

Original Article

Growth and physiology of seedlings of the tree species *Copaifera langsdorffii* Desf. treated with haloxyfop-p-methyl

Crescimento e fisiologia de mudas da espécie arbórea *Copaifera langsdorffii* Desf. tratadas com haloxyfop-p-metil

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Abstract

One of the biggest challenges in reforestation or restoration with native tree species has been the presence of invasive grasses. Chemical control can be a solution as long as the impacts of herbicides are adequately evaluated through studies of the effects of these products on the environment and non-target organisms. In this sense, the objective was to evaluate the effect of doses of the graminicide haloxyfop-p-methyl on seedlings of the *Copaifera langsdorffii* Desf. The experiment was installed in a climate-controlled greenhouse of the Department of Forestry Engineering of the Federal University of the Jequitinhonha and Mucuri Valleys and conducted in a randomized block design (RBD) with 60 healthy individuals of *C. langsdorffii*. The experiment was installed in May 2018 and conducted for approximately 120 days. The treatments comprised herbicide doses (0, 30, 60 and 120 g ha⁻¹). The variables analyzed were visual intoxication, total chlorophyll, chlorophyll *a* fluorescence, number of leaves and leaflets, increases in height and diameter, and survival. No symptoms of intoxication were observed in the plants throughout the experiment period. Haloxyfop-p-methyl did not influence plant physiology or growth. *C. langsdorffii* seedlings were not sensitive to the herbicide. The plants were tolerant to the graminicide haloxyfop-p-methyl. Therefore, this herbicide can be used to control grasses in areas under forest restoration without harming the tree species studied.

Keywords: copaíba, chemical control, graminicide, forest restoration.

Resumo

Um dos maiores desafios no reflorestamento ou restauração com espécies arbóreas nativas tem sido a presença de gramíneas invasoras. O controle químico pode ser uma solução desde que os impactos dos herbicidas sejam adequadamente avaliados por meio de estudos dos efeitos destes produtos no meio ambiente e em organismos não alvo. Neste sentido, objetivou-se avaliar o efeito de doses do graminicida haloxyfop-p-metil em mudas da espécie *Copaifera langsdorffii* Desf. O experimento foi instalado em casa de vegetação climatizada do Departamento de Engenharia Florestal da Universidade Federal dos Vales do Jequitinhonha e Mucuri e conduzido em delineamento de blocos casualizados (DBC) com 60 indivíduos sadios de *C. langsdorffii*. O experimento foi instalado em Maio de 2018 e conduzido por cerca de 120 dias. Os tratamentos consistiram em dosagens de herbicida (0, 30, 60 e 120 g ha⁻¹). As variáveis analisadas foram intoxicação visual, clorofila total, fluorescência da clorofila *a*, número de folhas e folíolos, aumentos em altura e diâmetro e sobrevivência. Não foram observados sintomas de intoxicação nas plantas durante todo o período do experimento. O haloxyfop-p-metil não influenciou a fisiologia ou o crescimento das plantas. As mudas de *C. langsdorffii* não foram sensíveis ao herbicida. As plantas foram tolerantes ao graminicida haloxyfop-p-metil. Portanto, este herbicida pode ser usado para controlar gramíneas em áreas em restauração florestal sem prejudicar as espécies arbóreas estudadas.

Palavras-chave: copaíba, controle químico, graminicida, restauração florestal.

1. Introduction

The anthropogenic pressure that natural ecosystems have suffered over time has caused the loss and fragmentation of these habitats, which is considered one

of the main threats to biodiversity (Newbold et al., 2015; Fletcher Junior et al., 2018). This scenario favored the arrival and establishment of several species with invasive

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Received: August 28, 2024 – Accepted: February 10, 2025



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behavior (Pagad et al., 2018) and currently constitutes one of the main bottlenecks for ecological restoration (Souza et al., 2012).

