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ACTION OF THE PRE-EMERGENT HERBICIDE ISOXAFLUTOL ON THE INITIAL ESTABLISHMENT OF FIVE FOREST SPECIES

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Resumo

Ação do herbicida pré-emergente isoxaflutol no estabelecimento inicial de cinco espécies florestais. O objetivo deste trabalho foi avaliar a tolerância de espécies nativas do sul de Minas Gerais utilizadas em programas de restauração florestal por meio de semeadura direta ao herbicida pré-emergente isoxaflutol. As espécies utilizadas foram: *Cássia grandis, Anadenanthera macrocarpa, Cedrela fissilis, Hymenaea courbaril e Sesbania virgata*. Os tratamentos com o herbicida constituíram de uma dose de dosagens 0, 50, 100, 200, 300 gramas de ingrediente ativo por hectare (g.i.a.ha^{-1}), do herbicida. Avaliaram-se os sintomas de fitotoxidade durante o processo germinativo das sementes e posterior crescimento em altura, diâmetro, massa seca aérea e massa seca radicular. O delineamento experimental foi o inteiramente casualizado, com oito repetições conduzidos em vaso e casa de vegetação, em que cada vaso foi considerado uma parcela experimental. As espécies *C. grandis, A. macrocarpa, C. fissilis* apresentaram elevada sensibilidade ao herbicida sendo a dose de 50 g.i.a.ha^{-1} provocou sintomas visíveis de fitotoxidade e reduziu os incrementos dos parâmetros avaliados quando comparadas com a dose de 0 g.a.i.ha-1, enquanto que a dose de 300 g.a.i.ha^{-1} provocou 100% de mortalidade. Já para as espécies *H. courbaril e S. virgata* a dose de 50 g.i.a.ha^{-1} favoreceu o incremento dos parâmetros avaliados quando comparada com a dose controle (0 g.i.a.ha^{-1}). Já a dose de 300 g.i.a.ha^{-1}, provocou sintomas visíveis de fitotoxidade às plantas. Contudo, não foi suficiente para promover mortalidade das mesmas, indicando tolerância de ambas as espécies ao herbicida nas doses testadas.
Palavras-chave: Recuperação de áreas degradadas, seletividade de herbicida, semeadura direta.

Abstract

The objective of this study was to evaluate the tolerance of native species from southern Minas Gerais state, commonly used in forest restoration programs through direct seeding, to the pre-emergent herbicide isoxaflutole. The species used were *Cassia grandis*, *Anadenanthera macrocarpa*, *Cedrela fissilis*, *Hymenaea courbaril* and *Sesbania virgata*. The herbicide treatments consisted of five doses: 0, 50, 100, 200, and 300 grams of active ingredient per hectare (g.a.i.ha^{-1}). The phytotoxicity symptoms were assessed during the germination process as well as the subsequent plant growth in height, stem diameter, shoot dry mass, and root dry mass. The experimental design was completely randomized, with eight replicates, conducted in pots under greenhouse conditions, with each pot considered an experimental unit. The species *C. grandis*, *A. macrocarpa*, and *C. fissilis* showed high sensitivity to the herbicide, with the 50 g.a.i.ha^{-1} dose causing visible phytotoxic symptoms and reductions in all evaluated parameters when compared to the 0 g.a.i.ha^{-1} dose, while the 300 g.a.i.ha^{-1} dose resulted in 100% mortality. In contrast, for *H. courbaril* and *S. virgata*, the 50 g.a.i.ha^{-1} dose promoted an increase in the evaluated parameters compared to the control (0 g.a.i.ha^{-1}). Although the 300 g.a.i.ha^{-1} dose caused visible phytotoxic symptoms in these species, it was not sufficient to induce the plant mortality, indicating tolerance of both species to the tested herbicide doses.
Keywords: recovery of degraded areas, herbicide selectivity, direct seeding.

INTRODUCTION

The weed competition with forest species negatively impacts forest restoration projects and the poor or absent weed control in the first years after planting affects the seedling survival and development. In general, weeds are more efficient at obtaining resources from the site than forest species, causing high mortality rates and reduced stem height and diameter, all of which jeopardize the reforestation projects (FARIA *et al*., 2017).

According to Gonçalves *et al.* (2017), the high costs of degraded area restoration projects are due to production, planting, and seedling management, in addition to weed control. Inefficient weed control is responsible for much of the increased cost of restoration projects, generating increased labor demand, the need for replanting, and systemic reentry for the weed control.

