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Original Article

Pre-emergent indaziflam can enhance forest seed germination in direct seeding?

Pré-emergente indaziflam pode melhorar a germinação de sementes florestais na semeadura direta?

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Abstract

Pre-emergent herbicides can contribute to the control of weed competition in direct seeding restoration, however it is necessary to evaluate their effects on seeds of native tropical forest species. The aim of the study was to assess the potential impact of the herbicide indaziflam on the germination of 17 forest species. For this, a dosage of 180 mL of the product in 200L of water was compared to the control without herbicide. The degree of sensitivity of each species was calculated by a ratio between the percentage of germination with herbicide (G_H) and the control without herbicide (G_C) classifying them as: extremely sensitive (ES= (G_H/G_C) <0.25), sensitive (S=0.25< (G_H/G_C) <0.50), low sensitivity (LS=0.50< (G_H/G_C) <0.75), indifferent (I=0.75< (G_H/G_C) <1.0) and potentiated (P= (G_H/G_C) >1). The herbicide promoted a significant reduction in mean germination in 35% (n=6) of the species and 59% (n = 10) were sensitive or extremely sensitive to indaziflam, and only three did not germinate. On the other hand, 29.4% (n=5) showed low sensitivity or indifference to the herbicide, while seed germination was slightly increased by indaziflam to 11.7% (n=2). Pre-emergent indaziflam can be recommended in direct seeding restoration, as only 17.6% (n=3) of the species were inhibited by pre-emergent. However, the effect of indaziflam varies by species and requires further studies to support large-scale use in direct seeding.

Keywords: herbicide, restoration, forest species, Atlantic Forest.

Resumo

Herbicidas pré-emergentes podem contribuir para o controle da competição de plantas daninhas em restauração por semeadura direta, porém é necessário entender seus efeitos em sementes de florestais nativas. O objetivo do estudo foi avaliar o potencial impacto do herbicida indaziflam na germinação de 17 espécies nativas de florestas tropicais. Para isso, uma dosagem ligeiramente acima da recomendada (180 mL do produto em 200 L de água) foi comparada com a testemunha sem herbicida. O grau de sensibilidade de cada espécie, foi calculado pela razão entre a porcentagem de germinação com herbicida (G_H) e a testemunha sem herbicida (G_C) e classificando-as como: extremamente sensível (ES= (G_H/G_C) <0,25), sensível (S=0,25< (G_H/G_C) <0,50), baixa sensibilidade (LS=0,50< (G_H/G_C) <0,75), indiferente - I=0,75< (G_H/G_C) <1,0) e potencializado (P= (G_H/G_C) >1). O herbicida promoveu redução significativa na média de germinação em mais de 35% (n=6) das espécies avaliadas e 59% (n=10) foram sensíveis ou extremamente sensíveis ao indaziflam e apenas três espécies não germinaram. Por outro lado, 29,4% (n=5) apresentaram baixa sensibilidade ou indiferença ao herbicida, enquanto a germinação das sementes foi levemente aumentada pelo indaziflam para 11,7% (n=2). O indaziflam pré-emergente pode ser recomendado na restauração de semeadura direta pois apenas 17,6% (n=3). O indaziflam pré-emergente pode ser recomendado na restauração de semeadura direta pois apenas 17,6% (n=3) das espécies foram inibidas pelo pré-emergente. No entanto, o efeito do indaziflam variou em função da espécie e requer mais estudos para apoiar o uso em larga escala na semeadura direta.

Palavras-chave: herbicida, restauração, espécies florestais, Floresta Atlântica.

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1. Introduction

Direct seeding is a viable option in ecological restoration programs, as it allows greater control of the species composition, richness and plant density in restored sites (Stanturf et al., 2014). Besides, it also reduces the costs, nursery operations for the seedlings production, the use of equipment and permits minimum operational plans (Grossnickle and Ivetić, 2017). Many areas for restoration by direct sowing are abandoned or degraded pastures dominated by fast-growing exotic grass species, such as *Urochloa* spp. which inhibit the forest species growing and the natural regeneration (Resende and Leles, 2017).