African grasses of the genus *Urochloa* were widely spread in Brazil for the purpose of forming pastures. However, these species find it easy to occupy niches of native species, as they have greater efficiency in extracting nutrients from the soil, using water and high photosynthetic capacity, resulting in greater competitive potential (Pereira et al., 2014; Medeiros et al., 2016).

The establishment of native species is greatly hampered in areas previously used as pastures (Santos et al., 2018). As an aggravating factor, the majority of areas destined for forest restoration in Brazil are made up of inadequately managed (degraded) or abandoned pastures (Resende and Leles, 2017), which makes human intervention a necessary tool for converting these pastures back into forests (Santos et al., 2018).

In recent years, the search for methods and techniques that can control invasive species efficiently, with the lowest possible environmental impact and at low cost, has intensified (Flórido, 2015). In this context, the use of systemic and low-toxicity herbicides can provide a new perspective on the process of ecosystem restoration (Brancalion et al., 2009; Pereira et al., 2015).

The herbicide haloxyfop-p-methyl belongs to the group of ACCase inhibitors (aryloxyphenoxypropionates) and has great potential for controlling *Urochloa* after its emergence (Oliveira et al., 2019). It is a selective herbicide for broadleaf species, and the most evident symptoms on target plants are leaf chlorosis and necrosis between one and three weeks after application. Herbicides in this class are a good alternative for controlling grasses in forest restoration areas, as their mechanism of action allows eudicot plants not to be harmed by the procedure (Oliveira et al., 2019).

The decision to chemically control grasses in areas where tree species are planted must consider the tolerance of the species to the product, as plants in their initial stages may be more susceptible to herbicides (Oliveira Junior, 2011). In addition, herbicides used incorrectly may affect other organisms, such as the soil microbial community and non-target insects (Santos et al., 2021; Souza et al., 2021). Therefore, it is necessary to study the effect of using haloxyfop-p-methyl on native tree species in order to ensure that its use does not negatively affect the restoration focus.

Copaifera langsdorffii Desf. (Fabaceae) does ecological group classify a species as late secondary and has a wide distribution in the national territory, occurring both in cerrado areas and in forests (Queiroz et al., 2015). The species performs symbiosis with soil microorganisms. This set of characteristics favors its wide use in forest restoration projects, and there are no reports regarding the effect of using the active ingredient haloxyfop-p-methyl for this species.

Thus, the objective of the work was to evaluate the sensitivity of seedlings of the native species *Copaifera langsdorffii* Desf. to the herbicide haloxyfop-p-methyl, with a focus on forest restoration.

2. Material and Methods

2.1. Obtaining and preparing seedlings

The seedlings used in this study were produced in the municipality of Conceição do Mato Dentro (18°54'31.39"S and 43°24'21.86"W), Minas Gerais, by the mining company Anglo American Minério de Ferro Brasil, using tubes of 180 cm³. In January 2018, 60 seedlings, with an average height of 21cm, were transported to the Department of Forestry Engineering (DEF) of the Federal University of Vales do Jequitinhonha and Mucuri (UFVJM), in Diamantina, Minas Gerais, and then each individual was transplanted into a 2.8 L pot, filled with a substrate composed of sifted subsoil soil (Red-yellow Oxisol), plus 1.91 g of MAP; 0.3 g of KCl; 0.64 g of ammonium sulfate; 1.17 g of FTE and 1.68 g of limestone. After transplanting, the seedlings were taken to a climate-controlled greenhouse, where they remained for approximately 120 days, receiving manual irrigation once a day.

The experiment was installed in May 2018 in a climate-controlled greenhouse of the Agronomy Department (DAG) of UFVJM, located in the municipality of Diamantina, Minas Gerais, with an altitude of 1400 m. Location 18°12'11.52"S and 43°34'17.25"W. During the experiment, the greenhouse maintained an average humidity of 70% of field capacity and an average temperature of 32 °C (±7 °C).