Therefore, reducing costs is essential to the viability of environmental restoration projects. In this sense, direct seeding is an alternative that has gained ground among other restoration techniques, since it eliminates the need for the seedling production, facilitates transportation, favors implementation in hard-to-reach areas, and facilitates the mechanization of operations, once the topographic conditions permit (CAMPOS-FILHO *et al*., 2013). However, the initial plant establishment using direct seeding is slower compared to seedling planting, since the initial development stage of forest species, previously carried out in the nursery, will now be entirely in the field. Hence, the weed control is a major challenge to be overcome in areas where this technique is implemented (AGUIRRE *et al*., 2015).

Therefore, the use of chemical weed control in forest restoration areas is one of the most efficient weed control methods, as it offers greater control effectiveness and lower costs compared to mechanical and manual weeding. These factors allow its use over large areas, increasing the operational yield and, consequently, reducing costs. However, most studies on the influence of herbicides in reforestation are related to commercial species, such as eucalyptus and pine, leaving a gap for studies with native species (AGUIAR *et al*., 2015; RESENDE; LELES, 2017).

The herbicides can be classified according to their application method, which are divided into post-emergent and pre-emergent. The former are applied after weed emergence, while pre-emergent are applied directly to the soil, with a residual effect, controlling the soil seed bank. An example of a pre-emergent is isoxaflutole, a systemic herbicide with residual effects indicated for controlling weed seed banks in the soil, with herbicidal effect on both monocotyledonous and dicotyledonous seeds (FERREIRA *et al*., 2010).

According to Carvalho *et al*. (2013), the plants can exhibit biological mechanisms intrinsic to their metabolism to withstand the herbicide applications. These can be divided into two groups: tolerant and resistant plants. Tolerance is an innate characteristic of a species, meaning it is capable of surviving and reproducing after the herbicide treatment. Resistance, on the other hand, is a characteristic acquired by specific biotypes of a plant population, capable of surviving certain herbicide treatments that, under normal conditions, control members of that population (CORREIA; STREK, 2023).

However, most herbicides that exhibit selectivity for agricultural crops are used empirically in reforestation projects, with no understanding of the actual interactions between these chemicals and native species (ARALDI *et al*., 2015; AGUIAR *et al*., 2016). Therefore, identifying native species that exhibit tolerance to certain chemical groups of herbicides will enable more effective weed control methods, expanding the management and implementation possibilities of forest restoration projects. So, the aim of this study was to evaluate the tolerance of the forest species *Cassia grandis*, *Anadenanthera macrocarpa*, *Cedrela fissilis*, *Hymenaea courbaril*, and *Sesbania virgata* to different doses of the herbicide isoxaflutole, applied pre-emergence.

MATERIAL AND METHODS

The experiment was conducted in a greenhouse at the Federal University of Lavras, Minas Gerais state, located at latitude 21°13.652'S and longitude 44°58.143'W. The substrate used was collected from the topsoil of a Red Latosol on the university campus and then packaged in 4 dm³ plastic pots.

The seeds of the studied species were collected in the permanent preservation area of the Federal University of Lavras, located at latitude 21°13'35.98"S and longitude 44°58'6.78"W, during the months of August and September. After collection, the seeds were processed and subjected to morphological selection to use only those considered viable.

For the species exhibiting dormancy, pre-germination treatments were applied to minimize uneven seedling emergence. For the dormant species, pre-germination treatments were adopted to standardize and accelerate the seedling emergence. In the case of *Cassia grandis*, Hymenaea courbaril, and Sesbania virgata, which exhibit physical dormancy due to the impermeability of their seed coats, a mechanical scarification was performed by superficial sanding of the region opposite the hilum, exposing the inner layer, to promote water and gas absorption by the seeds. Seeds of *Cedrela fissilis* and *Anadenanthera macrocarpa* did not require specific pre-germination treatment, as they do not exhibit significant dormancy.

The sowing was carried out directly in the pot, with four seeds per plot. Along with the forest species, 4 g of Urochloa decumbens seeds were sown per pot to validate the control of herbicide application and action, as this species is susceptible to the herbicide isoxaflutole, according to the product label. Irrigation was sufficient to maintain soil moisture without saturating the field capacity, thus avoiding water loss through runoff in the pot and, consequently, herbicide leaching.

The five treatments consisted of varying doses of the commercially available herbicide Fordor 750 WG Bayer®, with isoxaflutole as the active ingredient. The dosages were applied at 0, 50, 100, 200, and 300 grams of active ingredient per hectare ($g.a.i.ha^{-1}$). The herbicide was applied pre-emergence after sowing. The herbicide application was calculated based on a spray volume of 200 liters per hectare and recalculated based on the pot surface area of 0.025 m². Using a graduated pipette, the pre-emergent herbicide spray volume was applied to the pots and the first irrigation was carried out immediately after the application.

Seedling emergence was assessed daily until no new seedlings emerged, aiming to assess the potential damage caused by the herbicide's phytotoxicity to the germination process of the species. Sixty days after herbicide application, seedlings were assessed for: signs of phytotoxicity caused by the herbicide; height measured from the soil to the apical bud using a graduated ruler; stem diameter using a digital caliper; shoot dry weight; and root dry weight.