The initial emergence in direct seeding depends on seed density and quality, and losses are concentrated in the emergence phase, occurring mainly up to 90 to 180 days after sowing (Ferreira et al., 2009). Seed density is around 250,000 to 500,000 seeds per hectare (Campos-Filho et al., 2013), however, seedling establishment in the field is low, ranging from 1,400 to 13,000 plants per hectare (Meli et al., 2018). Increasing the efficiency of direct seeding restoration, therefore, is directly linked to soil preparation and the control of post-emergence factors, such as weed control (Silva and Vieira, 2017). Even management practices such as manual or mechanized weeding can cause physical damage to young seedlings (Khaliq et al., 2013), making it necessary to seek management alternatives in the pre- and post-emergent phase.

Invasive species control is one of the most important practices in ecological restoration since weed species can hinder the development of planted species (Prior et al., 2018) and interfere in the establishment of plant community structure and assembly (Weidlich et al., 2020). The use of herbicides is the most used practice to control weed competition in restoration, but they can also impact regeneration or even the development of planted or sown species.

Herbicides are classified by selectivity (selective or non-selective for a particular crop), time of application (pre- or post-emergent), translocation (contact or systemic) and mechanism of action (Silva et al., 2009). Pre and/or post-emergence herbicides are used in direct seeding of agricultural crops (Mann et al., 2007; Khaliq et al., 2011), whose success depends on the dosage and persistence of the product in the field. The residual effect of a herbicide is related to its composition, which prevents the development of competition with weeds and, at the same time, does not cause damage to the soil seed bank or plants of more sensitive species (Dan et al., 2012).

The herbicide indaziflam (Esplanade®, Bayer CropScience) has a low leaching in any type of soil (Guerra et al., 2016) and is a member of the chemical family called nitriles (HRAC Group L). It acts by inhibition of cellulose biosynthesis (Tompkins, 2010) interfering in the growth of roots in the newly germinated seedlings (Clark, 2019). The herbicide has low solubility in water L^{-1} (2010 mg L^{-1}) and low Kow (2.0 at pH=2.0 at pH=2.0 and pH=9). In Brazil, the use of indaziflam is recent and restricted to agricultural crops and silviculture of exotic tree species, presenting a broad spectrum of action in the control of monocots and eudicots, applied as pre-emergent and/or post-emergent (Brosnan et al., 2011, 2012).

In order to assess the selectivity of a pre-emergence herbicide in direct seeding restoration, it is necessary to use mathematical attributes to understand the dynamics of the seed germination process and to evaluate the quality of the seeds. One of the most important attributes is the germination percentage (G%) that allows identifying whether the seeds are capable of forming a healthy plant under favorable conditions (ISTA, 2022). Furthermore, the germination speed index (GSI) and the mean germination time (MTG) indicate the seed vigor expressed by the relationship between the number of germinated seeds and the germination time. In addition, the synchrony test measures germination synchrony (Z) and expresses the degree of germination overlap, with Z values equal to zero showing seeds that distribute germination over time (Ranal and Santana, 2006).

Pre-emergent herbicide is an effective method to control weed and invasive species (Holfus et al., 2021), however can cause injury or adverse effects on emergent seedlings (Davies et al., 2014). The use of herbicides must avoid damage to the germination process or to the soil seed bank, reducing losses in the emergence phase and ensuring the establishment of post-emergence plants. Indaziflam is a broad-spectrum pre-emergent approved for forestry, restoration and other cultures to control invasive grasses (Sebastian et al., 2016) and it has been studied by Brazilian researchers, mainly in relation to long-term effects for perennial crops (Guerra et al., 2013). The herbicide indaziflam has a long permanence in the soil, and as the sown seeds can be exposed to the herbicide for up to 180 days, we hypothesize that it can interfere with the seed germination process, regardless of the sown species. Due to the relevance of weed control for the restoration by direct seeding, the present study aimed to evaluate the potential impact of the indaziflam on the seed germination of tropical forest native species.