2.2. Experimental design

The experiment was conducted in a randomized block design (RBD) consisting of 3 blocks, four treatments and five replications per treatment. The treatments consisted of four dosages of the herbicide haloxyfop-p-methyl: T1 = No herbicide application; T2 = 30 g ha⁻¹; T3 = 60 g ha⁻¹ (commercial dose); T4 = 120 g ha⁻¹. The experimental unit consisted of one seedling per pot.

2.3. Application of haloxyfop-p-methyl

The commercial product Verdict R (containing 60 g/L of haloxyfop-p-methyl) was applied at the rates specified for each treatment, mixed with the adjuvant Assist (containing 786 g/L of mineral oil) at a concentration of 0.5% v/v. The herbicide was applied to the aerial part of the plants using a knapsack electric sprayer (Yamaha FT5®, with a capacity of 5 L), whose application displacement assumed a constant speed of 1 m s⁻¹, with a final solution volume of 150 L ha⁻¹. After application, the plants did not have contact with rainwater or irrigation, aiming to avoid washing the product molecules from the surface of the leaves for up to 10 hours.

2.4. Visual intoxication, height, diameter, survival, number of leaves and leaflets

Intoxication was assessed visually at 7, 14 and 21 days after application of the herbicide (DAA), using a percentage scale of 0-100%, where 0 is the absence of injury and 100 is the death of the plant (SBCPD, 1995). The height and diameter variables were collected before the application of the herbicide and at 60 DAA, obtaining the increase. To obtain the height, the distance between the neck of the plant and the insertion of the last expanded leaf was

measured with the aid of a graduated ruler, and to obtain the diameter of the collar, a digital caliper with precision in millimeters was used. Survival was achieved at 60 DAA. The number of leaves and leaflets was obtained at 21 DAA.

2.5. Total chlorophyll and chlorophyll a fluorescence

Total chlorophyll (TC) readings were taken on leaves from the middle third, fully expanded, during the morning, using the portable ClorofiLOG device (mod1030, Falker), at 14 and 21 DAA. Chlorophyll a fluorescence was evaluated using a portable fluorometer (MINI-PAM-II, Walz) at night (7-8 pm), at 14 and 21 DAA. The variables obtained were the initial fluorescence (Fo), the maximum fluorescence (Fm), the potential quantum yield (Fv/Fm) and the electron transport rate (ETR).

2.6. Statistical analyzes

The data obtained were subjected to analysis of variance (ANOVA), and when significant, the Tukey test was performed at 5% significance. All analyses were performed using the R software (R Core Team, 2018).

3. Results

During the entire experimental period (60 days), no symptoms of toxicity were observed in *Copaifera langsdorffii* plants due to the application of the herbicide haloxyfop-p-methyl (Figure 1). Overall survival was 97% at 60 DAA.

The doses of the herbicide haloxyfop-p-methyl did not affect the increases in height (p-value = 0.75) and diameter (p-value = 0.51), as well as the survival (p-value = 0.48) and

number of leaves (p-value = 0.44) and leaflets (p-value = 0.43) of *C. langsdorffii* seedlings (Table 1).

At 14 DAA, only total chlorophyll was influenced by the doses (p-value = 0.04), being higher for the dose of 60 g ha⁻¹ and lower for the dose of 30 g ha⁻¹ (Table 2). The variables Fo (p-value = 0.78), Fm (p-value = 0.33), Fv/Fm (p-value = 0.65) and ETR (p-value = 0.09) showed no statistical difference in relation to the treatments tested (Table 2).

At 21 DAA, only the ETR (p-value = 0.04) showed a statistical difference for the doses tested, being higher in the treatment that did not receive herbicide and lower in the treatment with twice the commercial dose (120 g ha⁻¹) (Table 2). TC (p-value = 0.70), Fo (p-value = 0.78), Fm (p-value = 0.13) and Fv/Fm (p-value = 0.65) ratio showed no statistical difference at 21DAA (Table 2).

Table 1. The average number of leaves and leaflets (21 DAA), average increase in height and diameter and survival of *C. langsdorffii* seedlings (60 DAA).