The shoots of the seedlings remaining in the pots until the time of assessment were cut close to the soil, and all the material obtained was placed in paper bags. The roots were carefully separated from the soil using directed jets of water, removing the soil. They were then dried with paper towels and placed in paper bags. The root and shoot samples were placed in a forced air circulation oven at a fixed temperature of 60°C for 96 hours, until a constant weight was obtained.

A completely randomized experimental design was adopted with five treatments and eight replicates, with each pot considered an experimental unit. Data on height, stem diameter, and shoot and root dry mass were subjected to analysis of variance using the Sisvar 5.6 program (Ferreira, 2019). When significant at a 5% probability of error, regression models were tested using the CurveExpert Pro: 2.7.3 statistical program (Hyams, 2022), selecting the equation models that best fit the data.

RESULTS

Regarding *Brachiaria decumbens*, a species sown in all pots as a control, the germination only occurred in the pots where the herbicide dose was 1000 g.a.i.ha^{-1}. Therefore, it is possible to conclude that all other herbicide doses were sufficient to control the grass development, a result similar to that found by Idziak and Woznica (2014), in addition to ensuring that all other treatments in the experiment received the herbicidal action of the applied chemical.

The species began the germination process between the fourth and the ninth days after sowing. However, phytotoxic effects were observed in the species *Anadenanthera macrocarpa, Cedrela fissilis,* and *Cassia grandis* immediately after emergence at a dose of 50 g.a.i.ha^{-1}, which worsened as they were exposed to increasing doses of the pre-emergent.

The main symptoms observed in the development of forest species were wilting of the apical buds and leaves, a change in the green color of the leaves, hypocotyls, and cotyledons to white, as well as necrosis and leaf drop, indicative of phytotoxicity caused by the herbicide isoxaflutole. As the herbicide doses increased, the lesions in the leaf tissues became more severe, occupying a larger area of the leaves, in addition to causing malformation of these vegetative organs, such as wilting of the leaves and apical buds.

All doses containing the pre-emergent caused a reduction in height at 60 days after herbicide application for the species *C. grandis, A. macrocarpa,* and *C. fissilis* when compared to the 0 g.a.i.ha^{-1} dose (Figure 1).

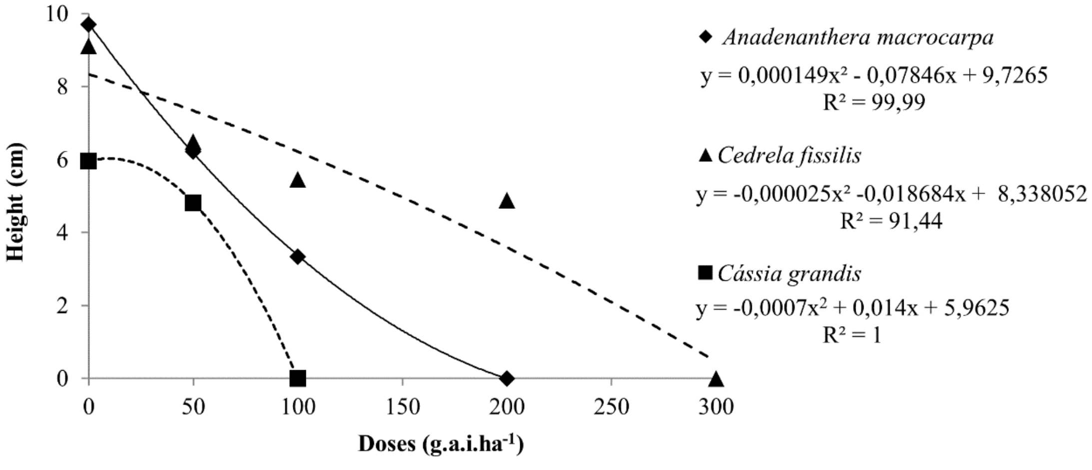

Figure 1. Development of the height parameter of the species, *A. macrocarpa*, *C. fissilis* and *C. grandis* as a function of different doses of isoxaflutole herbicide.

Figura 1. Desenvolvimento do parâmetro altura das espécies, *A. macrocarpa, C. fissilis e C. grandis* em função de diferentes doses do herbicida isoxaflutol.

The phytotoxicity of the herbicide also affected the diameter development at 60 days after application for the species *A. macrocarpa, C. fissilis* and *C. grandis* when compared to the dose of 0 g.a.i.ha^{-1} (Figure 2).