2. Material and Methods

Native forest species used in restoration by direct seeding were selected, based on our previous field experience (Table 1). We evaluated the resistance of the species to the pre-emergent Esplanade® with the active ingredient indaziflam, adopting a dosage of 180 mL of the product (20% above recommended by the package leaflet) in 200L of water compared to a control without herbicide. Seed lots were purchased from suppliers recently stored and evaluated according to the general methods of the International Seed Testing Association (ISTA, 2022). We determined the number of seeds.kg^{-1} and the mass of 1,000 seeds by purity test, and assessed seed germination with four replicates of 25 seeds per species by treatments without (control-C) and with indaziflam (herbicide-H), and we adopted dormancy-overcoming when necessary (Table 1).

In the germination tests of larger seeds, we used as substrate two sheets of filter paper pre-moistened with 5mL of distilled water (control-without indaziflam) or 5mL of the herbicide solution. Smaller seeds (<100,000 seeds/kg)

Table 1. Species and families with the description of the conditions for the germination test following the recommendation of the manual Instructions for Analysis of Seeds of Forest Species (Brasil, 2013) and International Seed Testing Association (ISTA, 2022).

Species	Family	Subst	T (°C)	Overc. Dorm.	Final count (days)
Aegiphila integrifolia (Jacq.) Moldenke	Lamiaceae	EV	30	No dormancy	30
Anadenanthera colubrina (Vell.) Brenan	Fabaceae	EV	20-30	No dormancy	10
Apeiba tibourbou Aubl.	Malvaceae	EV	30	Immersion in hot water at 90°C, without heating, for 10 minutes	20
Astronium urundeuva (M.Allemão) Engl.	Anacardiaceae	SP	25	No dormancy	25
Cordia trichotoma (Vell.) Arráb. ex Steud.	Boraginaceae	SP	30	Scarification	36
Dalbergia nigra (Vell.) Allemão ex Benth.	Fabaceae	SP	25	No dormancy	14
Gallesia integrifolia (Spreng.) Harms	Phytolaccaceae	SP	25	No dormancy	17
Hymenaea courbaril L.	Fabaceae	EV	25	Scarification	28
Mimosa bimucronata (DC.) Kuntze	Fabaceae	SP	30	Immersion in water at 80°C for 24 hours	14
Peltogyne confertiflora (Mart. ex Hayne) Benth.	Fabaceae	SP	25	No dormancy	28
Peltophorum dubium (Spreng.) Taub.	Fabaceae	SP	25	Immersion in water at temperature 80°C, without heating, for 5 minutes	14
Poecilanthe parviflora Benth	Fabaceae	EV	30	No dormancy	28
Psidium cattleyanum Sabine	Myrtaceae	SP	30	No dormancy	90
Psidium guajava L.	Myrtaceae	EV	30	No dormancy	90
Pterogyne nitens Tul.	Fabaceae	SP	25	Scarification	14
Schinus terebinthifolia Raddi	Anacardiaceae	SP	25	No dormancy	18
Senegalia polyphylla (DC.) Britton & Rose	Fabaceae	SP	25	Immersion in water at temperature environment for 2 hours	14

Subst = Substrate (EV = Between vermiculite; SP = On paper); T (°C) = temperature; Overc. Dorm. = overcoming dormancy; Final count (days).

were placed in 10 cm diameter petri dishes with 2.5 mL of water (control) or 2.5 mL of herbicide solution incorporated. Species whose recommendation was vermiculite (Brasil, 2013; ISTA, 2022), we filled 11x11x3.5 cm germination-type boxes (gerbox) to half of their height with a fine-grained substrate and moistened with 100mL of distilled water (control) or the same volume of herbicide solution.