	Doses (g ha ⁻¹)				CV (%)
	0	30	60	120	
Sheets (n°)	7.66 ^{ns}	7.33 ^{ns}	6.93 ^{ns}	6.40 ^{ns}	24.72
Leaflets (n°)	36.40 ^{ns}	33.80 ^{ns}	30.13 ^{ns}	31.33 ^{ns}	18.73
Height (cm)	4.47 ^{ns}	4.41 ^{ns}	4.36 ^{ns}	4.75 ^{ns}	10.58
Diameter (mm)	0.70 ^{ns}	0.95 ^{ns}	0.79 ^{ns}	0.63 ^{ns}	19.70
Survival (%)	93.33 ^{ns}	93.33 ^{ns}	100 ^{ns}	100 ^{ns}	6.90

^{ns}Non-significant by the F-test, at 5% de significance. CV: coefficient of variation.



Figure 1. *Copaifera langsdorffii* seedlings 16 days after application of different doses of the herbicide haloxyfop-p-methyl. T1 = No herbicide application; T2 = 30 g ha⁻¹; T3 = 60 g ha⁻¹ (commercial dose); T4 = 120 g ha⁻¹.

Table 2. Total chlorophyll (TC), initial fluorescence (Fo), maximum fluorescence (Fm), potential quantum yield (Fv/Fm) and electron transport rate (ETR) at 14 and 21 days after herbicide application (DAA) in seedlings from *C. langsdorffii*.

	Doses (g ha ⁻¹)				CV (%)
	0	30	60	120	
14 DAA					
TC	41.78 ^{ab}	39.44 ^b	46.92 ^a	40.02 ^{ab}	6.29
Fo	491.13 ^{ns}	499.93 ^{ns}	453.20 ^{ns}	445.80 ^{ns}	16.51
Fm	2974.27 ^{ns}	3065.87 ^{ns}	3098.13 ^{ns}	2792.87 ^{ns}	6.74
Fv/Fm	0.83 ^{ns}	0.83 ^{ns}	0.85 ^{ns}	0.83 ^{ns}	3.60
ETR	44.85 ^{ns}	40.71 ^{ns}	39.90 ^{ns}	42.61 ^{ns}	4.89
21 DAA					
TC	39.81 ^{ns}	37.99 ^{ns}	41.12 ^{ns}	37.57 ^{ns}	10.33
Fo	343.60 ^{ns}	509.33 ^{ns}	418.20 ^{ns}	512.13 ^{ns}	23.72
Fm	2884.53 ^{ns}	2889.07 ^{ns}	2821.87 ^{ns}	2716.13 ^{ns}	3.26
Fv/Fm	0.88 ^{ns}	0.83 ^{ns}	0.85 ^{ns}	0.80 ^{ns}	4.29
ETR	42.32 ^a	40.91 ^{ab}	39.71 ^{ab}	36.74 ^b	4.38

^{ns}Non-significant by the F-test, at 5% significance. For significant variables, means followed by the same lowercase letter per line do not differ by the Tukey test, at 5% significance. CV: coefficient of variation.

4. Discussion

The absence of visual symptoms of intoxication in *C. langsdorffii* seedlings during the 60 days of experimentation confirms the selectivity of the herbicide for this tree species, even at the highest doses tested. Haloxypop-p-methyl is a herbicide that acts by inhibiting the ACCase enzyme (acetyl-coenzyme A carboxylase), preventing the production of phospholipids necessary for cell growth (Shaner, 2014). The selectivity of the herbicide for dicotyledonous plants is due to the presence of prokaryotic (chloroplast) and eukaryotic (cytoplasm) ACCase, unlike monocotyledons that only have eukaryotic ACCase, the herbicide's site of action (Shaner, 2014). Similar behavior has already been reported for other species such as *Trema micrantha*, *Schinus molle*, *Apuleia leiocarpa*, *Toona ciliata*, *Myracrodruon urundeuva* (Oliveira et al., 2019; Oliveira et al., 2008a). For the *Paullinia cupana* species, small visible changes (discoloration, deformation) were observed in some plants, assuming EWRC (1964) toxicity scale 2 (very low) (Santos, 2018).