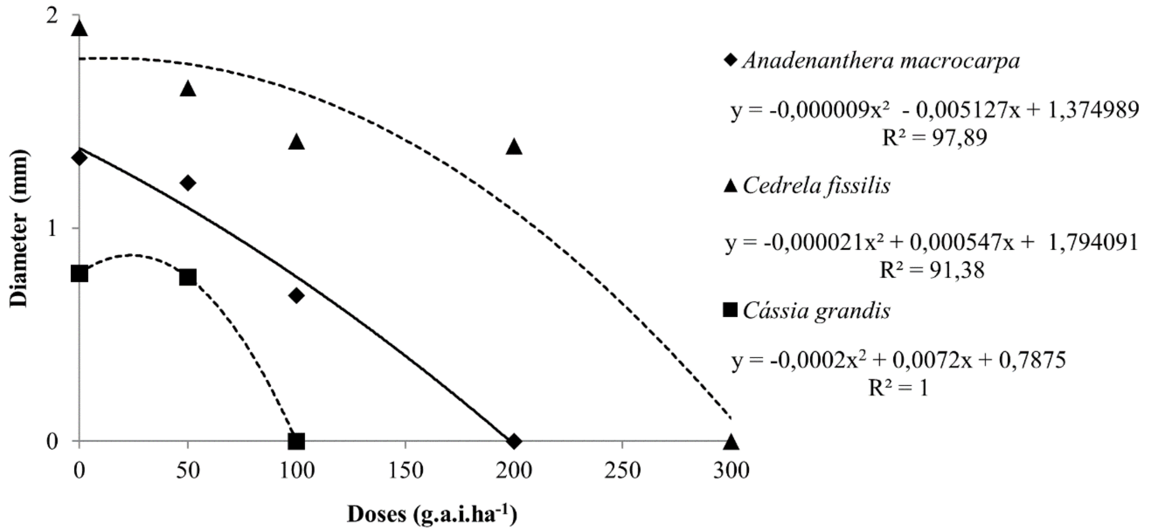

Figure 2. Development of the diameter parameter of the species, *A. macrocarpa*, *C. fissilis* and *C. grandis* as a function of different doses of isoxaflutole herbicide.

Figure 2. Development of the diameter parameter of the species, *A. macrocarpa*, *C. fissilis* and *C. grandis* as a function of different doses of isoxaflutole herbicide.

The herbicidal action of the pre-emergent isoxaflutole negatively affected the development of both height and diameter of the species *A. macrocarpa*, *C. fissilis,* and *C. grandis*, resulting in a lower accumulation of shoot dry mass when compared to the dose of 0 g.a.i.ha^{-1} (Figure 3). In addition, one of the phytotoxicity symptoms caused by the herbicide is precisely the cause of necrosis and leaf abscission, corroborating the reduction in the accumulation of aerial biomass in addition to the reduction of the photosynthetic area of sensitive species, resulting in reduced development.

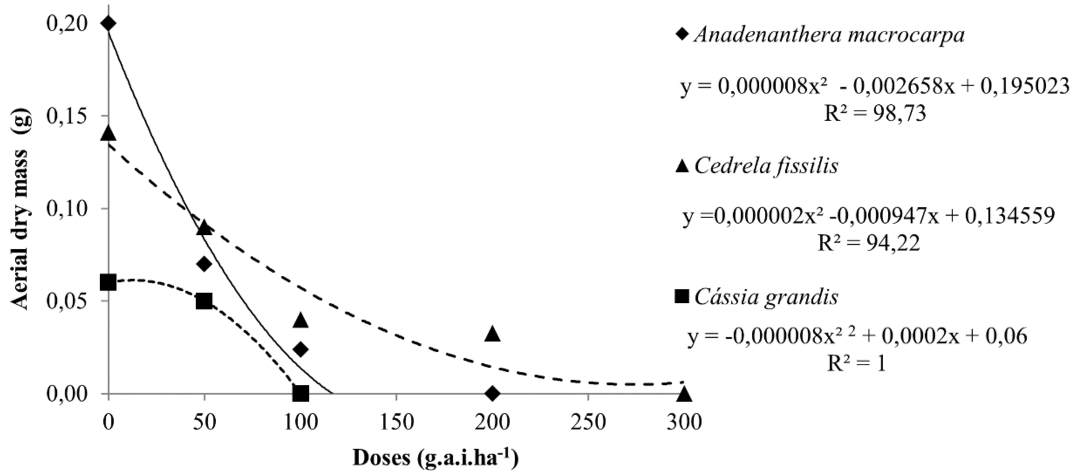

Figure 3. Development of the aerial dry mass parameter of the species *A. macrocarpa, C. fissilis and C. grandis* as a function of different doses of isoxaflutole herbicide.

Figure 3. Development of the aerial dry mass parameter of the species *A. macrocarpa, C. fissilis and C. grandis* as a function of different doses of isoxaflutole herbicide.

