

TOLERANCE TO THE HERBICIDE CLOMAZONE AND POTENTIAL FOR CHANGES OF FOREST SPECIES

USO DE ESPÉCIES FLORESTAIS PARA DIMINUIÇÃO DO RESÍDUO DE CLOMAZONE NO AMBIENTE

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ABSTRACT: Clomazone has excelled among Brazilian non-target-site herbicides with high environmental impact. Its high solubility in water can result in leaching, contaminating groundwater and watercourses with possible riparian forest degradation. This situation can be mitigated by phytoremediation process. This study aimed to identify tree species tolerant to clomazone aiming to use them in bioremediation programs. Twelve forest species were evaluated: *Inga marginata* Willd, *Handroanthus serratifolius* (A.H. Gentry) S. Grose, *Jacaranda puberula* Chan, *Cedrela fissilis* Vell, *Calophyllum brasiliense* Cambess, *Psidium myrsinoides* Berg, *Tibouchina granulosa* Cogn, *Caesalpinia ferrea* Mart ex. Tul, *Caesalpinia pluviosa* DC, *Terminalia argentea* Mart & Zucc, *Schinopsis brasiliensis* Eng and *Schizolobium parahyba* (Vell). The statistical analysis was performed in a completely randomized block design with four replications. Three clomazone applications were made each 20 days (60, 80 and 100 days after planting); each application was equivalent to one-half of the recommended rate (2.0 L ha⁻¹). The evaluated parameters were plant height, stem diameter, leaf number, leaf area and dry biomass. The forest species survived the clomazone application; and *I. marginata*, *C. ferrea* and *S. brasiliensis* showed increased tolerance to this herbicide, demonstrating potential for phytoremediation of areas contaminated by clomazone.

KEYWORDS: Bioremediation. Herbicide. Leaching.

INTRODUCTION

The use of *in situ* vegetation for purposed of contaminated soil treatment is named phytoremediation. This technique provides notable advantages, and early, may enable areas for plantation of species non-tolerant to some compounds (KOPTSIK, 2014). Phytoextraction and phytostimulation have highlighted among the remediation techniques; however, root degradation seems to be a major route for these pesticide residues in Brazilian soil (SANTOS et al., 2007).

Herbicides stand out as important pesticides for agriculture management due to high use in recent years, being an effective and economically viable weed control alternative (Inoue, et al., 2011). Nonetheless, problems related to herbicide behavior in soil have been evidenced and leaving toxic waste within soil (COBBUCI; MACHADO, 1999), which can be leached into groundwater or contaminate watercourses by runoff, making it necessary to mitigate this pollution.

The clomazone is the active ingredient of Gamit® that is an important herbicide in Brazil, and is recommended for irrigated rice, soybeans, corn, tobacco, cotton, sugarcane and cassava (MAPA,

2014) applied for pre-emergence control of several weed species. Despite its moderate persistence in soil, the product is highly soluble in water (1,100 mg L⁻¹ at 25 °C) making it dangerous in abundant water environment (ESTEVEZ et al., 2008). Thus, clomazone molecule properties indicate high potential for displacement in the environment near to water streams during irrigation and drainage, which may cause intoxication to sensitive plants downstream pesticide application areas.

Clomazone belongs to isoxazolidinon chemical group, and acts by inhibiting carotenoid biosynthesis (FOROUZESH et al., 2013), being absorbed by plant through apical meristem (preferably in roots) (SCHREIBER et al., 2013). Herbicides such as hexazinone, imazapyr and sulfentrazone, clomazone, ametryn, amicarbazone and diuron were detected in semiartesian wells and springs region of the Córrego Rico-SP microbasin. Among these, clomazone was the most frequent herbicide, being detected in more than 60% of the samples (SANTOS et al., 2015). Similar evaluations were carried out in rivers and agricultural waters of the central region of Rio Grande do Sul, finding in 90% of samples collected residues of clomazone (NALINI et al., 2016).

Phytoremediation with tree species is an alternative for rehabilitation of degraded areas by herbicide contamination; since in general, these plants produce high biomass and extensive root systems (ASTIER et al., 2016). Studies on phytoremediation of herbicide-contaminated areas using fast-growing annual species are already available in national literature (SANTOS et al., 2007; KOPTSIK, 2014). Contrarily, tree species investigations are scarce and it has been increasingly greater the search for information on these plants to subsidize restoration projects, especially with species of riparian areas, thereby constituting as a barrier to the herbicides.

Riparian zones are essential for natural hydrological processes and are located along the banks of watercourses, reservoirs and river sources (JYVÄSJÄRVI et al., 2014). These areas present high water saturation, at least for most of the year, due to the proximity of the groundwater or water courses. Some of the species that compose a riparian forest are able to reach deep layers in the soil profile due to the greater growth of their roots, reaching groundwater (ROOD et al., 2015). Thus, riparian species with remediated capacity can assist in the decontamination of soil and water located at greater depths.