Chlorophylls are green pigments present in plant cells and indicate the intensity of photosynthesis (Roca et al., 2024). The amount of pigments is related to the plant organism's reaction to growth conditions, such as the presence of herbicides (Zheryakov and Zheryakova, 2021). Under stress-free conditions, light energy is absorbed by chlorophyll and used in photochemical processes. However, a proportion of this energy is released in the form of heat and fluorescence. When electron transport in the photosynthetic system is partially or completely blocked due to a stress factor, the intensity of the emitted fluorescence increases, reflecting less use of photosynthetically active radiation by plants (Rastogi et al., 2019).

The toxicity of pesticides converges in reducing chlorophyll levels and the photosynthetic efficiency of

plants (Sharma et al., 2019). In this study, the lack of toxicity symptoms demonstrated the tolerance of the species analyzed to haloxypop-p-methyl. It corroborated the fact that the herbicide did not influence practically all the other variables measured (height, diameter, number of leaves and leaflets, chlorophyll total - 21DAA and chlorophyll a fluorescence - 14 DAA).

Furthermore, the Fv/Fm ratio is an estimate of the maximum quantum efficiency of the photochemical activity of photosystem II (Baker and Rosenqvist, 2004). It has been used to detect perturbations in the photosynthetic system caused by stress (Percival and Fraser, 2001). All treatments maintained Fv/Fm values between 0.75 and 0.85 quantum-1 electrons, indicating that the photosynthetic apparatus remained intact (Reis and Campostrini, 2011), that is, with no physiological stress. The tolerance of tree species to this herbicide has already been reported for *Toona ciliata* and *Eucalyptus* sp. (Oliveira et al., 2008b; Assis et al., 2015).

The fact that the plants that had contact with the herbicide showed growth in height and diameter, as well as the number of leaves and leaflets, similar to the seedlings that did not receive haloxypop-p-methyl (Table 1), demonstrates the normality pattern of the new tissues formed by individuals subjected to the herbicide. It was considered that the meristematic region is the first to be affected in sensitive plants, as the synthesis of lipids for the formation of membranes is compromised (Oliveira Júnior, 2011).

As for the electron transport rate, there was a decrease in this variable at the highest dose tested, which may be related to the fact that even tolerant species, at higher doses, can be minimally affected by the presence of a herbicide. Environmental conditions such as temperature, radiation, water deficiency, salinity, presence of insects or fungi, among others, can cause small fluctuations in

the rate of electron transport since the photosynthetic capacity of plants can be altered by biotic or abiotic stresses. (Bown et al., 2002; Oliveira et al., 2002). However, this change was not significant enough to reduce the growth of *C. langsdorffii*.

The set of variables evaluated in this study can be used as a group of sensitive and early indicators of the physiological state of plants under stress (Krause and Weis, 1991). Considering that the herbicide haloxyfop-p-methyl did not influence most variables, it can be an alternative for the efficient control of grasses in forest restoration areas without harming the native species *C. langsdorffii*. However, more studies need to be carried out to expand the range of native species covered, as herbicides from this chemical group can have different effects on the same plant species (Shang et al., 2017).

5. Conclusion

The seedlings of the species *C. langsdorffii* were tolerant to the tested doses of haloxyfop-p-methyl, indicating that this herbicide can be applied to seedlings of this species without causing physiological stress or negative effects on their growth.

Acknowledgements

To the “National Council for Scientific and Technological Development (CNPq - processes: 311438/2021-0)”, “Coordination for the Improvement of Higher Education Personnel (CAPES) - Financial Code 001” and, “Foundation for Research Support of the State of Minas Gerais (FAPEMIG)”.

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