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POTENTIAL ALLELOPATHIC EFFECT OF *Pinus elliottii* FRESH NEEDLES IN *Lactuca sativa* SEEDS GERMINATION

Efeito potencial alelopático em acículas frescas de *Pinus elliottii* na germinação de sementes de *Lactuca sativa*

Paula Helena Pereira O'Connor[1], Américo Wagner Júnior[2], Daniela Cleide Azevedo de Abreu[3], Bruna Elisa Trentin[4], Bruna Bighi Thompson[5], Fernando Campanhã Bechara[6]

[1] Discente da University of Hawai'i Maui College, Maui, USA. E-mail: paulahp@hawaii.edu
[2]Docente da Universidade Tecnológica Federal do Paraná, Dois Vizinhos, Brasil. Pós Doutor em Fitotecnia pela Universidade Federal de Viçosa, Viçosa, Brasil. Orcid: https://orcid.org/0000-0001-5081-5281 , e-mail: americowagner@utfpr.edu.br
[3]Docente da Universidade Tecnológica Federal do Paraná, Dois Vizinhos, Brasil. Doutora em Agronomia pela Universidade Estadual Paulista Júlio de Mesquita Filho, Jaboticabal, Brasil. Orcid: https://orcid.org/0000-0002-6127-3198 , e-mail: daniela.abreu.utfpr@gmail.com
[4]Doutoranda em Ciências Florestais na Universidade Estadual do Centro Oeste, Guarapuava, Brasil. Orcid: https://orcid.org/0000-0003-1823-6030 , e-mail: brunaelisatrentin@gmail.com
[5]Discente em Engenharia Florestal pela Universidade Tecnológica Federal do Paraná, Dois Vizinhos, Brasil. Orcid: https://orcid.org/0000-0001-8053-0674 , e-mail: brunabthompson@gmail.com
[6]Docente da Universidade Tecnológica Federal do Paraná, Dois Vizinhos, Brasil. Pós Doutor em Recuperação de Áreas Degradadas pela University of Hawai`i at Manoa, EUA. Orcid: https://orcid.org/0000-0001-7573-2124 , e-mail: bechara@utfpr.edu.br

ABSTRACT

There is a vast knowledge about the management of American pines plantations in Brazil, but not much about the factors that contribute to its invasion dynamics in natural ecosystems. Allelopathy studies is a gap in the Ecology of pines invasion and could help to better understand its invasive capacity. We aimed to evaluate the allelopathic potential of fresh green needles of *Pinus elliottii* on germination of *Lactuca sativa*. We tested extraction with distilled water in concentrations of 5%, 10%, 15%, 20% and control (0%). Extraction was carried out also in ethanol but dissolved in Tween 20 solution. The allelopathic effect of green needles of *P. elliottii* was detected since we observed the increasing concentration (in the two types of extraction) resulting in lower germination of both root and hypocotyl length. Although this could be a competitive advantage of *P. elliottii* in relation to tropical native plants, the implications in the development and establishment of native seedlings in a natural environment should be better studied.
Keywords: Allelopathy, biological invasion, pines.

RESUMO

Há um vasto conhecimento sobre a gestão de pinheiros americanos plantados no Brasil, mas não muito sobre os fatores que contribuem para a sua dinâmica de invasão nos ecossistemas naturais. Os estudos de alelopatia são uma lacuna na Ecologia da invasão dos pinheiros americanos que poderia ajudar a compreender melhor a sua capacidade invasora. O nosso objetivo foi avaliar o potencial alelopático das acículas frescas e verdes de *Pinus elliottii* na germinação de *Lactuca sativa*. Testamos a extração com água destilada em concentrações de 5%, 10%, 15%, 20% e controle (água destilada pura). A extração foi realizada também em etanol nas mesmas concentrações, mas dissolvido em solução Tween 20 a 4% (0% para o controle). O efeito alopático das acículas verdes de *P. elliottii* foi detectado uma vez que observamos o aumento da concentração (nos dois tipos de extração) resultando em menor germinação e comprimento de raiz e hipocótilo. Embora isso possa ser uma vantagem competitiva de *P. elliottii* em relação às plantas nativas tropicais, as implicações no desenvolvimento e estabelecimento de plântulas nativas em ambiente natural deve ser mais bem estudada.
Palavras Chaves: Alelopatia, invasão biológica, pinheiros.

