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Germination potential of *Libidibia ferrea* (Mart. ex Tul.) L.P. Queiroz var. *ferrea*) seeds in contact with petroleum hydrocarbons

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Abstract

Background: *Libidibia ferrea* belongs to the Fabaceae family, an early secondary species known as jucá, its propagation occurs mainly through the seeds. Several vital functions of the plants are affected by the pollutants and due to these, visible damages can occur, depending on the influence of both the environment and the characteristics of the species itself, oil is one of the pollutants that has been causing impacts to nature, mainly in areas destined to the planting of tree seeds, affecting the germination and emergence of these species. **Objetive:** The objective of this study was to evaluate the germination of *L. ferrea* seeds over time when subjected to contact with petroleum, based on the following treatments: witness (intact seed), intact and scarified seeds, submerged in oil for 1 h and 24 hours.It is important to emphasize that there are no previous experimental studies on the germination of Caatinga tree species submitted to contamination by petroleum hydrocarbons, and the species presenting good results in this investigation could be used for phytoremediation studies and possibly be a alternative in the use in productive soils contaminated by petroleum, which are generally characterized by having a low productivity. **Results:** The submerged oil seeds, followed or not by scarification, showed differences in the germination curves and the germinative behavior over time. The maximum germination (G_{max})estimated for the witness was 69%, followed by the intact seeds in contact with oil for 1 h of 54% and 24 h of 66%, whereas for seeds physically scarified, the G_{max} estimated was lower for exposure times of 1 h (47%) and 24 h (47%). **Conclusions:** There were differences in germination of the seeds over time between the evaluated treatments, with each treatment having a faster initial growth phase, followed by stabilization. Scarified seeds at both oil contact times showed slower germination over time, but at the end of the process the values were higher when compared to mechanically scarified seeds.

Key words: Forest seeds; toxicity; mathematical model; Fabaceae

INTRODUCTION

Libidibia ferrea belongs to the Fabaceae family, an early secondary species known as jucá, is a Brazilian native species of multiple uses due to its attributes of wood, landscape, medicines and fodder, also prescribed for the recovery of degraded areas (Santana *et al*. 2011). Its propagation occurs mainly through the seeds, these presenting dormancy, caused by the impermeability of the integument to water.

Seed germination is an important stage of plant growth, where soil sensitivities to soil contaminants occur (Banksand Schultz 2005; Masakorala *et al*. 2013). In this case, the seeds are sown in contaminated soil, and the number of seedlings after a short time is counted, and compared the number of seedlings grown in the contaminated soil and in the witness (Banks and Schultz 2005; Inckot *et al*. 2011). Another important phase is the identification of sensitive species for use as bioindicators of toxicity of a soil contaminant (Vazquez-Luna *et al*. 2010; Pérez-Hernández *et al*. 2013).

Oil is one of the major energy sources in the world, and its use has greatly contributed to economic and social development (Mohsenzade *et al*. 2009). With the increase in the demand for exploration of oil and its derivatives, spills tend to be more frequent, affecting the fauna and flora that surrounds the exploration area. Petroleum hydrocarbons (TPH's) are highly toxic to plants, micro-organisms and invertebrates and cause various health problems and ecological impacts on contaminated ecosystems (Al-Mutairi *et al*. 2008).

The toxicity of petroleum in plants may occur by direct contact or absorption of some compound, as recorded by Alkio *et al*. (2005), where the authors verified that the presence of phenanthrene (compound present in the oil) in the interior of *Arabidopsis thaliana* acting in the reduction of the roots growth, decrease in the size and number of leaves, deformation of trichomes, and necrotic areas with cell death.

The expression of toxicity and tolerance to TPH's is variable in the plant kingdom, even among members of the same botanical genus (Adamand Duncan 2002b). Where some plant species have the capacity to germinate and develop in soil contaminated by TPH, it has been used in bioassays to identify the degree of tolerance and the ability to degrade hydrocarbons, considering their use in phytoremediation techniques.

It is important to emphasize that there are no previous experimental studies on the germination of Caatinga tree species submitted to contamination by petroleum hydrocarbons, and the species presenting good results in this investigation could be used for phytoremediation studies and possibly be a alternative in the use in productive soils contaminated by petroleum, which are generally characterized by having a low productivity. The objective of this study was to evaluate the germination and germination potential of *L. ferrea* seeds when submitted to contact with petroleum hydrocarbons.

