

Biology – Zoology

Influence of *Atta sexdens rubropilosa* on seeds germination of *Copaifera langsdorffii*

Influência de *Atta sexdens rubropilosa* sobre a germinação de sementes de *Copaifera langsdorffii*

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ABSTRACT

Considering the information gaps on the effect of ant action on germination, this study aimed to evaluate the influence of ants of the species *Atta sexdens rubropilosa* in overcoming seed dormancy of *Copaifera langsdorffii* comparing the results obtained with traditional methodologies, in addition to evaluating the presence of coumarin in the seeds. For each germination treatment (7) 100 seeds were used, with 4 replications: (1) control, (2) seeds without aril, (3) seeds with immersion in acid for 5 minutes, (4) seeds with immersion in acid for 10 minutes, (5) sanded seeds, (6) collected seeds with presence of fissures on the forehead and, (7) seeds with partial cotyledons. The seeds were maintained at a temperature of 25 °C, being evaluated the germination and vigor. Data were subjected to analysis of variance and means were compared by the Tukey test at 5% probability. The best germination was obtained in treatment 6 (seeds with fissures caused by ants), which also provided the best germination speed index and the shortest germination time. The chemical analysis of the seeds also indicated the presence of coumarins in the aril and in the external structure (forehead) of the seeds, demonstrating the presence of germination inhibitors, indicating the importance of this ant species in the overcome dormancy process of seeds of *Copaifera langsdorffii*.

Palavras-chave: Overcome dormancy; Saúva-limão; Pau-d'óleo; Coumarins

RESUMO

Considerando as lacunas de informação sobre o efeito da ação de formigas na germinação, este trabalho teve como objetivo avaliar a influência de formigas da espécie *Atta sexdens rubropilosa* na superação da dormência de sementes de *Copaifera langsdorffii*, comparando os resultados obtidos com metodologias tradicionais, além de avaliar a presença de cumarina nas sementes. Para cada tratamento de germinação (7) foram utilizadas 100 sementes, com 4 repetições: (1) controle, (2) sementes sem arilo, (3) sementes com imersão em ácido por 5 minutos, (4) sementes com imersão em ácido por 10

minutos, (5) sementes lixadas, (6) sementes coletadas com presença de fissuras na testa e, (7) sementes com cotilédones parciais. As sementes foram mantidas à temperatura de 25 °C, sendo avaliados a germinação e vigor. Os dados foram submetidos à análise de variância e as médias comparadas pelo teste de Tukey a 5% de probabilidade. A melhor taxa de germinação foi obtida no tratamento 6 (sementes com fissuras causadas por formigas), que também proporcionou o melhor índice de velocidade de germinação e o menor tempo de germinação. A análise química das sementes também indicou a presença de cumarinas no arilo e na estrutura externa (testa) das sementes, demonstrando a presença de inibidores de germinação e indicando a importância desta espécie de formiga no processo de quebra de dormência de sementes de *Copaifera langsdorffii*.

Keywords: Superação de dormência; Saúva-limão; Pau-d'óleo; Cumarinas

1 INTRODUCTION

Some species present seeds with exogenous dormancy, a strategy used to germinate only when environmental conditions are more favorable, increasing survival rates Bradford & NONOGAKI (2007). On the other hand, this feature is a limiting factor and means that overcoming the dormancy, either chemical or mechanical, are needed BEWLEY *et al.* (2013). In the laboratory, overcoming dormancy is usually accomplished through several methods and the success of these treatments depends on the type of dormancy, variable among species BRADFORD & NONOGAKI (2007). The two most commonly used methods are chemical or mechanical scarification, although the use of acid by seedling producers should be avoided, due to the risks caused by its use BRASIL (2009).

The seeds of Fabaceae usually present exogenous integumentary dormancy, a factor that hinders germination and can be a limiting for the production of a greater number of individuals CARVALHO (2003). One of the species of Fabaceae with dormant seeds is *Copaifera langsdorffii* Desf. (Caesalpinioideae) popularly known as 'copaiba' or 'pau-d'óleo'. It is classified as a climax and reaches up to 35 m in height, being used in reforestation programs and found in transition areas from the Cerrado to broadleaf forest CARVALHO (2003).

