (2017) The effects of ant nests on soil fertility and plant performance: a meta-analysis. *Journal of Animal Ecology*, **86**, 866–877.

Farwig, N. & Berens, D.G. (2012) Imagine a world without seed dispersers: A review of threats, consequences and future directions. *Basic and Applied Ecology*, **13**, 109–115.

Fernandes, T.F., Paolucci, L.N., Carmo, F.M.S., Sperber, C.F. & Campos, R.I. (2018) Seed manipulation by ants: an experiment disentangling the effects of three main ant behaviors on seed germination. *submited to Ecological Entomology*.

Filomeno, C.A., Barbosa, L.C.A., Pereira, J.L., Pinheiro, A.L., Fidêncio, P.H. & Montanari, R.M. (2016) The Chemical Diversity of Eucalyptus spp. Essential Oils from Plants Grown in Brazil. *Chemistry & Biodiversity*, **13**, 1656–1665.

Frazer, S., Canham, C.D. & Lertzman, K. (1999) Gap light analyzer (GLA). Users manual and programme documentation, Version 2.0.

Giladi, I. (2006) Choosing benefits or partners: a review of the evidence for the evolution of myrmecochory. *Oikos*, **3**, 481–492.

Gómez, C. & Espadaler, X. (1998) Myrmecochorous dispersal distances: a world survey. *Journal of Biogeography*, **25**, 573–580.

Gómez, C. & Espadaler, X. (2013) An update of the world survey of myrmecochorous dispersal distances. *Ecography*, **36**, 1193–1201.

Handel, S.N. (1976) Dispersal Ecology of Carex pedunculata (Cyperaceae), a New North American Myrmecochore. *American Journal of Botany*, **63**, 1071.

Hölldobler, B. & Wilson, E.O. (1990) *The Ants*. *The Belknap Press of Harvard University Press*.

Horvitz, C.C. (1981) Analysis of how ant behaviors affect germination in a tropical myrmecochore Calathea microcephala (P. & E.) Koernicke (Marantaceae): Microsite selection and aril removal by neotropical ants, Odontomachus, Pachycondyla, and Solenopsis (Formicidae). *Oecologia*, **51**, 47–52.

Howe, H.F. & Smallwood, J. (1982) Ecology of Seed Dispersal. *Annual Review of Ecology and Systematics*, **13**, 201–228.

Hurtado, A., Escobar, S., Marina Torres, A. & Armbrecht, I. (2012) Exploring the role of the generalist ant Solenopsis geminata (Formicidae: Myrmicinae) on seed germination of Senna spectabilis (Fabaceae: Caesalpinioideae). *Caldasia*, **34**, 127–137.

Imbert, E. (2006) Dispersal by ants in Centaurea corymbosa (Asteraceae): What is the elaiosome for? *Plant Species Biology*, **21**, 109–117.

Janzen, D. H. (1970) Herbivores and the number of tree species in tropical forests.

American Naturalist.**104**, 501-528.

Kaspari, M. (1996) Worker size and seed size selection by harvester ants in a neotropical forest. *Oecologia*, **105**, 397–404.

Kwit, C., Marcello, G.J., Gonzalez, J.L., Shapiro, A.C. & Bracken, R.D. (2012) Advantages of seed dispersal for a myrmecochorous temperate forest herb. *American Midland Naturalist*, **168**, 9–17.

Leal, I.R., Leal, L.C. & Andersen, A.N. (2015) The benefits of myrmecochory: A matter of stature. *Biotropica*, **47**, 281–285.

Leal, I.R., Wirth, R. & Tabarelli, M. (2007) Seed dispersal by ants in the semi-arid caatinga of north-east Brazil. *Annals of Botany*, **99**, 885–894.

Leal, I.R., Wirth, R. & Tabarelli, M. (2014a) The multiple impacts of leaf-cutting ants and their novel ecological role in human-modified neotropical forests. *Biotropica*, **46**, 516–528.

Leal, L.C., Andersen, A.N. & Leal, I.R. (2014b) Anthropogenic disturbance reduces seed-dispersal services for myrmecochorous plants in the Brazilian Caatinga. *Oecologia*, **174**, 173–181.

Lengyel, S., Gove, A.D., Latimer, A.M., Majer, J.D. & Dunn, R.R. (2009) Ants sow the seeds of global diversification in flowering plants. *PLoS ONE*, **4**, 1–6.

Levin, S.A., Muller-Landau, H.C., Nathan, R. & Chave, J. (2003) The ecology and evolution of seed dispersal: a theoretical perspective. *Annual Review of Ecology Evolution and Systematics*, **34**, 575–604.

Liland, K.H. (2018) mixlm: Mixed Model ANOVA and Statistics for Education. R Package Version 1.2.3.

Lorenzi, H. (2000) Árvores brasileiras: Manual de identificação e cultivo de plantas arbóreas nativas do Brasil. 3th ed. Plantarum. Nova Odessa, BR.

Madureira, M.S., Schoereder, J.H., Teixeira, M.C. & Sobrinho, T.G. (2013) Why does Atta robusta (Formicidae) not change soil features around their nests as other leaf-cutting ants do? *Soil Biology and Biochemistry*, **57**, 916–918.