The current study aimed to evaluate the tolerance of twelve forest species to clomazone, so as they might be used in phytoremediation programs to recover areas contaminated by this herbicide.

MATERIAL AND METHODS

The experiment was carried out in a greenhouse of the Graduate Program in Forest Science in the Universidade Federal dos Vales do Jequitinhonha e Mucuri – UFVJM (Federal University of Jequitinhonha and Mucuri Valleys – UFVJM). The statistical design was a completely randomized block with four replications. Twelve forest species of trees were evaluated: *Inga marginata* Willd (Fabaceae), *Handroanthus serratifolius* (A.H. Gentry) S.Grose (Bignoniaceae), *Jacaranda puberula* Chan (Bignoniaceae), *Cedrela fissilis* Vell (Meliaceae), *Calophyllum brasiliense* Cambess (Calophyllaceae), *Psidium myrsinoides* Berg (Myrtaceae), *Tibouchina granulosa* (Desr.) Cogn (Melastomataceae), *Caesalpinia ferrea* Mart. ex Tul (Fabaceae), *Caesalpinia pluviosa* DC

(Fabaceae), *Terminalia argentea* Mart & Zucc (Combretaceae), *Schinopsis brasiliensis* Eng (Anacardiaceae) and *Schizolobium parahyba* (Vell.) Blake, (Fabaceae). Seedlings were acquired from the Forest Garden of the State Forest Institute (IEF) in Diamantina-MG, Brazil. Subsequently, they were grown in 5-L pots (0.0314 m²) filled with properly fertilized substrate. Three herbicide applications with clomazone at a 20-day interval were performed (60, 80 and 100 days after planting); each application was equivalent to one-half of the recommended rate (2.0 L ha⁻¹). Applications were carried out with the aid of micropipette with a manual adjustment directly on water-holding dishes under the pots, simulating water absorption by roots from a contaminated groundwater. For irrigation throughout experiment, water was placed in the dishes and absorbed by the plants through capillary, and substrate moisture kept close to 80% field capacity.

One-hundred days after the first application, plant parameters were evaluated. The assessed variables were plant height (PH), measured from plant base up to the last leaf insertion; stem diameter (SD), measured with a caliper at two-centimeter height; leaf number (LN) and area (LA). The leaf area was determined by scanning leaf limbs in a flatbed scanner attached to a computer. Leaf area was analyzed through an image processing software with advanced measuring - IMAGE PRO-PLUS version 4.1 for Windows®. Moreover, all collected plant material was split into roots, stems and leaves and, subsequently, dried in oven with forced air circulation at 65 °C until constant weight to determine dry mass, which was weighed in a precision balance.

Data were transformed into percentage related to control treatment, since they come from different species with different growth characteristics. Afterwards, variance analysis was performed and means, when significant, were grouped by Scott-Knott test at 5% probability.

RESULTS AND DISCUSSION

Growth analysis of the twelve species showed significant differences for stem diameter (SD), plant height (PH), leaf number (LN) and leaf area (LA) on average of 70.8% for tested species (Table 1).

Table 1. Plant height (PH %), stem diameter (SD %), leaf number (LN %), and total leaf area (LA %) of plants submitted to clomazone applications compared to control (100%).

Species	PH %		SD %		LA %		LN %	
<i>Inga marginata</i>	113.26	a	94.07	b	72.94	a	106.50	b
<i>Handroanthus serratifolius</i>	92.04	b	89.09	b	48.54	b	71.43	b
<i>Jacaranda puberula</i>	110.05	a	115.43	a	83.23	a	245.83	a
<i>Cedrela fissilis</i>	89.01	b	110.06	a	89.18	a	66.00	b
<i>Calophyllum brasiliensis</i>	112.90	a	95.89	b	70.59	a	75.00	b
<i>Psidium myrsinoides</i>	88.78	b	79.34	b	40.65	b	65.34	b
<i>Tibouchina glandulosa</i>	91.53	b	92.84	b	89.01	a	112.16	b
<i>Caesalpinia ferrea</i>	86.23	b	97.93	b	60.48	b	251.40	a
<i>Caesalpinia pluviosa</i>	83.21	b	113.99	a	40.59	b	84.78	b
<i>Terminalia argentea</i>	73.46	b	88.65	b	30.94	b	24.39	c
<i>Schinopsis brasiliensis</i>	83.64	b	105.56	a	79.56	a	127.30	b
<i>Schizolobium parahyba</i>	93.77	b	120.48	a	79.49	a	58.33	b
VC%	20.00		17.31		37.64		82.67	

* Means followed by the same lower case letter in the column do not differ from each other by Scott knott test at 5% probability.