Once the photosynthetic apparatus of the species *A. macrocarpa, C. fissilis* and *C. grandis* was compromised by the herbicide isoxaflutole, a lower development of root dry mass was observed when compared to the dose of 0 g.a.i.ha^{-1} (Figure 4).

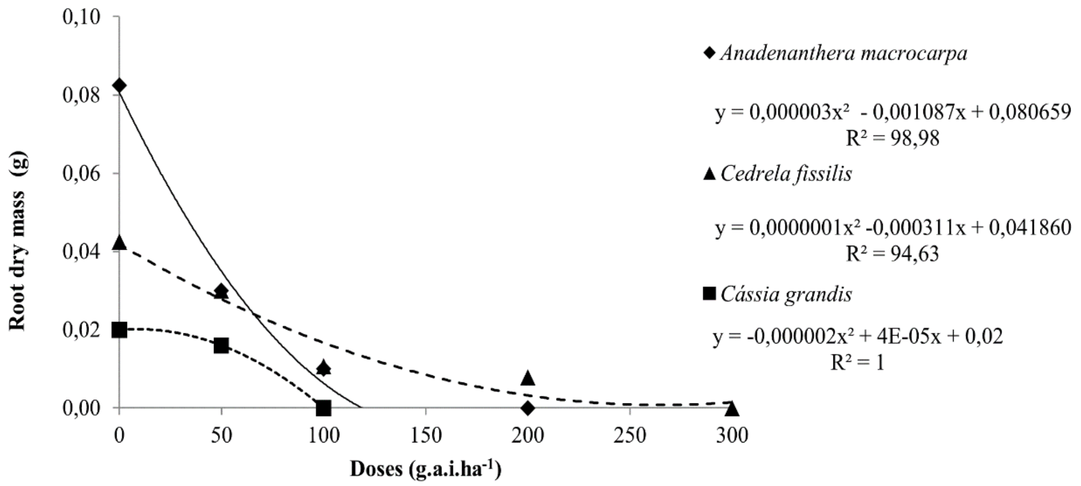

Figure 4. Development of the root dry mass parameter of the species *A. macrocarpa*, *C. fissilis* and *C. grandis* as a function of different doses of isoxaflutole herbicide.

Figura 4. Desenvolvimento do parâmetro massa seca radicular das espécies, *A. macrocarpa, C. fissilis e C. grandis* em função de diferentes doses do herbicida isoxaflutol.

Although the species demonstrated sensitivity to the pre-emergent, different levels of tolerance can be observed among the species. For *C. grandis*, a dose of 100 g.a.i.ha^{-1} would be sufficient to cause 100% mortality, while for *A. macrocarpa*, 200 g.a.i.ha^{-1} would be necessary to produce the same effect. For *C. fissilis*, a dose of 300 g.a.i.ha^{-1} caused damage soon after the emergence of the first pairs of leaves, rapidly progressing to the death of all plants.

It is noteworthy that the species *Hymenaea courbaril* and *Sesbania virgata* exhibited different behavior than *A. macrocarpa*, *C. fissilis,* and *C. grandis* in terms of dose-response to the herbicide. Visual emergence assessments showed no deleterious effects on the germination process of either species, such as apical bud wilting, color changes, necrosis, and leaf drop of the first pairs, or color changes in the hypocotyls and/or cotyledons.

The applications of different herbicide doses did not affect the height development of H. courbaril. For *S. virgata*, the 50 g.a.i.ha^{-1} dose resulted in a 7.45% increase in the observed height averages compared to the 0 g.a.i.ha^{-1} dose, with the increase decreasing with increasing herbicide concentration (Figure 5).

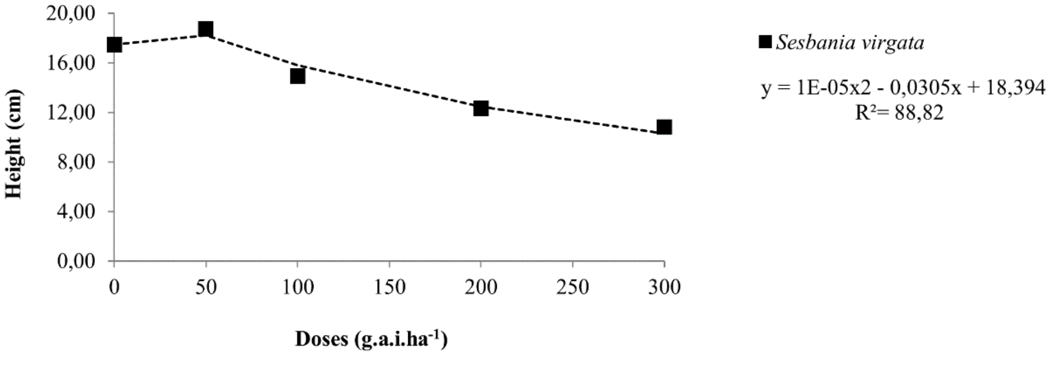

Figure 5. Development of the height parameter of the species *S. virgata*, as a function of different doses of isoxaflutole herbicide.