The action of animals, such as birds and mammals, on the processes of seed dispersal and induction of germination, are well known. However, few studies are reported on the importance of ants in these processes. The Formicidae family is

distributed in almost all terrestrial ecosystems, highlighting the genus *Atta*, known as 'saúva' or cutter ant (subfamily Myrmicinae, Attini tribe), insects typical of the Americas, covering an area that extends from the southern United States to northern Argentina SOLOMON *et al.* (2008).

In Brazil there are several species of *Atta* cataloged and of these, *Atta sexdens rubropilosa* Forel, 1908, popularly known as 'saúva-limão' stands out, found in almost all the territory SOUZA *et al.* (2009). According to Carvalho *et al.* (2012), 'saúvas' may decrease or delay the process of forest regeneration, as well as accelerate it, due to the high selective predation, depending on the existing plants being more or less palatable. This situation was reported by Tavares *et al.* (2016), demonstrating that *Atta laevigata* Smith (1858) acted primarily as predators and their actions may interfere in the native vegetation regeneration. According to Santos *et al.* (2008), 'saúvas' are very abundant in altered areas, and can become an environmental problem. However, studies also demonstrate that species of this genus act as dispersers and/or inducers of germination of several plants, dispersion called myrmecoria LEITE *et al.* (2013); SILVA & SOUZA (2014).

The process in which the ants interfere in the germination is linked to the removal of the aryl by the foraging, where lesions can occur in the integument that promotes the overcoming of dormancy, inducing a more significant germination SILVA & SOUZA (2014). Souza *et al.* (2015), in a study with *Copaifera langsdorffii* seeds in the laboratory, simulating aryl removal by ants, obtained about 50% germination while seeds with aryl did not germinate. Eira and Martins Netto (1998) write that in the aril there may be the presence of germination inhibitors, such as coumarin, which would reduce the percentage of germination.

Considering the value attributed to this species in forest programs and its usefulness in folk medicine, besides being considered in danger of extinction Carvalho (2003), this work aimed to: evaluate the effect of the aril removal of *Copaifera langsdorffii* seeds by ants of the species *Atta sexdens* in the germination process, comparing with the methods used in the analysis of seeds of the species under laboratory conditions.

2 MATERIAL AND METHODS

2.1 Study area

The collection occurred in forest área ('cerradão'), in the municipality of Terenos (Latitude S 20°26'32 ", longitude 54°51'37", alt 437 m.), Mato Grosso do Sul. On site ants *Atta sexdens* were viewed moving in trails, transporting seeds and also collecting aril. Species was identified in the field through a characteristic lemon odor exhaled when the head of a worker is cut, with confirmation of identification in the laboratory.

Figure 1 – Seeds of *Copaifera langsdorffii*, predated and whole, found in different forest locations, with some being transported by *Atta sexdens* to their nests, Terenos, Mato Grosso do Sul



Source: The authors (2020)

The seeds of *Copaifera langsdorffii* found on the ground, under the canopy of the matrix trees (8) and in the trails, in the middle of the forest, were collected (Figure 1). Also, open fruits were harvested, adhered to the trees, containing seeds that had already reached the physiological maturity, indicated by the orange coloration of the aryl. The collected seeds, with an average length of 15 mm (± 2 mm), were packed in paper bags and transported to the Research Laboratory of Environmental Systems and Biodiversity, Anhanguera-Uniderp University, Campo Grande.

2.2 Evaluation of the presence of coumarins in seeds

The seeds were evaluated for the presence of coumarins in the aril, external structure (forehead) of seeds and reserve structure (starch), according to Matos (2009) and Simões *et al.* (2017) (10% NaOH solution and 10% KOH solution and UV light test).

2.3 Evaluation of aryl

In the laboratory, the percentage of aryl consumed was evaluated visually by dividing the seeds into 5 groups: 1 to 25% of aryl consumed, 26 to 50%, 51 to 75% and 76 to 100% of aryl consumed, plus 0% (seeds with intact aryl).