Plant height was moderately reduced for most species by herbicide application, and *T. argentea* was the most negatively affected with a drop of 26.7% in height. In contrary, *I. marginata*, *J. puberula* and *C. brasiliense* showed a significant increase of 12% on average ($p < 0.05$) for this variable (Table 1). Nevertheless, this feature by itself does not reflect a better development since height increase without stem diameter raise, which can frequently affect plant shoot support and lateral branch development weakening stem strength (STEINGRAEBER, 1982).

H. serratifolius, *C. brasiliense*, *P. myrsinoides*, *C. ferrea*, and *T. argentea* had negative results with respect to stem diameter, which reduced in 8.9%. By contrast, *C. fissilis*, *C. pluviosa*, *S. brasiliensis* and *S. parahyba* had stem diameter increased, but it was not followed by height increase. *S. parahyba* showed an increase in stem diameter and height growth statistically close to control. It was observed a slight trend in diameter reduction for *I. marginata*, with no statistical significance together with height growth. As in *J. puberula*, an increase for both variables was observed (Table 1). Larger SDs suggest great availability of photo-assimilated compounds within shoot (SCALON et al., 2001). This availability indicate seedling survival potential, growth and greater adaptability, due to their high capacity of new root formation and growth (SCALON et al., 2002).

Leaf number (LN) among species was different when the clomazone was applied on plants. *T. argentea* was the most adversely affected

compared to other species, with a less LN value, followed by *S. parahyba*, *P. myrsinoides*, *C. brasiliense*, *C. fissilis* and *H. serratifolius* (Table 1). In contrast to those results, the average increasing of 148.6% for *J. puberula* and *C. ferrea* (Table 1) might come from a drastic response to stress caused by herbicide. The same was observed for *I. marginata*, *T. granulosa* and *S. brasiliensis*, but to a lesser extent, with an average increase of 15.3% (Table 1). Leaf number increase might occur due to changes in plant metabolism stimulus.

Recent studies show that the number of leaves produced by plants is determined by resource allocation as compensation (LONNIE, 2012), i.e., species that produce lower mass leaves produce them in greater number per plant, in a corresponding scale. Plants with smaller leaves have greater "foliar intensity" (MILLA; REICH, 2011). The same can occur among different plants within the same species (SCOTT ; AARSSSEN, 2012). The more the leaf are produced, the greater the "gem stock" is, which means more axillary meristems per plant (WHITMAN; AARSSSEN, 2010). This mechanism will be available for a strategic deployment, for example, expression of plasticity architecture/growth (including branching intensity), or as a "supply" for survival and compensation after tissue loss by herbivory or disorders (LONNIE, 2012). In any above-mentioned situation, it is proved the effect of clomazone residue on vegetation downstream fields with herbicide application.

Leaf area stands out among the growth parameters once it plays on light interception and

absorption, and plant photosynthetic capacity (SEVERINO et al., 2004), and plant-environment gas exchanges (PEREIRA et al., 1997). All assessed species were negatively affected by clomazone. *H. serratifolius*, *P. myrsinoides*, *C. pluviosa* and *T. argentea* presented the highest LA reduction rates, followed by LN decrease, limiting light absorption area and, consequently, affecting the photosynthetic apparatus (Table 1). The same was true for the other species except for *I. marginata*, *T. granulosa*, *S. brasiliensis*, *J. puberula* and *C. ferrea* that despite had reduced LA showed a significant increase in LF, which seems to be a compensation strategy to keep the photosynthetic rate (Table 1). With respect to *J. puberula* and *C. ferrea*, there is a LA decrease

of 30% despite of increase by 1.5 times of NL. The reduced leaf area is an early cellular response to low expansion, reducing the surface exposed to the sun, leaving plants less subjected to elevated transpiration and temperature increase (KLICH, 2000).

Concerning dry biomass, the leaf growth was significantly affected by clomazone (Table 2), with an average reduction of 38.4%, for most species. Corroborating results for LA, *T. argentea* stood out negatively with 71.2% reduction in LDM. However, *C. ferrea* and *S. brasiliensis* showed increased LDM accumulation in accordance with LN, but contrary to LA data.