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Pines are native to the Northern Hemisphere and they have been mostly invading tropical environments in marginal areas with harsh and restricted conditions, such as: mountain tops, acidic soils, low fertility, arid and cold habitats (RICHARDSON and REJMÁNEK, 2004). Some factors that contribute to its high invasion capacity are mycorrhizal associations, seed dispersal by wind, high germination and reproduction rates, and production of large quantities of seeds (RICHARDSON and HIGGINS, 1998; RICHARDSON, 2006; BECHARA et al., 2013).

Many pines promote biological invasion in the Northern Hemisphere and Southern Hemisphere, where 19 species are invasive (RICHARDSON and REJMÁNEK, 2004; BECHARA et al., 2013)

The genus *Pinus* was introduced in Brazil in the 1950s and has since spread throughout the country, especially in the southern region, where we can find extensive *Pinus elliottii* L. and *Pinus taeda* L. stands for pulp (paper). They were both indicated by Rejmanek and Richardson (1996) as the most invasive pines. Pines invasion resulted in biodiversity losses in Brazil (ABREU and DURIGAN, 2011; FALLEIROS et al., 2011; BECHARA et al., 2013, 2014; CAZETTA and ZENNI, 2020)

Pines can produce secondary metabolites causing allelopathy affecting the seed germination and seedling growth. Tolerance to allelochemicals effects may be variable, and some species are more susceptible than others, developing an important role in tropical plants natural regeneration (RICE, 1984; FERREIRA and AQUILA, 2000; GATTI et al., 2004; WANDSCHEER and PAASTORINI, 2008; WANDSCHEER et al., 2011; PRICHOA et al., 2013). We aimed to evaluate the allelopathic potential of fresh (green) needles extract of *P. elliottii* in the germination and early growth of *Lactuca sativa* (lettuce).

We used extracts of *P. elliottii* fresh (green) needles to evaluate the allelopathic effects on *Lactuca sativa* seeds under controlled conditions of temperature, humidity, and light. The collection of material was held in a 15-y old plantation, located in the municipality of

Francisco Beltrão, southwestern region of Paraná state, Brazil. Fresh needles were collected in the treetops of 10 individuals on the stand edge.

To produce the raw extract, 125g of fresh needles were triturated in 500 ml of distilled water using a blender. After filtering it, the extract was centrifuged at 3,000 rpm for 5 min, selecting the supernatant. The supernatant was considered as the raw extract at a 100% concentration. From the raw extract, the dilutions were made in distilled water to get solutions with concentrations of 5%, 10%, 15% and 20%. The effect of these four concentrations was compared with pure distilled water, considered as a control (0% concentration). We used Gerbox® plates with six replications of 25 seeds placed on the Germitest® germination paper moistened with the different solutions with volume equal to twice the weight of the paper.

For extraction in ethanol, the fresh needles were dried in a forced-air oven at 40°C. Afterwards, the material was submitted to three successive extractions with ethanol in room temperature (one extraction each 2 days). We used 44g of dry mass in 0.75 L of ethanol p.a. for the extraction. After each extraction the material was filtered and put in a vacuum rotary evaporator at a temperature of 55°C. From the gross extract, we diluted 2g in 50 ml of 4% Tween 20, of which tests were taken in the concentrations of 5%, 10%, 15% and 20%. The effect of these four concentrations was compared to the Tween 20 solution at 0.08%, considered as a control (0% concentration). Gerbox® plates were used in six replications of 25 seeds placed on the Germitest® germination paper. Assays were placed in BOD chambers regulated at a temperature of 20°C and a photoperiod of 8 h-darkness and 16 h-light. The seeds were pre-cooled to 10°C for three days to overcome dormancy. Evaluations started 96 h after sowing, and daily measurements were taken at the same time. We determined the percentage of germination (G%), germination speed index (GSI), average germination time (t), seedlings dry mass and length. Calculations were performed according to Labouriau and Valadares (1976): 1) Germination percentage: (G%)=(N/A) X 100, where N = number of germinated seeds, and A = total number of germinated seeds; 2) Germination Speed Index (GSI): GSI = $\Sigma(ni/i)$, where ni = number of germinated seeds per day, and i = number of days after sowing; 3) Average germination time (t) = (Σ niti)/Σni, where: ni = number of germinated seeds per day, and ti = incubation time.