2.4 Germination test

The seeds water content was carried out using the oven method at 105 °C Brasil (2009), using four replicates, with 20 seeds. The experimental design was completely randomized, with four replicates of 25 seeds, and the treatments consisted of:

(1) intact seeds (with aryl), collected directly from the matrices (**control**); (2) seeds without aryl (manually removed), collected directly from the matrices (**without aryl**); (3) seeds without aryl (manually removed), collected directly from the matrices and subjected to overcoming dormancy by chemical method, by immersion in sulfuric acid (PA) for five minutes and subsequent washing for three minutes (**5 minutes acid**); (4) seeds without aryl, collected directly from the matrices and subjected to

overcoming dormancy by chemical method, by immersion in sulfuric acid (PA) for ten minutes and subsequent washing for three minutes (**10 minutes acid**); (5) seeds without aryl (manually removed), collected directly from the matrices and subjected to overcoming dormancy by means of scarification using water sandpaper, granulometry 120, on the opposite side of the embryo (**sandpaper**); (6) seeds without aryl, collected directly from the ground, with the presence of fissures in the forehead, observed to the naked eye and through a stereomicroscope, due to the consumption of aril by ants (**fissures**); and, (7) seeds without aryl (removed by ants), collected on the ground and with part of damaged cotyledons (**damaged**).

The seeds which have not undergone chemical treatment were disinfected superficially by sodium hypochlorite (5%) for three minutes. Then washed for one minute in running water to avoid contamination by pathogens.

After the seeds preparation, they were wrapped in two sheets of germitest paper (paper roll), previously moistened with 0.1% deconyl® fungicide and placed in polyethylene bags. The plastic bags were kept at a temperature of 25° C in a germination chamber with a 12-hour white photoperiod (four fluorescent lamps of 20 W, ± 660 lux).

The germination evaluation was performed every 24 hours and finished on the 14th day. Germinated seeds were considered the ones that presented primary root with 2 mm of length, taking into account the physiological criteria of germination Labouriau (1983).

After the end of the experiment, the non-germinated seeds were subjected to the viability test of 1% tetrazolium in aqueous solution Brazil (2009). The seeds were placed in darkened plastic boxes (the seeds of the "control" and "aryl-free" treatments were previously scarified in acid), between two sheets of paper towel soaked with the solution and kept in the dark at 25 °C for 24 hours. After this period, they were sectioned and evaluated by stereomicroscope observation.

2.5 Statistical treatment

The percentage of germination (root emission) and the vigor measured indirectly by the mean germination time (GMT) were evaluated, quantifying germination from the kinetic point of view Labouriau (1983) and by germination speed index (GSI) MAGUIRE (1962). The data were subjected to the Shapiro-Wilk test for normality and Levene for homogeneity of the variances. Based on these two assumptions, the analysis of variance (ANOVA) was applied, followed by the Tukey test for comparisons among the means ($\alpha = 0.05$), using the statistical program Bioestat 5.0.

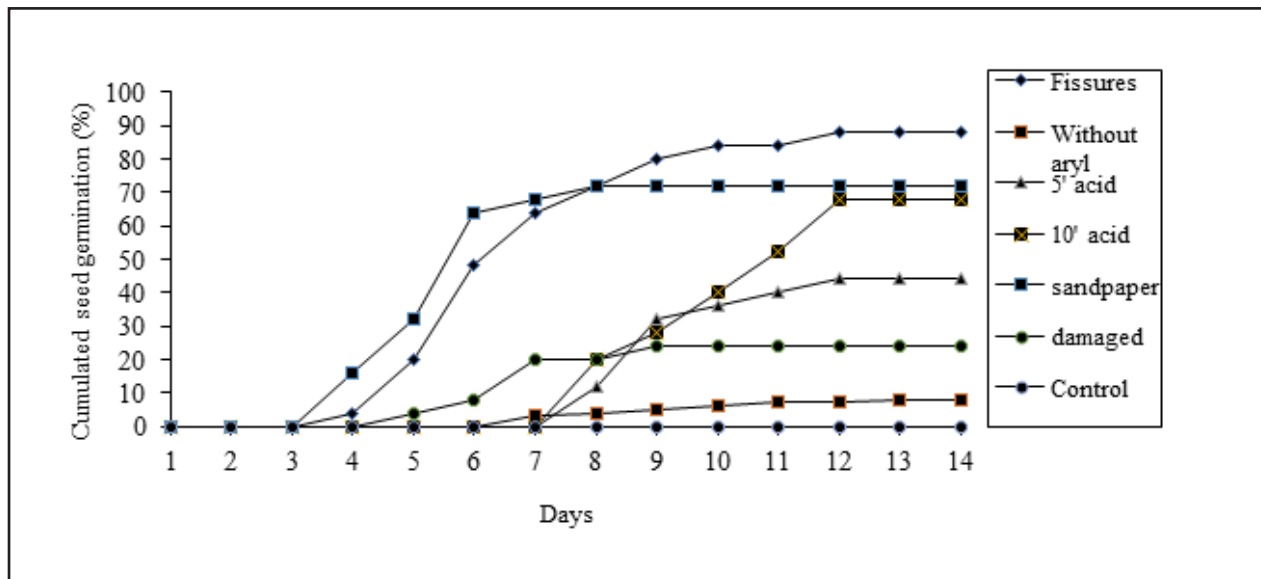
The relative frequency of germination LABOURIAU & VALADARES (1976) and cumulative germination (accumulated data) were evaluated, obtained by adding the seeds germinated daily, from the protrusion of the primary root.