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MATERIAL AND METHODS

Collection, processing and storage of seeds:

The work was conducted with jucá (*L. ferrea*) seeds collected from 20 matrix trees located in the municipality of Mossoró - RN. At the time of collection, the fruits were packed in nylon bags and kept for seven days in a greenhouse to induce natural drying, after which the processing was carried out, eliminating the small, cracled and malformed seeds. In the sequence they were stored under refrigeration at a temperature of 7 ± 2°C until the experiment was set up.

Treatments used:

The treatments used were: witness (intact seeds) (T_1), mechanically scarred seeds submerged in oil for 1 h (T_2), mechanically scarred seeds submerged in oil for 24 h (T_3), intact seeds submerged in oil for 1 h (T_4) and intact seeds submerged in oil for 24 h (T_5). Prior to the installation of the experiment, part of the seed sample was mechanically scarified with sandpaper N° 80, rubbing the side opposite the thread until the endocarp appeared, then submerged in oil, inside a glass container for the periods pre-determined (1 and 24 h). After this period, they were removed with the aid of forceps and placed on absorbent paper to remove excess oil. Afterwards, the seeds were submitted to the germination test.

Germination test:

The germination test was performed with four replicates of 20 seeds, for each treatment, and seeded in plastic trays (0.40 x 0.40 x 0.11 m) containing washed and sterilized sand. Emerging seedlings were counted daily, followed preferably at the same time. The test was conducted in a laboratory environment, without temperature and humidity control, until the number of seedlings was already stabilized, considering normal ones with characteristics consistent with those prescribed by the Seed Analysis Rules (Brasil 2009). Irrigations were done daily to maintain substrate moisture.

Mathematical models for the evaluation of germination as a function of time:

To evaluate seed germination as a function of time, the model proposed by Maia *et al*. (2009), according to equation 1, where, G and $G_{max}$is the percentage of germination at time t and the estimated maximum, respectively, $\alpha$and *n* are parameters of the model adjusted by non-linear regression methodology, withα in day^{-1} and*n*is the form factor and dimensionless.

$$G = G_{\max} - \frac{G_{\max}}{1+(\alpha \cdot T)^n} \quad (1)$$

The absolute (TGA) and relative (TGR) germination rates were estimated by equations 2 and 3, respectively. The time for the maximum TGA ($T.TGA_{max}$), the germination time of 50% of the maximum ($T.G_{50\%}$) and the maximum absolute germination rate (TGA_{max}), were calculated by equations 4, 5 and 6, respectively.

$$TGA = \frac{G_{max} \cdot n \cdot \alpha^n \cdot T^{n-1}}{\left[1+(\alpha \cdot T)^n\right]^2} \quad (2)$$

$$TGR = \frac{n}{T \cdot \left[1+(\alpha \cdot T)^n\right]} \quad (3)$$

$$T.TGA_{max} = \frac{1}{\alpha}\left[\frac{n-1}{n+1}\right]^{1/n} \quad (4)$$

$$T.G_{50\%} = \frac{1}{\alpha} \quad (5)$$

$$TGA_{max} = \frac{G_{max}.\alpha^n.(n+1)^2}{4.n}.(T.TAG_{max})^{n-1} \quad (6)$$

Experimental design and data analysis:

For the statistical analysis, the treatments were distributed in the completely randomized design, the germination data being submitted to the analysis of variance and the means of treatments compared by the Tukey's test at 5% probability.

RESULTS AND DISCUSSION

The results presented in Table 1 indicate that the highest estimated maximum germination value (G_{max})occurred in the witness, with 69%, followed by the intact seeds in contact with oil for 1 and 24 hours, with 54% and 66%, whereas, for mechanically scarified seeds, the $G_{max}$was lower for 1 h (47%) and 24 h (46%), showing that the oil acts to reduce the germination of jucá seeds when submitted to mechanical scarification, regardless of the contact time.