The gerbox and petri dishes were placed in Mangelsdorf-type germinators under the recommended temperature conditions for the species (Table 1). The evaluation time for the germination test followed the instructions for analysis of seeds of forest species (Brasil, 2013). Weekly, we evaluated germination based on the primary root protrusion with at least 2 mm. After seedling growth or seed death, we classified seed germination as high (G>50%), medium (50% ≥ G >20%) or low (G>20%), and performed a variance coefficient per species for each treatment.

We assessed herbicide selectivity by the germination percentage (G%), and the seed vigor by estimating: (a) **germination speed index**- GSI = Σ (ni/ti), in which: ni = number of seeds that germinated at time ‘i’; ti = time after test installation; (b) **mean germination time** - MTG = $(\Sigma n_i t_i)/\Sigma n_i$, in which: n_i = number of germinated seed per day; t_i = test duration time; (c) **germination synchrony** - Z = $\Sigma Cn_{i,2}/N$, where $Cn_{i,2} = n_i(n_i - 1)/2$ and $N = \Sigma n_i(\Sigma n_i - 1)/2$, where: $Cn_{i,2}$ = combination of seeds that germinated in time ‘i’, two by two; n_i = number of seeds that germinated at time “*i*” (Ranal et al., 2009).

We evaluated the effect of the herbicide on the mean germination time (MGT) by the ratio between the number of days to start germination without (C_{MGT}) and with the herbicide (WH_{MTG}). Therefore, ratio values greater than 1.0 ($C_{MGT}/H_{MGT} > 1$) indicated that the herbicide delayed (D) germination, increasing the average time; if $C_{MGT}/H_{MGT} = 1$, the species was indifferent (I) to the herbicide or showed accelerated (A) ($0<(C_{MTG}/H_{MTG})<1$), or inhibited (IH) ($C_{MGT}/H_{MGT} = 0$) germination in response to the herbicide. To compare the grade of sensibility for each species, we calculated a ratio between the percentage of germination with herbicide (G_H) and the control without herbicide (G_C) and classified species as: extremely sensible – ES ($G_H/G_C<0.25$), sensible– S ($0.25<(G_H/G_C) < 0.50$), low sensibility - LS ($0.50< (G_H/G_C) <0.75$), indifferent- I ($0.75< (G_H/G_C) < 1.0$) and potentialized - P ($G_H/G_C >1$).

In order to verify whether there was a phylogenetic response of the species in relation to germination as a function of the herbicide application, we used the taxonomic distinction index (Δ^*) (Clarke and Warwick, 2001). We applied the index since phylogenetic metrics make it possible to identify the accumulation of phenotypic and genetic as well as phenological response at different taxonomic levels (Morelli et al., 2018).

The non-parametric Mann-Whitney test was applied to compare the effect of the herbicide for each studied variable plotted in a box-plot, both performed in R Program (R Development Core Team, 2021). The taxonomic distinction index was calculated using the PAST 4.05 program (Hammer et al., 2001).

3. Results

The herbicide promoted a significant reduction (W= 901; p= 7.699^{e-10}) in the average germination in more than 80% (n=15) of the species, with a coefficient of variation close (83%) to the control treatment (82%) (as shown in Table 2; see Figure 1).

Among the species studied (N=17), only seven showed high germination (G>50%) but the majority (n= 8; 47%) had medium quality (50% ≥ G >20%), and only *Psidium guajava* L. and *Peltophorum dubium* (Spreng.) Taub. were classified as low-quality seeds (as shown in Table 1). Regardless of seed quality, germinability was reduced by the herbicide (see Figure 1), except for *Pterogyne nitens* and *Hymenaea courbaril* (see Table 2). Nevertheless, some species demonstrated more sensibility to indaziflam than others (as shown in Table 3).