Figura 5. Desenvolvimento do parâmetro altura da espécie *S. virgata*, em função de diferentes doses do herbicida isoxaflutol.

Regarding the diameter parameter, the dose of 50 g.a.i.ha^{-1} favored the increase in the observed averages for both the species *H. courbaril*, providing an increase of 1.25%, and for the species *S. virgata* of 7.13% in relation to the dose of 0 g.a.i.ha^{-1} (Figure 6).

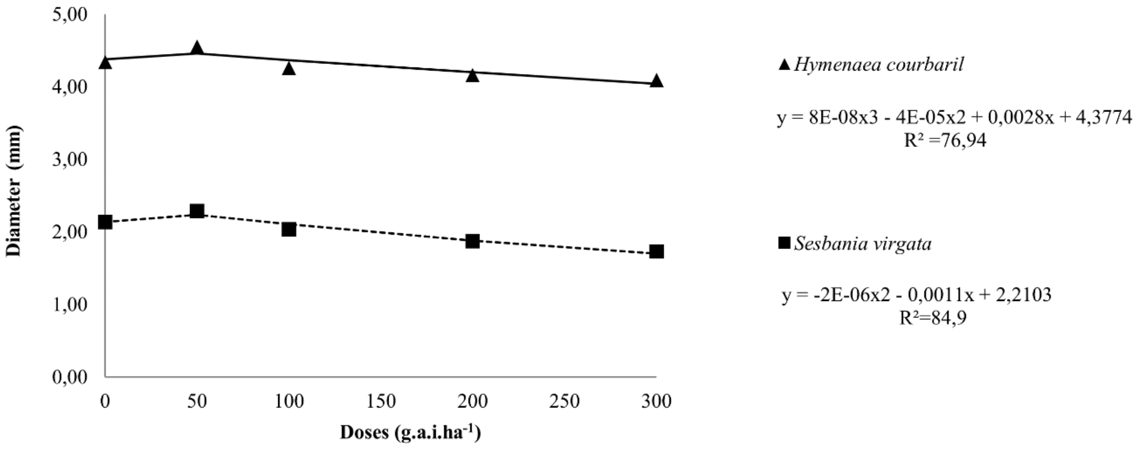

Figure 6. Development of the diameter parameter of the species *H. courbaril* and *Sesbania virgata* as a function of different doses of the herbicide isoxaflutole.

Figura 6. Desenvolvimento do parâmetro diâmetro das espécies *H. courbaril* e *S. virgata* em função de diferentes doses do herbicida isoxaflutol.

The dry mass of the aerial part for both *H. courbaril* and *S. virgata* species was positively influenced by the application of 50 g.a.i.ha^{-1}, favoring the increase of this parameter when compared with the other applied doses. The observed increases were 6.13% for *H. courbaril* and 39.48% for *S. virgata* when compared to the values found at the dose of 0 g.a.i.ha^{-1} (Figure 7).

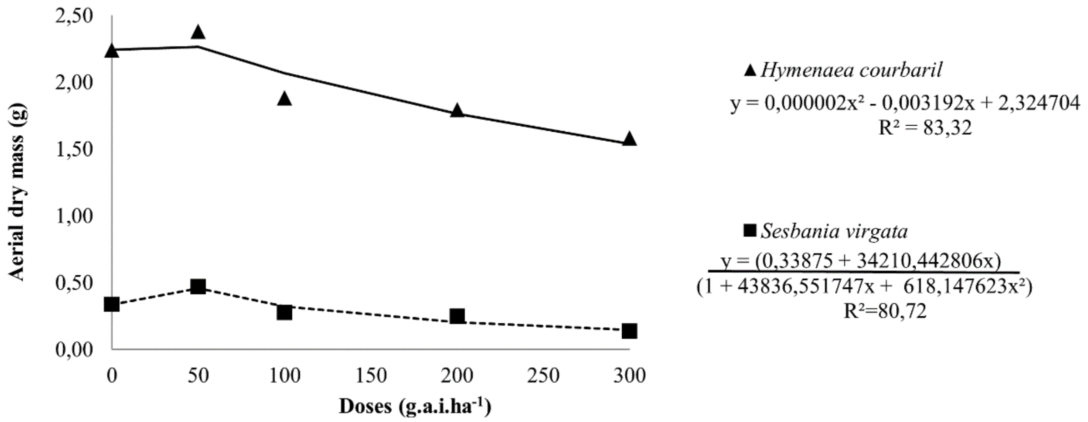

Figure 7. Development of the aerial dry mass parameter of *H. courbaril* and *S. virgata* species as a function of different doses of isoxaflutole herbicide.