3 RESULTS AND DISCUSSION

The water content in the seeds of *Copaifera langsdorffii* was 7.2%. The seeds of *Copaifera langsdorffii* presented water content within the percentage found in most plant species, between 5 and 20% Bewley *et al.* (2013). Similar values were also cited by Souza *et al.* (2013), 10.3% and Salomão *et al.* (2003), 10%, who considered the seeds of the species as orthodox.

According to Bewley *et al.* (2013), *Copaifera langsdorffii* seeds supports dehydration from 5 to 7%, without losing viability, and this tolerance is acquired during the maturation process. According to Piña-Rodrigues and Aguiar (1993), the degree of the seeds moisture at maturation can vary depending on the environmental conditions, decreasing until reaching stability with the environment. The data found by this work indicated that the seeds of *C. langsdorffii* used have adequate moisture content, which, according to Bewley *et al.* (2013) corresponds to a certain loss of moisture without damage to the germination process.

Figure 2 – Cumulated seed germination of *Copaifera langsdorffii* collected in Cerrado of Mato Grosso do Sul, undergoing different treatments to overcome dormancy



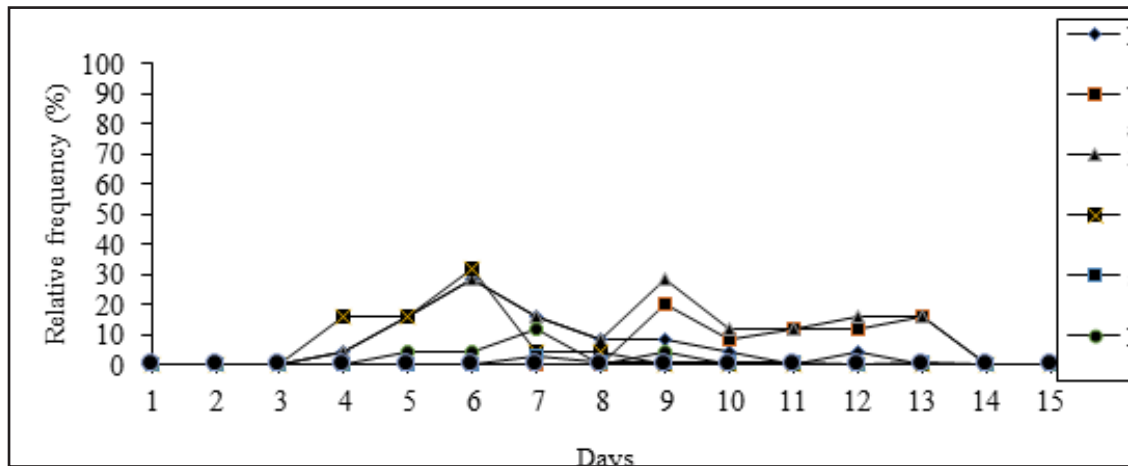
Source: The authors (2020)

In relation to the accumulated germination, the data demonstrated that the process occurred in almost all the treatments. In “fissures” and “sandpaper”, the germination occurred from the 4th day; in “damaged”, day 5 and “without aryl” and “acid 5 and 10 minutes”, from the 7th and 8th day, respectively. Only in the control treatment germination did not occur (Figure 2).