From the seventeen species tested, 59% (n = 10) were sensitive or extremely sensitive to indaziflam, but only three species did not germinate, probably due to the herbicide (as shown in Table 3). On the other hand, 29.4% (n = 5) demonstrated low sensitivity or indifference to the herbicide, while seed germination was slightly potentialized by indaziflam to *Hymenaea courbaril* and *Pterogyne nitens* (as shown in Table 3).

The use of indaziflam affected significantly (W= 1636.5; *p*-value= 0.003256) the mean time of seed germination (see Figure 1) (MGT_C=12.2±8.6 days; MGT_H= 11.7±8.5 days), notwithstanding several species 57% (n=14) presented lower MGT without herbicide (see Table 1). Since *G. integrifolia, P. confertiflora* and *P. cattleyanum* did not germinate when exposed to the herbicide, they were not included in this analysis, as they would cause bias and misinterpretation. Despite the longer germination time (>20 days) observed to *Dalbergia nigra*, *Poecilanthe parviflora*, *Cordia trichotoma, H. courbaril, Aegiphila integrifolia*, *Psidium guajava*, the treatment with indaziflam concentrated germination in less than 15 days for *Senegalia polyphylla*, *P. parviflora*, *Astronium urundeuva*, *Apeiba tibourbou*, *C. trichotoma* and *Peltophorum dubium* (as shown in Table 2).

Table 2. Germination percentage (G%) and standard deviation, mean germination time (MGT), germination speed index (GSI) and synchronization index (Ē) of the Seasonal Semideciduous Forest species tested with the pre-emergent herbicide indaziflam (H) and control (C).

Species	G%		MGT (days)		GSI		Ē (bits)	
	C	H	C	H	C	H	C	H
Aegiphila integrifolia (Jacq.) Moldenke	22±6.9a	4±2.2b	22±1.6a	21±14.0a	0.25	0.04	0.65	0.00
Anadenanthera colubrina (Vell.) Brenan	79±11.0a	22±5.1b	10±1.9a	6±0.0b	2.49	0.92	1.32	0.00
Apeiba tibourbou Aubl.	39±17.4 a	7±2.0b	6±0.0b	12±0.0a	1.63	0.15	0.21	0.41
Astronium urundeuva (M.Allemão) Engl.	51±18.9a	28±8.6a	4±0.4a	7±1.1a	3.05	1.07	1.78	0.70
Cordia trichotoma (Vell.) Arráb. ex Steud.	36±5.6a	15±11.9b	14±0.3b	23±4.8a	0.64	0.17	0.68	0.70
Dalbergia nigra (Vell.) Allemão ex Benth.	79±14.7a	43±34.6a	20±4.0a	b13±0.0	1.18	0.83	0.76	0.00
Gallesia integrifolia (Spreng.) Harms	27±10.0a	0b	13±3.4a	0b	0.61	0.00	1.18	0.00
Hymenaea courbaril L.	25±6.8a	31±6.8a	25±1.7a	16±3.5b	0.26	0.49	0.83	0.62
Mimosa bimucronata (DC.) Kuntze	49±8.8a	11±8.2b	8±0.8b	15±4.9a	1.77	0.35	14.51	0.54
Peltogyne confertiflora (Mart. ex Hayne) Benth.	87±5.0a	0b	30±1.1a	0b	0.73	0.00	0.82	0.00
Peltophorum dubium (Spreng.) Taub.	15±6.8a	10±4.0a	11±2.4a	14±0.0a	0.38	0.18	0.66	0.28
Poecilanthe parviflora Benth	66±17.4a	52±16.3b	8±0.4b	21±6.0a	2.12	0.70	0.74	0.44
Psidium cattleyanum Sabine	52±5.6a	0a	16±0.3a	0b	0.84	0.00	0.99	0.00
Psidium guajava L.	20±3.3a	1±2.0b	28±5.6a	6±12.0b	0.20	0.01	0.73	0.52
Pterogyne nitens Tul.	52±13.1a	54±17.4a	6±0.3a	6±1.2a	2.63	2.52	1.39	0.35
Schinus terebinthifolia Raddi	45±21.7a	7±3.8b	14±3.2a	6±0.0b	0.92	0.29	1.36	0.00
Senegalia polyphylla (DC.) Britton & Rose	94±5.1a	81±6.0b	4±0.0b	5±0.0a	5.875	4.05	0	0
Mean	34	35.8	12.2	11.7	1.01	1.1	1	0.9
Standard deviation	28.1	29.2	8.6	8.5	1.24	1.3	3.1	3.1
CV%	83%	82%	71%	73%	123%	119%	311%	318%