Our data showed significant effects of extract concentrations compared to to control. But the same was not true for the dry mass, which may be explained by the lack of precision in our weighing scale. The average germination time was not significant.

Table 1**.** Effects of different fresh needle extracts of *Pinus elliottii* on germination percentage (G), Germination Speed Index (GSI), root length and aerial part of lettuce seedlings (*Lactuca sativa*).

Treatments	G (%)	GSI (seedlings . day^{-1})	Root length (cm)	Seedlings length (cm)
Distilled Water Extraction				
Control	94 a	3.3 a	0.48 a	0.9 a
5%	92 a	3.1 a	0.38 a	0.79 a
10%	90 a	3.07 a	0.38 a	0.78 a
15%	83 b	2.93 a	0.37 ab	0.74 a
20%	58 c	1.45 b	0.24 b	0.59 b
CV(%)	17.28	15.83	20.69	13.97
Ethanol Extraction				
Control	98 a	3.54 a	0.49 a	0.72 a
5%	80 b	2.28 b	0.36 ab	0.63 ab
10%	58 bc	1.49 c	0.27 bc	0.55 bc
15%	43 c	1.04 c	0.27 bc	0.49 cd
20%	12 d	0.29 d	0.15 c	0.42 d
CV(%)	23.13	20.26	25.09	18.07

OBS: Means followed by the same letter do not differ in each variable, by Tukey test at 1% probability of error.

The pH obtained was 6.17 in water extraction and 4.68 for the ethanol extraction. The osmotic potential was -0.19 MPa and - 0.081 MPa, respectively. The pH and osmotic potential are important, since the allelopathy may be in osmotically active substances such as amino acids, sugars and organic acids that can influence the ion concentration (FERREIRA and AQUILA, 2000; FERREIRA and BORGHETTI, 2004). *Lactuca sativa* accepts a wide pH range 3-7 for germination (BASKIN and BASKIN, 1998).

For Gatti et al. (2004), solutions with osmotic potential not exceeding -0,2 MPa will not significantly interfere on the seeds and/or seedlings and mask the allelopathic effect. Hence, our results were probably related to effects due to the presence of inhibitory substances present in the *P. elliottii* extract.

The germination percentage adjusted to a quadratic equation for the extraction in distilled water and a linear equation for extraction in ethanol is in Figure 1. The increase of the extract concentration of *P. elliottii* led to a decrease in germination percentage values. Based on our results obtained from the aqueous extracts with extraction in distilled water, we noted that 20% of concentration produced a significant inhibitory effect, inhibiting the germination of *L. sativa* seeds by 40%. Additionally, the extraction in ethanol at concentrations of 10%, 15% and 20% showed significant difference, where concentrations of 15% to 20% caused a greater negative impact on germination, inhibiting by 56% and 87% germination seeds, respectively, when compared to the control.

Our results contradict Ferreira et al. (2007), who reported that the ethanol extract of dried needles of *P. elliottii* in different concentrations showed no allelopathic effect for the variables germination and early growth to *L. sativa* and *Bidens pilosa*. On the other hand, Sartor et al. (2009) tested the inhibitory effect of aqueous extract of *Pinus taeda* fresh (green) needles on oat (*Avena strigosa*) seeds and detected that the germination was delayed in the presence of extract. These authors also concluded that needles in decomposition did not present allelopathy.

Our fresh extract of green needles probably caused greater allelopathic effect on *L. sativa* seed germination since these fresh needles are likely to contain higher concentrations of inhibitory substances compared to needles in decomposition (DA SILVA RODRIGUES-CORREA et al., 2017).