Confirming the results obtained, Guerra *et al.* (2006) observed that the germination of *Copaifera langsdorffii* seeds, treated with sulfuric acid, started on the 5th day.

The relative frequency of germination demonstrates that it is not perfectly synchronized and is distributed in a short incubation time (Figure 3). This slightly heterogeneous frequency, similar to almost all treatments, would indicate a lower synchronization and, consequently, the distribution of germination for longer periods of time Labouriau (1983).

Figure 3 – Relative frequency of seed germination of *Copaifera langsdorffii* collected in the Cerrado of Mato Grosso do Sul, subjected to different treatments to overcome dormancy



Source: the authors (2020)

According to Rossato and Kolb (2010), for some species, a greater synchronization of germination (which would occur under optimal temperature conditions) would be a strategy related to a rapid colonization of the environment. On the other hand, according to Labouriau (1983), the lower synchronization could favor a greater percentage of seedling survival at certain moments, taking into account the germination distribution over time.

The short period of germination with heterogeneous distribution is probably related to the favorable environmental conditions of the region; the season of seed dispersal occurs in October, beginning of the rainy season. This facilitates the seedlings establishment. Germination peaks, for all treatments, occurring between the sixth and ninth day, confirm that the species can settle quickly in the environment. According to Carvalho (2003), up to 365 trees per hectare can be found in certain areas, indicating their colonization capacity.

Studies with other species, such as *Anadenanthera colubrina* (Vellozo) Brenan, peak of germination on the 1st day, *Acacia polyphylla* DC., maximum germination on the 3rd day Arruda et al. (2015) and *Swartzia recurva* Poep., beginning of germination

on the 3rd day Santos *et al.* (2015), demonstrate that this may be a strategy for some species of this family.

In relation to the highest percentage of germination, the seeds of the treatment “fissures” (greater number of seeds germinated in less time) presented the highest averages followed by “acid 10 minutes” and “sandpaper”. Regarding seed vigor (GSI and MGT), the results also indicated the best average in the treatment “fissures”, followed by “sandpaper” (Table 1). Salomão *et al.* (2003) reported between 85-95% of germination with the removal of aryl and scarification, both results similar to this work.

Table 1 - Germination (G%), germination speed index (GSI), mean germination time (MGT) in days, number of viable and dead seeds, and coefficients of variation (CV%) of *Copaifera langsdorffii* seeds collected in Cerrado of Mato Grosso do Sul, undergoing different treatments to overcome dormancy

Treatment	G (%)	GSI	MGT (days)	Viable seeds (%)	Dead seeds (%)
Control	0	-	-	95 a	5 a
Without aryl	8 e	0.9 e	9.0 c	88 b	4 a
Acid 5'	44 c	4.8 c	9.3 c	51 c	5 a
Acid 10'	68 b	7.0 b	9.9 c	28 d	4 a
Sandpaper	72 b	13.6 a	5.8 a	26 d	2 b
Fissures	88 a	13.8 a	6.4 a	8 f	4 a
Damaged	24 d	3.6 d	6.4 a	14 e	62 c
CV (%)	72.4	82.6	46.9	73.2	164.9

*Means followed by the same letter in the column did not differ statistically from each other, Tukey test ($p < 0.05$).

Source: Authors' organization

The germination results indicated that germination did not occur in “control” treatment, factor related to the presence of coumarins found in the aryl and external structure (forehead) of the seeds. In the reserve structure (starch) coumarins were not found. Although the “control” and “without aryl” treatments did not show adequate germination rates, the non-germinated seeds, for the most part, were not dead, with the tetrazolium test demonstrating their viability.