Equal letters on the same line for each variable (%G and MGT) do not differ from each other by the Mann-Whitney test with p<0.05. CV = Coefficient of variation.

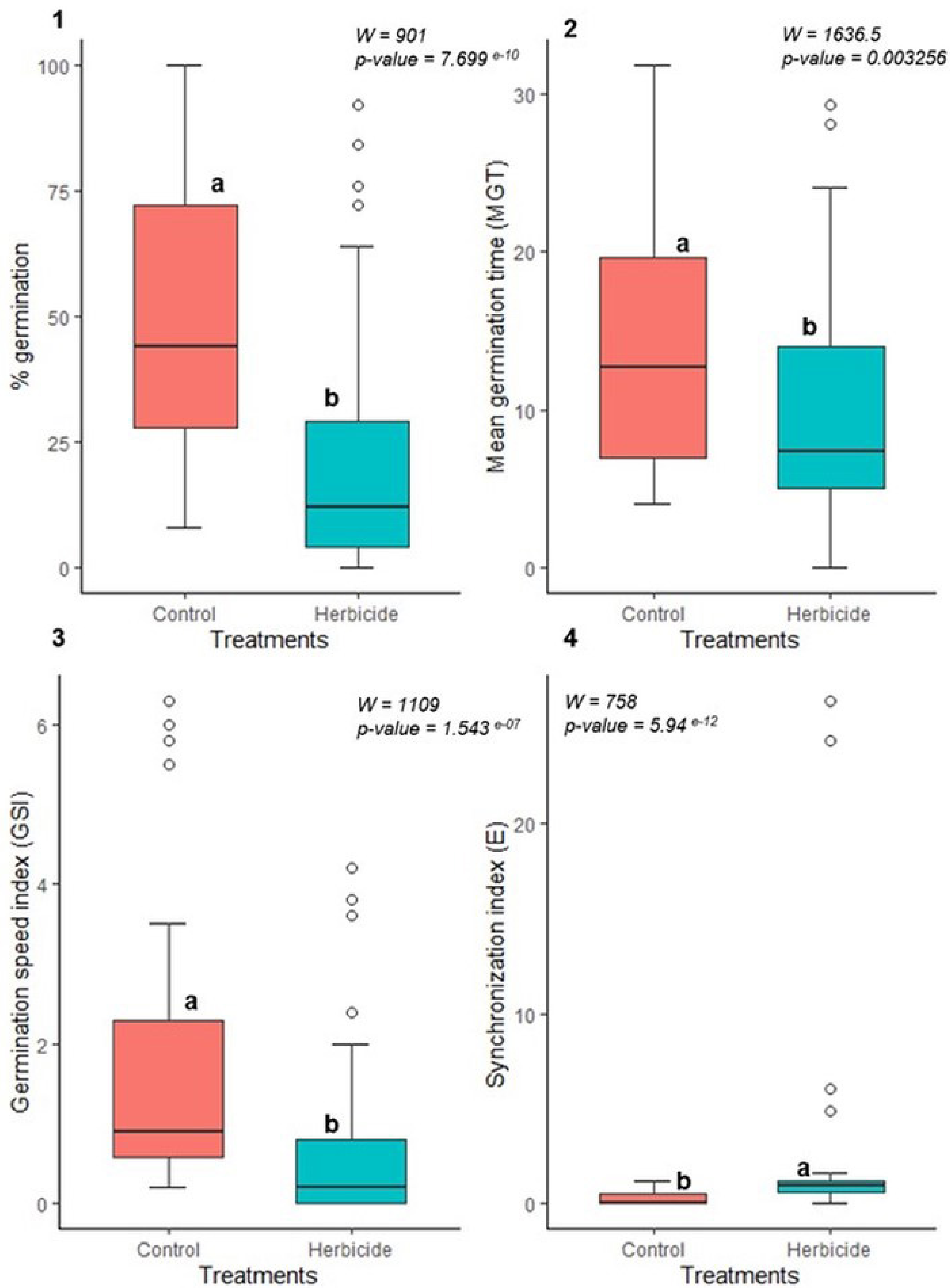

Figure 1. Box-plot of germination percentage (1). average germination time (2). germination speed index (3) and synchronization index (4) of potential species for direct seeding. treated without (control) and with the herbicide pre-emergent Indaziflam (herbicide). Different letters differ by the Mann-Whitney test ($p < 0.05$).

The pre-emergent indaziflam reduced significatively (*W*=1109; *p-value*=1.543^{e-07}) the speed of seed germination in 47.7% compared to the control (GSI_H= 0.7; GSI_C= 1.5) (as shown in Table 2), however affected the synchrony of germination significantly (*W*= 758; *p-value*=5.94^{e-12}) in relation to control ($\bar{E}_H$= 0.3 bits; $\bar{E}_C$= 1.7 bits) (as shown in Table 1; see Figure 1). Meanwhile, the taxonomic diversity index was higher for seed germination without herbicide (Δ^*= 2.711; lower limit=2.657; upper limit= 2.696) than for those with the herbicide (Δ^*= 2.597; lower limit=2.628; upper limit= 2.722) indicating that germination response is less influenced by indaziflam at the taxonomic level.

Table 3. Species with values for the grade of sensibility (S) and their classification (Class). and the values of the effect on the mean germination time (E_{MGT}) and their classification.