Table 1: Adjusted model parameters (G_{max}, αand n), determination coefficient (R^2), time to germinate 50% of G_{max} ($TG_{50\%}$), time for maximum germination rate ($T.TAG_{max}$) and estimated maximum germination rate (TAG_{max}) of the jucá seeds, in the evaluated treatments (T_1 - witness - intact seeds); (T_2 - scarified seeds submerged in oil for 1 h); (T_3 - scarified seeds submerged in oil for 24 h); (T_4 - intact seeds submerged in oil for 1 h); (T_5 - intact seeds submerged in oil for 24 h)

	T_1	T_2	T_3	T_4	T_5
G_{max}	69	47	46	54	66
α	0,0709	0,0892	0,0544	0,0395	0,0422
n	4,0467	8,8863	3,8295	6,9569	6,2017
R^2	0,9630	0,9835	0,9810	0,9901	0,9921
$T.G_{50\%}$	14,11	11,22	18,38	25,31	23,69
$T.TAG_{max}$	12,46	10,93	15,98	24,28	22,47
TAG_{max}	5,29	9,52	2,58	3,81	4,43

Even with the G_{max} in the highest non-scarified seeds, the time required to germinate 50% of the seeds ($TG_{50\%}$) was higher (25.31 and 23.69 days) when compared to the control (14.11 days), inferring that, although the time required to germinate 50% of the seeds was higher in these treatments, a longer time was required, which may be related to the physical impediment that the oil makes on the integument, making it difficult to absorb water and delay the germination process. In this sense, Adam and Duncan (1999a, 2002b) investigated the effects of diesel oil on the growth of several plants, and observed delay in germination speed and percentage of seed germination and a reduction in plant growth, the authors attributed these results to the physical impediment of the oil, which, in turn, makes it difficult to transfer water and oxygen to the seeds.

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Kramer and Koslowski (1960) argue that either the lack of water in the soil or its excess can reduce or inhibit germination due to reduced oxygen supply. Sealing of the seed tegument due to the presence of a contaminant, such as petroleum, can also be a factor that interferes with the absorption of water. However, if the roots grow in an oil polluted environment, the soil particles are covered with a hydrophobic layer that reduces the availability of water. This causes anoxic stress and water stress, and then the hydrophobic nature of the pollutant also causes chemical stress (Peña-Castro *et al*. 2006).

Besides the delay in the germination of the non-scarified seeds, the oil also caused the increase in the time to the maximum germination rate ($T.TGA_{max}$), proving that it interfered in this characteristic, in contrast, the seed coat acted as a protective barrier to the hydrocarbons. The estimated maximum germination rate (TGA_{max}) for the evaluated treatments was higher in the scarified seeds in contact with oil for 1 h (9.52% per day^{-1}).

The scarification of the seeds increased its area of contact with oil, facilitating the absorption of the TPHs and retarding the germination, a fact that can be explained by Masakorala *et al*. (2013), where they mention that, during germination under normal conditions, carbohydrate hydrolysis of the seeds is initiated with imbibition, which reduces the osmotic potential of the cells causing a rapid increase in water uptake and volumetric vacuolar growth characterizing germination. In the presence of TPH, these compounds may enter into the soaked seeds. Then, as a consequence of the toxic influence, inhibition in the hydrolysis and mobilization of hydrolyzed products may occur, resulting in inhibition of germination or changes.