Meyer, S.T., Leal, I.R., Tabarelli, M. & Wirth, R. (2011) Performance and fate of tree seedlings on and around nests of the leaf-cutting ant Atta cephalotes: Ecological filters in a fragmented forest. *Austral Ecology*, **36**, 779–790.

Ohkawara, K. (2005) Effect of timing of elaiosome removal on seed germination in the ant-dispersed plant, Erythronium japonicum (Liliaceae). *Plant Species Biology*, **20**, 145–148.

Ohkawara, K. & Akino, T. (2005) Seed cleaning behavior by tropical ants and its anti-fungal effect. *Journal of Ethology*, **23**, 93–98.

Ortiz-Pulido, R. & Rico-Gray, V. (2000) The effect of spatio-temporal variation in understanding the fruit crop size hypothesis. *Oikos*, **91**, 523–527.

Padilla, D.P., González-Castro, A. & Nogales, M. (2012) Significance and extent of secondary seed dispersal by predatory birds on oceanic islands: The case of the Canary archipelago. *Journal of Ecology*, **100**, 416–427.

Peel, M.C., Finlayson, B.L. & McMahon, T.A. (2007) Updated world map of the Köppen-Geiger climate classification. *Hydrology and Earth System Sciences Discussions*, **4**, 439–473.

Peternelli, E.F.D.O., Barbosa, L.C.A. & Lucia, T.M.C. Della. (2008) Isolation of compounds attractive to the leaf-cutting ant *Atta sexdens rubropilosa* forel (Hymenoptera: Formicidae) from Mabea fistulifera elaiosome. *Quimica Nova*, **31**, 475–478.

Peternelli, E.F.D.O., Lucia, T.M.C. Della & Martins, S.V. (2004) Espécies de formigas que interagem com as sementes de Mabea fistulifera Mart.

(Euphorbiaceae). *Revista Árvore*, **28**, 733–738.

Peternelli, E.F.O., Delia Lucia, T.M.C., Peternelli, L.A. & Moreira, N.C. (2009) Seed transport and removal of the elaiosome of *Mabea fistulifera* by workers of *Atta sexdens rubropilosa* (Hymenoptera: Formicidae). *Sociobiology*, **53**, 275–290.

Prior, K.M., Saxena, K. & Frederickson, M.E. (2014) Seed handling behaviours of native and invasive seed-dispersing ants differentially influence seedling emergence in an introduced plant. *Ecological Entomology,* **39**, 66–74.

R Core Team. (2016) R Development Core Team. *R: A Language and Environment for Statistical Computing*.

Schupp, E.W. (1995) Seed-seedling conflicts, habitat choice, and patterns of plant recruitment. *American Journal of Botany*, **82**, 399–409.

Schupp, E.W., Jordano, P. & Gómez, J.M. (2010) Seed dispersal effectiveness revisited: A conceptual review. *New Phytologist*, **188**, 333–353.

Servigne, P. & Detrain, C. (2010) Opening myrmecochory’s black box: What happens inside the ant nest? *Ecological Research*, **25**, 663–672.

Silva, C.A., Vieira, M.F. & Amaral, C.H. Do. (2010) Floral attributes, ornithophily and reproductive success of Palicourea longepedunculata (Rubiaceae), a distylous shrub in southeastern Brazil. *Revista Brasileira de Botânica*, **33**, 207–213.

Travest, A., Robertison, A.W. &Rodriguez-Pérez (2007) A review on the role of endozoochory in seed germination in: Dennis, A.J., Schupp, E.W., Green, R.J. & Westcott, D.A. (2007) Seed Dispersal: theory and its application in changing word. CABI. Oxfordshire, UK.

Tymen, B., Vincent, G., Courtois, E.A., Heurtebize, J., Dauzat, J., Marechaux, I., *et al.* (2017) Quantifying micro-environmental variation in tropical rainforest understory at landscape scale by combining airborne LiDAR scanning and a sensor network. *Annals of Forest Science*, **74**.

van der Pijl, L. (1982) *Principles of Dispersal in Higher Plants*. *Soil Science*. 3rd editio. Springer-Verlang, New York,EUA.

Wenny, D.G. (2001) Advantages of seed dispersal: A re-evaluation of directed dispersal. *Evolutionary Ecology Research*, **3**, 51–74.

Wenny, D.G. & Levey, D.J. (1998) Directed seed dispersal by bellbirds in a tropical cloud forest. *Proceedings of the National Academy of Sciences of the United States of America*, **95**, 6204–6207.

Whitney, K.D. (2002) Dispersal for distance? *Acacia ligulata* seeds and meat ants *Iridomyrmex viridiaeneus*. *Austral Ecology*, **27**, 589–595.

Wirth, R., Meyer, S.T., Almeida, W.R., Araújo, M.V., Barbosa, V.S. & Leal, I.R. (2007) Increasing densities of leaf-cutting ants (Atta spp.) with proximity to the edge in a Brazilian Atlantic forest. *Journal of Tropical Ecology*, **23**, 501–505.

Zettler, J.A., Spira, T.P. & Allen, C.R. (2001) Ant-seed mutualisms: Can red imported fire ants sour the relationship? *Biological Conservation*, **101**, 249–253.