Species	Family	S	Class	E_{MGT}	Class
Hymenaea courbaril	Fabaceae	1.2	P	1.6	D
Pterogyne nitens	Fabaceae	1	P	1	I
Senegalia polyphylla	Fabaceae	0.9	I	0.8	A
Poecilanthe parviflora	Fabaceae	0.8	I	0.4	A
Astronium urundeuva	Anacardiaceae	0.7	LS	0.6	A
Peltophorum dubium	Fabaceae	0.7	LS	0.8	A
Dalbergia nigra	Fabaceae	0.5	LS	1.5	D
Cordia trichotoma	Boraginaceae	0.4	S	0.6	A
Anadenanthera colubrina	Fabaceae	0.3	S	1.7	D
Mimosa bimucronata	Fabaceae	0.2	ES	0.5	A
Aegiphila integrifolia	Lamiaceae	0.2	ES	1.1	D
Apeiba tibourbou	Malvaceae	0.2	ES	0.5	A
Schinus terebinthifolius	Anacardiaceae	0.2	ES	2.3	D
Psidium guajava	Myrtaceae	0.1	ES	4.7	D
Gallesia integrifolia	Phytolaccaceae	0	ES	----	----
Peltogyne contertiflora	Fabaceae	0	ES	----	----
Psidium cattleianum	Myrtaceae	0	ES	----	----

P = potentiated; I = indifferent; LS = low sensitivity; ES = extremely sensitive; A = accelerated; D = delayed; ---- absence of germination with the use of herbicide.

4. Discussion

Among the most sensitive species, *P. guajava, G. integrifolia, P. confertiflora* and *P. cattleianum* stand out, which do not present physical dormancy and have a thinner tegument, which can influence herbicide absorption, reducing seed germination. As pre-emergent herbicides act directly on seeds, by inhibiting cell wall biosynthesis (Tompkins, 2010), indaziflam decreases growth by suppressing cellulose synthesis (Brabham et al., 2014), and so may reduce seed germination.