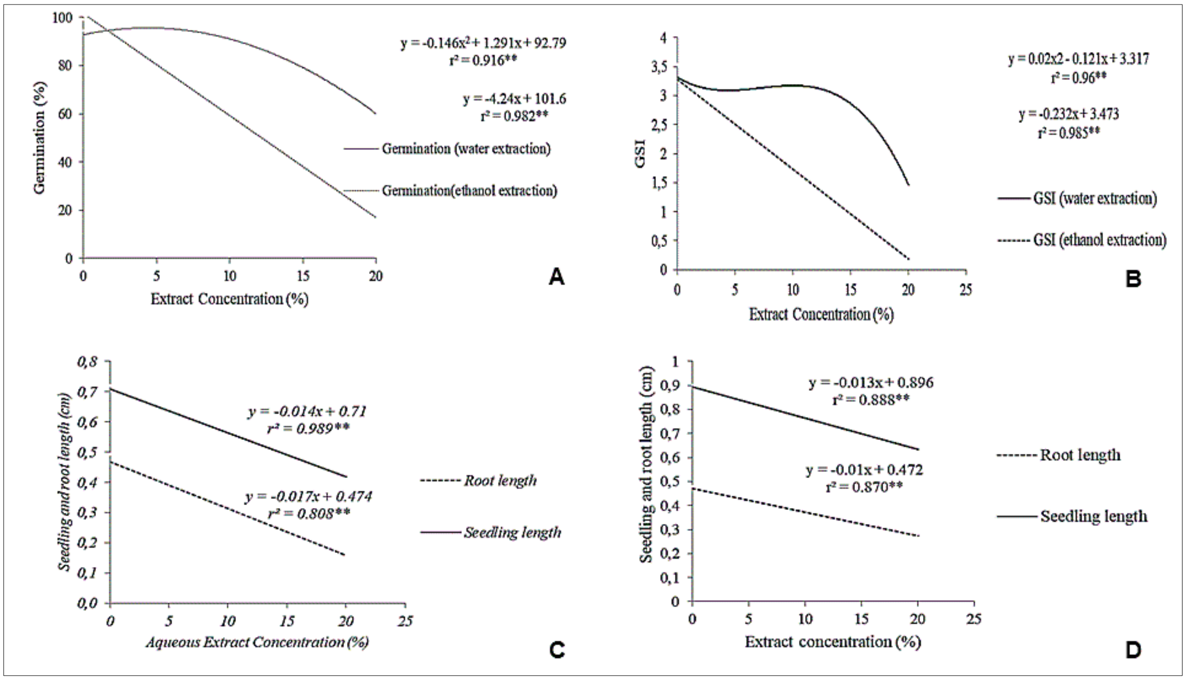

Figure 1 – A) Equation adjusted on germination of lettuce (Lactuca sativa) in different fresh needles extracts of Pinus elliottii. B) Equation adjusted on Germination Speed Index (GSI) of lettuce in different concentrations of fresh needles extract of P. elliottii. C) Adjusted equation on root and seedling length of lettuce in different concentrations of fresh needles extract of P. elliottii. D) Adjusted equation on root and seedling length of lettuce in different concentrations of fresh needles extract of P. elliottii. (** Significant values, 1% probability by the F test).

The Germination Speed Index (GSI) adjusted to a quadratic equation for extraction in distilled water and a linear equation for extracting in ethanol is in Figure 1. We noted a significant difference in the treatments compared to the control from the concentration of 20% with extraction in distilled water, and only 5% in ethanol.

The allelopathic effect is not limited to germination inhibiting, but also slowing the speed of germination (FERREIRA and BORGUETTI, 2004). Our results of GSI (Figure 1b) showed faster germination in the control than in concentrations of 5%, 10%, 15% and 20% extracts, especially in ethanol extraction. We noted a reduction in the lettuce germination rate index when increasing the extract concentration. This suggest that the vigor of lettuce seeds was affected by the allelopathic extracts.

According to the concentration of the allelopathic substances, time of germination may be delayed, and even if germination occurs, roots can absorb the allelopathic substances inhibiting or hindering the cells proliferation (PERIOTTO et al., 2004). Sartor et al. (2009) evaluating the effect of fresh needles of *Pinus taeda* on *L. sativa* seeds fond that the germination speed decreased according to the increase of the needles extract concentration.

Root and seedling growth was adjusted to a linear equation for both extractions, which were also negatively affected by the increase of the *P. elliottii* extract concentration (Figure 1). This was detected for the extraction in distilled water at concentrations from 15% and 5% for the ethanol.

The allelopathic effects were observed on our germination and seedling growth suggesting that the same laboratory effects could happen also in native ecosystems (INDERJIT and DASHINI, 1995).

In our laboratory study, extracts of fresh needles of *P. elliottii* produced inhibitory effects on the germination rate/speed and initial growth of lettuce seedlings. Field tests are recommended to check its effectivity in natural conditions in southern Brazil.

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