The absence of the germinative process in some treatments may be related to the presence of inhibitors of germination in the aryl Eira & Martins (1998), associated to exogenous tegumentary dormancy of the seed, not allowing the germination. Souza *et al.* (2015), using the same species, confirmed that the presence of aryl inhibits the germination process, demonstrating the possible presence of inhibitors in this structure. Pereira *et al.* (2009) describe as probable the presence of inhibitors such as coumarins in aryl, considering that they exist in other structures, such as the integument and cotyledons.

The results obtained by this research confirmed the presence of coumarins in the aril and in the seed husk, which would inhibit seed germination under natural conditions. However, the foraging of seeds by ants eliminates (or reduces) the structures that contain coumarins, allowing the beginning of the germination process, as observed in seeds without arils and submitted to scarification processes ("sandpaper" treatment).

The higher percentage of germination, found in the treatment "fissures", showed that the foraging of the ants, with the aryl removal, caused fissures in the seed forehead, assisting in the germination. The large number of viable seeds in the "control" and "without aryl" treatments indicated the need for scarification. It is known that when scarification is not adequate or nonexistent, certain seeds do not germinate; but can remain viable.

Evaluating all parameters, the treatment "fissures" probably allowed wear on the forehead of the seeds, uniformly, resulting in higher germination rate. However, for the formation of normal seedlings (root and shoot), the time of 14 days was not enough.

Souza *et al.* (2013), cited percentages of germination between 16 and 47%, without the use of scarification; but they do not mention whether they removed the aryl. Andreani Junior *et al.* (2014) described that the best treatment was with sulfuric acid for 10 minutes (61%), results similar to those found in this study (Acid 10' – 68%) although the "fissures" treatment achieved 88% germination. Whereas, Souza *et al.*

(2015), using acid scarification for 5 minutes, obtained about 38% of germination, similar to that found by this work and indicating that the time of 10 minutes or more is necessary to obtain better rates. The scarification, either chemical or mechanical, removes inhibitors of forehead germination, facilitating the water and gases absorption and increasing sensitivity to light and temperature Bewley *et al.* (2013).

The mechanical scarification ("sandpaper") presented satisfactory results, statistically equal to the "10-minute acid treatment". This is a very used type of treatment and safe for overcoming dormancy, causing fissures in the integument and increasing its permeability, leading to water imbibition and gas exchanges Rodrigues *et al.* (2009).

Oliveira *et al.* (2012), working with seeds of *Parkia gigantocarpa* Ducke, described that the mechanical scarification method was adequate, with 81% of germination, results similar to those found in this study. However, mechanical scarification, although efficient, is a time-consuming process, which makes it difficult to produce large quantities of seedlings. Costa *et al.* (2013), working with a species of the same family (*Canavalia rosea* (SW.) DC.), reported that the mechanical scarification presented satisfactory results, reducing the average germination time, with results similar to those observed with *C. langsdorffii*. They further describe that this method is safe, economical and viable. The "sandpaper" treatment also allowed to obtain a high germination vigor, statistically equal to the "fissures" treatment. Taking into account the germination values, it can be considered one of the most suitable methods for laboratory use.

Studies for the production of *C. langsdorffii* seedlings uses the removal of aryl as dormancy-breaking treatment Salomão *et al.* (2003). However, as evidenced in this research with intact seeds, only aryl removal was not enough to stimulate the germination process in a short period of time, and mechanical or chemical scarification was still necessary.

The ants, carrying out the aryl removal, facilitated the scarification of *C. langsdorffii* seeds. The results indicate the presence of coumarins on the seed forehead and demonstrate that not only removal from the aril is necessary, but also scarification

of the forehead. In this way, when the ants scarify the seeds, in addition to allowing gas exchange and water absorption, they reduce the amount of germination inhibitors present in the forehead, which can induce germination.

Silva and Souza (2014) observed that the seeds of *C. langsdorffii* foraged by *Atta sexdens* ants had a higher germination percentage (19%) than non-foraged seeds (1.7%). However, these values are lower than those found by this work, with the difference of values being related to the use of plots in the field. Whereas, Souza *et al.* (2015), with the same species, simulating aryl removal by ants, obtained about 50% of germination. The lower germination rate, compared to this work, may be related to the efficiency of scarification carried out by ants, in relation to the simulation carried out (manual removal of the aril by the researchers).