Tegument thickness is one of the most important factors to control water absorption in seeds (Souza and Marcos-Filho, 2001). For the species *Hymenaea courbaril* and *Pterogyne nitens*, which are considered large seeds (Souza and Válio, 2001; Pereira et al., 2013), they did not suffer a negative effect from indaziflam due to their size and the physical dormancy, as their seed tegument is thicker, making it difficult for the product to enter. Although *Senegalia polyphylla* is sensitive to glyphosate, imazapyr, sulfentrazone and metribuzin (Monquero et al., 2011), the species was indifferent to indaziflam, ($0.75< S< 1.0$) and exhibited high germination ($G > 50\%$) developing seedlings in both the presence and absence of the herbicide, even with a significant reduction in germination caused by the herbicide (see Table 3).

Although most species reduced the mean germination time with indaziflam, such as P. *parviflora, M. urundeuva, A. tibourbou, C. trichotoma* and *P. dubium* (see Table 2), their average germination was inhibited by the herbicide, indicating a compensatory and opposite effect between %G and MTG (see Table 2). At the same time, the indaziflam reduced the variation among species in the speed of germination (GSI) (see Figure 1) and synchronized germination in relation to the control (as shown in Table 2). It is important to note that the lower the index of synchrony, the more synchronized is the germination in relation to time (Ranal and Santana, 2006). In direct seed, to reduce the time of germination and synchronize seed germination can increase the probability of species establishment.

The seed field emergence in direct seeding ranges from the 30th to the 90th (Soares and Rodrigues, 2008) and, in some cases, arriving up to 150-180th day after sowing. So, although indaziflam is influencing seed germination of some species, it has reduced mean time of germination and synchronized emergence which can favor seedling establishment in direct seeding.

The exact mechanism of action of this herbicide is still not completely clarified. It is known that cell wall formation is inhibited, but the synthesis of polysaccharide polymers is not affected, and cell walls already completely formed are not affected by the active ingredient indaziflam (Griffin, 2005). However, indaziflam can potentially constrain the radicle development and root growth of newly germinated seedlings by preventing cellulose formation (Clark, 2019). As indaziflam is a new herbicide option for long-term control in natural areas, more studies should be done to understand the effect on different species, mainly its impact on forest seed emergence in restoration by direct seeding. Then, to understand the indaziflam impact on seed germination requires an extensive survey on traits related to the responses of the species.

The botanical family Fabaceae is one of the largest assembly of tree species of Angiosperms, with 2948 representatives throughout Brazil (JBRJ, 2022). Therefore, it is usually abundant in restoration programs with good results in direct seeding, especially for larger seed species (Palma and Laurance, 2015). The taxonomic diversity index indicated that there is a phylogenetic relationship higher for seeds without herbicide than those treated with indaziflam. The initial high quality of the seeds was more associated with the traits of the phylogenetic relationship of each species than with the germination responses in relation to the herbicide, which depends on other non-controlled factors. Confirming that, the species with the highest germination were concentrated in the Fabaceae family, as *P. contetiflora, A. colubrina, S. polyphylla* and *P. parviflora* (see Table 2). Although this family had the highest number of species, the taxonomic diversity index is not affected by the number of species tested at each taxonomic level (Gorenstein, 2009).

There is potential for the use of pre-emergent indaziflam in direct seeding restoration. Even at doses higher than those of exotic tree species, only the three natives *Pterogine contertiflora, Gallesia integrifolia* and *Psidium guajava* were inhibited by the pre-emergent. Despite a negative impact on seed germination of some species, we identified a compensatory effect reducing the mean time of germination and an increase of synchronization, favoring concentration of seed emergence in a short period. However, the effect of indaziflam was variable by species and requires more studies to support a large-scale use on direct seeding.

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