Figura 7. Desenvolvimento do parâmetro massa seca aérea das espécies *H. courbaril* e *S. virgata* em função de diferentes doses do herbicida isoxaflutol.

An increase in root dry mass was also observed for both *H. courbaril* and *S. virgata* species when subjected to a dose of 50 g.a.i.ha^{-1} compared to the other applied pre-emergent doses. The observed increases were 22.5% for *H. courbaril* and 15.28% for *S. virgata* when compared to the values found at the dose of 0 g.a.i.ha^{-1} (Figure 8).

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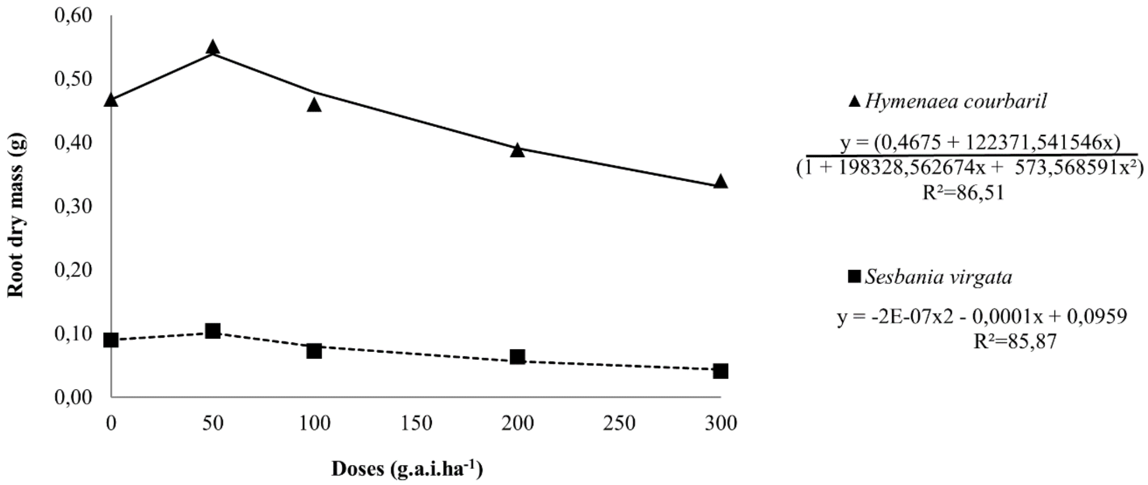

Figure 8. Development of root dry mass parameter of *H. courbaril* and *Sesbania virgata* species as a function of different doses of isoxaflutole herbicide.

Figura 8. Desenvolvimento do parâmetro massa seca radicular das espécies *H. courbaril* e *S. virgata* em função de diferentes doses do herbicida isoxaflutol.

However, as plants were exposed to doses above 50 g.a.i.ha^{-1}, the evaluated parameters decreased. Some plants exposed to a dose of 300 g.a.i.ha^{-1} showed color changes in some areas of the leaf margin, which progressed to localized necrosis. On the other hand, this damage was not sufficient to control the plants and kill them in either species, indicating tolerance to the active ingredient isoxaflutole at the tested doses.

DISCUSSION

Isoxaflutole is the active ingredient in herbicides that inhibit the enzyme 4-hydroxyphenylpyruvate dioxygenase (4-HPPD), preventing carotenoid production in plant cells. This leads to the photodegradation of chlorophyll, causing albinism in the leaves. This alteration causes the formation of free radicals in cells, reduced protein synthesis, ribosome degradation, blockage of electron transport in photosystem II, lipid oxidation, and loss of various functions in plant organelles, which can lead to death (CARVALHO, 2013).

Yet, some forest species may be resistant to the herbicide isoxaflutole, as described by Marchi et al. (2018), when evaluating the germination and development of the species *Platypodium elegans, Guazuma ulmifolia, Jacaranda micranta,* and *Anadenanthera colubrina.* The authors found that *Platypodium elegans* and *Jacaranda micranta* are tolerant to the herbicide. *Guazuma ulmifolia* and *Anadenanthera colubrina* are sensitive to the chemical compound.

Zhao *et al*. (2017) subjected 21 corn hybrids to different doses of the pre-emergent isoxaflutole and found that two hybrids were sensitive to the 110 g.a.i.ha^{-1} dose, showing symptoms of phytotoxicity and biomass reduction. At the 220 g.a.i.ha^{-1} dose, half of the tested hybrids were sensitive to the herbicide compared to the 0 dose. A similar result was found by Cavalieri *et al*. (2008), who also tested the selectivity of the herbicide isoxaflutole in different corn hybrids. This indicates that genetic variation is one of the main factors in plant tolerance to the herbicide.