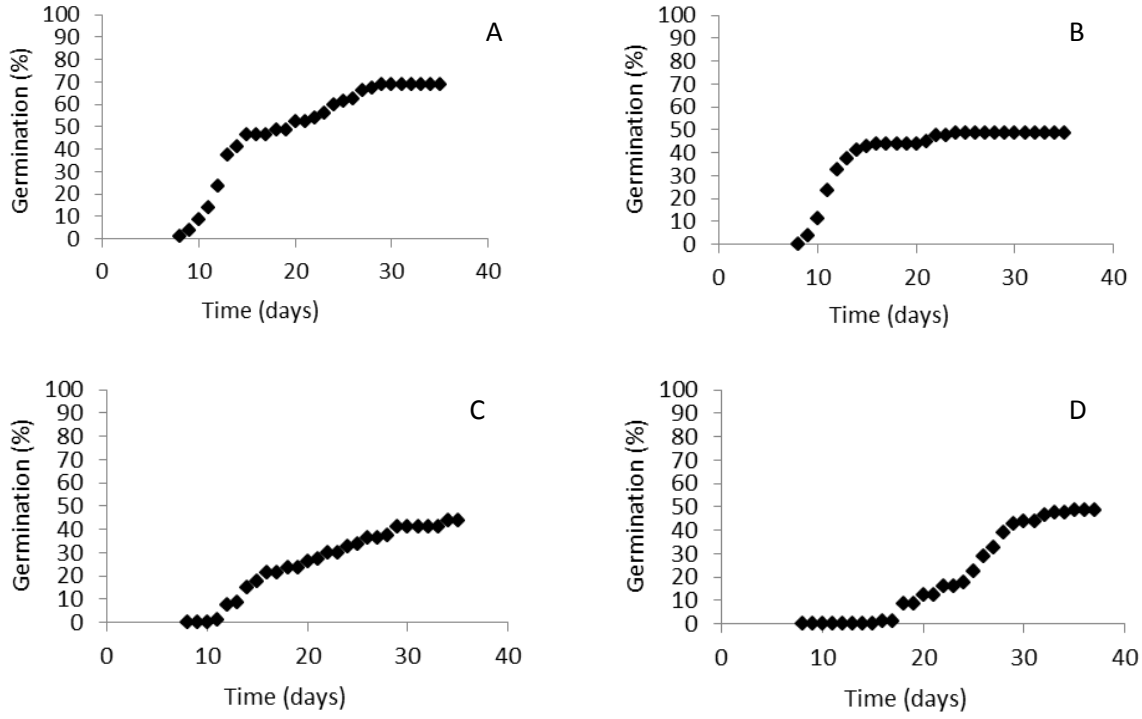
Taking into account the total germination, the control and the scarified seeds in contact with oil for 1 h, differed statistically; for the others, no significant differences were observed (Table 2). It is evidenced that the contact with the oil was able to reduce the germinative capacity of the seeds, especially when submitted to treatment to break dormancy, mechanical scarification, due to the greater contact and absorption, besides the hydrophobic character of the oil, where it can have there was difficulty in absorbing water, thus justifying the reduction of seed germination when compared to the control. According to Morley *et al*. (2005), polar organic compounds present in hydrocarbons are likely responsible for the water repellency of contaminated soils, thus limiting the plant's absorption of water and nutrients, a fact that may have occurred in seeds, since oil completely covered its tegument, making it difficult to absorb water.

Table 2: Germination of *L. ferrea* seeds in the evaluated treatments (T_1 - witness - intact seeds); (T_2 - scarified seeds submerged in oil for 1h); (T_3 - scarified seeds submerged in oil for 24h); (T_4 - intact seeds submerged in oil for 1h); (T_5 - intact seeds submerged in oil for 24h

Treatments	Germination (%)
T_1 – Witness/intact seeds	69 a
T_2 – Scarified seeds/1h	49 ab
T_3 – Scarified seeds/24h	44 b
T_4 – Intact seeds/1h	49 ab
T_5 – Intact seeds/24h	61 ab

* Averages followed by the same letter do not differ statistically ($\alpha = 0.05$)

It was possible to observe, from the germination curves (Fig. 1), differences in the germination behavior of seeds over time, among the evaluated treatments, having for each treatment a faster initial growth phase, followed by stabilization. In the intact seeds, it is noticeable that the initial germination phase occurred more slowly, but at the end of the process, the values were higher when compared to the scarified seeds.