Leal and Oliveira (1998) also confirmed that aryl removal by several genera of ants (*Cyphomyrmex*, *Mycetarotes*, *Mycocepurus*, *Myrmicocrypta*, *Sericomyrmex* and *Eachymyrmex*) increase and accelerate the seed germination process of Cerrado species. The authors cite *Ocotea pulchella* Nees et Mart. ex Nees (Lauraceae), *Prunus sellowii* Koehne (Rosaceae), *Ouratea spectabilis* (Mart. ex Engl.) Engl. (Ochnaceae), *Rapanea umbellata* Mart. (Myrsinaceae), *Psychotria stachyoides* Benth. (Rubiaceae), *Copaifera langsdorffii* (Fabaceae) and *Virola sebifera* Aubl. (Myristicaceae) as favored species.

Although the consumption of aril by ants can increase germination percentages by breaking integument dormancy and reducing the pathogens attack, such as fungi, the process can also cause damage. Cotyledons predation may occur, damaging the embryo and impairing germination, as observed, where the highest number of dead seeds was found in the treatment "damaged". Souza and Fagundes (2017) describe that predation can cause a significant reduction in the number of viable seeds of *Copaifera langsdorffii*.

The field observation indicated that levels of aryl consumption and predation are small. Only 20% of the seeds presented aryls consumed in different percentages, results similar to those found by Souza and Fagundes (2017), with seeds of the same

species, between 9 and 16%. These data indicate that other groups of the fauna should be active in the process of dispersion and breakage of dormancy, as reported by Carvalho (2003). The seeds presented arils consumed in different percentages, with most seeds having between 26 and 50% of removal. When the aryl consumption was between 76 and 100%, this meant that part of the cotyledons were also removed. In this case, the seeds had a lower percentage of germination (24%) (Table 1). According to Arruda *et al.* (2015), insect damage in species of Fabaceae are common in the natural environment, reducing the chances of the seeds germination.

Taking into account the percentage of seeds whose aryl was harvested with damage, it can be assumed that the use of aryl for fungi cultivation does not negatively impact *C. langsdorffii*. In addition, predation may also be an indication that, in addition to aryl, cotyledons may serve as food resources for ants. However, it should be noted that the percentages of predated seeds may be different depending on the environment, such as the number of trees present and/or ant colonies.

In addition, the seed dispersal process carried out by the ants may be a relevant factor. It was observed in the study area that the seeds, with aril removed, were found under the matrix trees and at irregular distances of the same, from 1 to 25 meters. However, few seeds were deposited around the anthills, indicating that the aryl removal could occur outside the colony, for the most part. According to Silva and Souza (2014), the action of the ants, dispersing the seeds away from the parent plant, decreases the inter and intraspecific competition and may increase the possibility of their deposition in more adequate micro sites, an important factor in a low fertility soils environment, as in the case of Cerrado biome. Leal & Oliveira (1998) confirm that the seeds dispersion by ants can be beneficial for the embryos survival and the seedlings establishment.

However, studies have also indicated that the action of this genus of ants can affect the regeneration of forest areas, providing conditions for certain plant species to be predominant in certain areas. Corrêa *et al.* (2010), Meyer *et al.* (2011) and Meyer

et al. (2013) have described that leaf-cutting ants can create clearings in nests areas, affecting the light regime, as well as impoverishing the soil, providing a favorable environment for the installation of pioneer plants.

Bearing in mind that large areas of Cerrado are being fragmented by anthropic action, the mode of action of *Atta sexdens* would facilitate the germination and establishment of *C. langsdorffii*, besides inducing the predominance of certain species. In addition, in the most fragmented areas, the number of individuals and colonies of the ant may be high due to their lower population control Wirth *et al.* (2007), (2008); Meyer *et al.* (2009), affecting vegetation dynamics and favoring some species, such as *C. langsdorffii*.

4 FINAL CONSIDERATIONS

In the natural environment, scarification is done naturally by the ants *Atta sexdens* with the aryl removal and promotion of cracks in the seed forehead, reducing the presence of coumarins, effective to promote the highest percentages of germination in less time of *C. langsdorffii*. However, the number of seeds that undergo this process is small, in terms of percentage, indicating the need of other groups of the fauna to break dormancy.

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