The increases observed in the regression evaluations for the species *H. courbaril* and *S. virgata* for the tested variables can be explained by the phenomenon known as hormesis, a term coined by Erlich in 1943. It hypothesizes that toxic substances applied at high doses can be beneficial when applied at low doses. Therefore, the application of herbicides at low doses can stimulate plant growth, particularly benefiting plants that already demonstrate tolerance to the chemical compound (CALABRESE, 2005).

Examples of hormesis can occur with various herbicides, as demonstrated by Pereira *et al*. (2015), who subjected *Psidium cattleyanum* and *Cedrela odorata* to the application of sub-doses of glyphosate, resulting in increases in plant dry matter of 37.8% and 106.9%, respectively, compared to a zero dose. Fiore *et al*. (2016), when applying sub-doses of the herbicides atrazine and 2,4-D pre-emergence, observed increases in height, diameter, and root dry matter in *Inga marginata* and *Schizolobium parahyba* seedlings compared to zero doses. Cabral *et al*. (2017) also observed increases in height and root dry matter in native plants tolerant to the pre-emergent clomazone. Hormetic effects can occur in agriculture, as demonstrated by Duke *et al*. (2006) for various crops, such as corn, soybean, rye, and sugarcane, in studies with various herbicides, which showed increases in dry matter, height, protein content, total sugar levels, among others.

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So, the hormetic concept is correlated with the dose-response capable of stimulating secondary metabolism in plants without causing injury. This metabolism is directly responsible for overcoming biotic or abiotic stress. Secondary metabolism acts directly on alterations in biochemical pathways, suppressing or stimulating the production of various biochemical compounds such as enzymes, aromatic amino acids, phenolic compounds, and others. It also converts toxic compounds into elements that can be stored in vacuoles or even used in plant metabolic processes (CALABRESE, 2005; DUKE *et al*., 2006; ROCKENBACH *et al*., 2018).

Based on this context, Pallet et al. (1998) observed that the tolerance of corn and sugarcane crops to the herbicide isoxaflutole is due to the ability to rapidly metabolize the chemical compound isoxaflutole to diketonitrile, which in turn is converted to benzoic acid, a chemical compound that has no herbicidal action, completing the cycle of conversion of the herbicide isoxaflutole into carbon dioxide, a compound that is non-toxic to the plant.

Thus, the biomass increase observed in *H. courbaril* and *S. virgata*, subjected to a dose of 50 g.a.i.ha^{-1} of the herbicide, may be associated with the neutralization of the chemical compounds and the increased concentration of carbon dioxide in the cell cytoplasm. The final phase of isoxaflutole metabolism consists of its conversion to carbon dioxide (CO_2), which is assimilated in the Calvin cycle, a process that occurs in the chloroplasts, promoting an increase in the photosynthetic rate and, consequently, plant growth.

C3 plant species are responsive to increasing carbon dioxide (CO_2) concentrations, exhibiting higher photosynthetic rates as environmental carbon dioxide concentrations increase. In a controlled environment with 550 ppm CO_2 concentrations, rice, wheat, and soybean cultivars achieved an average 27% increase in biomass and 29% in photosynthetic rate, compared to 380 ppm CO_2. Similar responses were observed by Pinto et al. (2001), who applied CO_2 fertigation to Yellow Valencian plants and obtained higher yields in treatments with CO_2 addition. Therefore, if *H. courbaril* and *S. virgata* are able to utilize the carbon dioxide generated by isoxaflutole metabolism, this higher concentration could stimulate the photosynthetic rate, resulting in growth gains, as observed in this study (LONG *et al*., 2006; PINTO *et al*., 2001).

Tolerance to the pre-emergent isoxaflutole, as observed in this study, by the species *H. courbaril* and *S. virgata* has potential for use in degraded area restoration projects, providing safe use of the herbicide for weed control, favoring the implementation of direct seeding projects. *A. macrocarpa, C. fissili,* and *C. grandis* should not be used due to their sensitivity to the herbicide. Direct seeding as a restoration technique utilizes a large number of seeds and forest species. Therefore, it is necessary to expand studies on the tolerance of forest species to pre-emergents so that this control method can be used effectively, representing an efficient alternative for grass management.

CONCLUSION

- The herbicide isoxaflutole applied pre-emergence negatively affected the germination and development of the species, *A. macrocarpa*, *C. fissilis* and *C. grandis*, which are sensitive to the herbicide.
- The species *H. courbaril* and *S. virgata* are tolerant to the herbicide isoxaflutole at all doses tested, and the application of 50 g.a.i.ha^{-1}, in addition to not causing injury to the plants, stimulated greater biomass production, both in the aerial and root parts.

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