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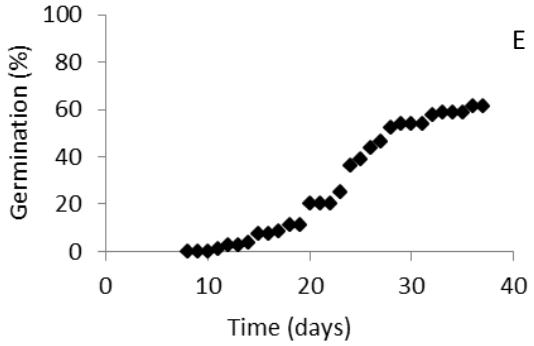
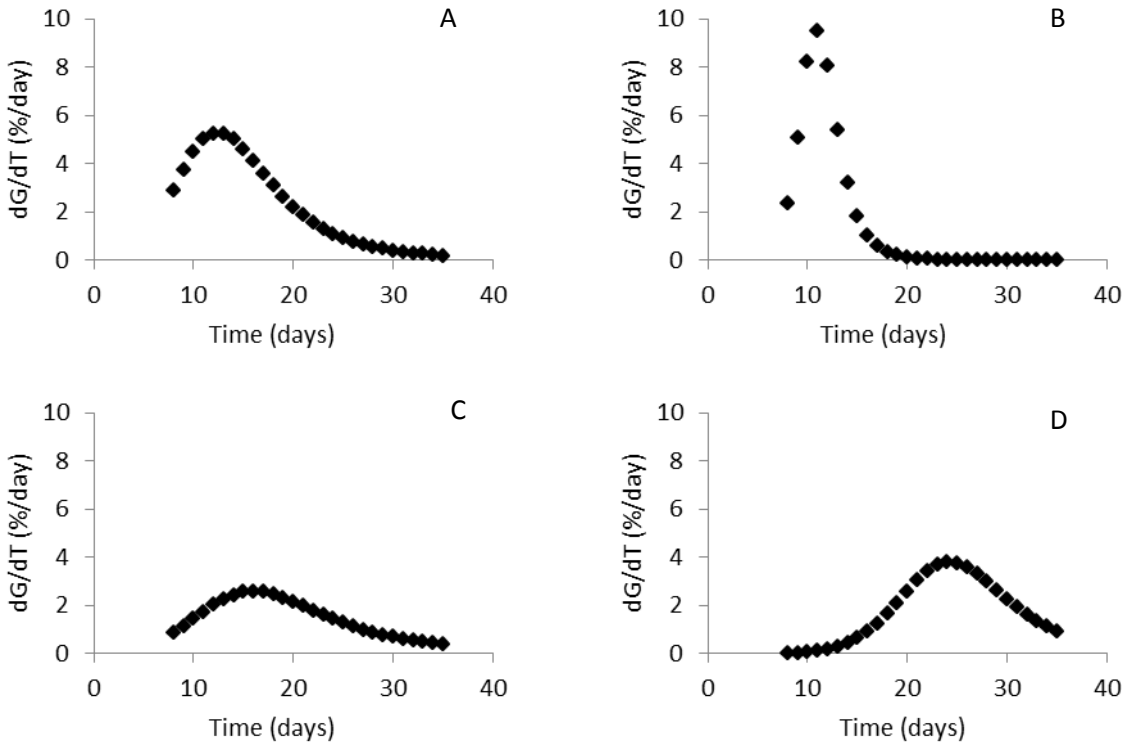


Fig. 1: Observed and estimated values of *L. ferrea* germination as a function of time for the evaluated treatments. A – T_1 (Witness - intact seeds); B –T_2 (scarified seeds submerged in oil for 1 h); C – T_3 (scarified seeds submerged in oil for 24 h); D –T_4 (intact seeds submerged in oil for 1 h); E –T_5 (intact seeds submerged in oil for 24 h)

Seed germination is a sensitive growth phase in plants and is useful for assessing the chronic effects of exposure to contaminants (Banksand Schultz 2005). Observing Fig. 1, it can be inferred that, although the time required for the stabilization of germination has been approximated, it is important to analyze the entire germination process, according to Gogosz *et al*. (2010) when they mention that, for a better understanding of the seed germination process, it is important to take into account not only the final percentage, but also the germination curves, which demonstrate the germination behavior over time. In the same sense, Santana and Ranal (2004) state that it is necessary to examine the germination curves in detail, since two or more treatments may have exactly the same final percentage of germination, but also have different germination curves, and such differences can have important implications for the interpretation of results.

Analyzing the absolute germination rate of jucá seeds, it was verified that the time to obtain the highest germination rate occurred for the intact seeds in direct contact with the oil for 1 h, with a rate of 9.51% per day^{-1} (Fig. 2B), confirming previous data, where the seeds scarified in direct contact with the oil affect the germinative potential, as well as the time required for such.



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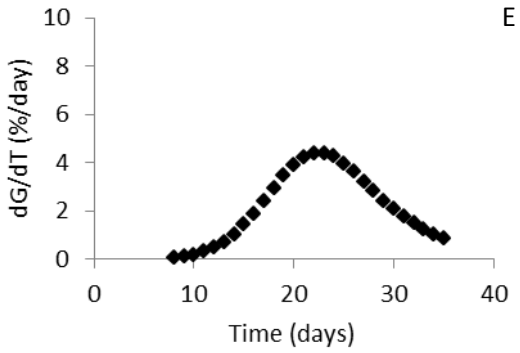

Fig. 2: Absolute growth rate of *L. ferrea* germination as a function of time for the evaluated treatments. A – T_1 (Witness/intact seeds); B – T_2 (Scarified 1h); C – T_3 (Scarified 24h); D –T_4 (Intact 1h); E – T_5 (Intact 24h)

Conclusions:

There were differences in germination of the seeds over time between the evaluated treatments, with each treatment having a faster initial growth phase, followed by stabilization.

Scarified seeds at both oil contact times showed slower germination over time, but at the end of the process the values were higher when compared to mechanically scarified seeds.

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