Table 2. Leaf dry mass (LDM %), root dry mass (RDM %), stem dry mass (SDM %) and total dry mass (TDM %) of forest species submitted to clomazone treatments compared with control (100%)

Species	LDM%		RDM%		SDM%		TDM%	
<i>Inga marginata</i>	88.57	b	141.77	a	140.49	a	121.88	a
<i>Handroanthus serratifolius</i>	44.54	c	82.44	b	80.77	b	74.49	b
<i>Jacaranda puberula</i>	68.80	c	137.24	a	95.24	b	110.81	a
<i>Cedrela fissilis</i>	59.62	c	115.66	a	90.15	b	96.66	b
<i>Calophyllum brasiliensis</i>	86.20	b	84.20	b	118.04	a	91.82	b
<i>Psidium myrsinoides</i>	37.49	c	68.42	b	50.37	b	49.76	c
<i>Tibouchina glandulosa</i>	93.65	b	88.25	b	95.78	b	91.29	b
<i>Caesalpinia ferrea</i>	148.86	a	128.04	a	92.85	b	120.25	a
<i>Caesalpinia pluviosa</i>	40.27	c	90.30	b	74.61	b	78.61	b
<i>Terminalia argentea</i>	28.71	c	71.36	b	50.75	b	53.18	c
<i>Schinopsis brasiliensis</i>	135.18	a	125.09	a	85.95	b	113.75	a
<i>Schizolobium parahyba</i>	67.31	c	85.35	b	156.76	a	146.62	a
VC%	51,03		27.19		31.87		23,91	

* Means followed by the same lower case letter in the column do not differ to each other by Scott knott test at 5% probability.

For stem dry mass, it was observed that *P. myrsinoides* and *T. argentea* were the most adversely affected with an average reduction of 49.4%, followed by *H. serratifolius*, *C. ferrea* and *S. brasiliensis*, which were affected in lower degree. Contrarily, *I. marginata*, *C. brasiliense* and *S. parahyba* had increased 38.4% for this variable (Table 2).

Root dry mass was moderately affected by the herbicide, with an increase of 29.6% for *I. marginata*, *J. puberula*, *C. fissilis*, *C. ferra* and *S. brasiliensis*; and an average reduction of 30.1% for *P. myrsinoides* and *T. argentea* and followed the other species (Table 2). Root system quality directly reflects plant survival in the field (MAFIA et al., 2005). In adaptation terms, root system imbalance can be harmful, since seedlings with well-developed roots have higher survival rates (LIMA et al., 2008).

The above cited biomass changes determine alterations in total dry matter (TDM), for which, *I. marginata*, *J. puberula*, *C. ferrea*, *S. brasiliensis* and *S. parahyba* had an increment of 23.4%, on average, by clomazone effect (Table 2). The same was not observed for the other species, which, in general, were negatively affected with an average reduction of 23.4% (Table 2).

The results have evidenced clomazone intoxication on forest species, reducing plant height, leaf area and dry biomass for both shoot and root; and they come from changes in photosynthetic process (TOMCO et al., 2013).

This may occur since this herbicide acts indirectly in photosynthesis by inhibiting deoxyxylulose phosphate synthase enzyme (DXP synthase), which is responsible for isoterpenoid synthesis, the basic carotenoid precursors

(SCHREIBER et al., 2013)) and inhibit chlorophyll photo-oxidation.

Luminosity enhances the herbicide effects, impairing species adaptation to light, which is especially important in early stages by conditioning morphogenetic and physiological modifications in structure and function (SULTAN, 2003), constituting a key factor for species establishment within a forest.

All species survived by the presence of clomazone molecule in the experiment conditions. Nevertheless, performing an associated analysis, *T. argentea*, *P. myrsinoides* and *H. serratifolius* showed results with more significant reductions ($p < 0.05$) for almost all parameters. These results suggest that these species are sensitive to clomazone, and care should be taken when using them in recovery programs for degraded areas. Moreover, the herbicide should not be recommended in areas where such species are protected, as in riparian forest or other forest fragments downstream crops with clomazone spraying.

I. marginata, *C. ferrea* and *S. brasiliensis* showed the best results regarding herbicide tolerance. Although *I. marginata* presented a slight trend in LA and LDM reductions, it has maintained the standards for the other variables, with significant increase in RDM and SDM, which are important parameters on field survival (MAFIA et al., 2005). The species must have been favored since it belongs to the Fabaceae family, which consist of plant species with nitrogen-fixing bacteria association, making them ideal for nutrient poor environments and regeneration areas (GEI AND POWERS, 2015)

and being often associated with riparian forests (RIBEIRO ; LIMA, 2009). *C. ferrea*, which is also from Fabaceae family, despite its LA reduction, it was successful in LN increase strategy, supporting the ability to maintain, at these conditions, a growth rate followed by *S. brasiliensis*, which is included in the Endangered Plant Species Official list as vulnerable (SNIF, 2012).

The above mentioned species were highlighted to preserve similar characteristics to control plants, even under stress conditions. Authors such as Pires et al. (2003c), Koptsik (2014), Santos et al. (2007) and Maladão et al. (2013) emphasized that species tolerant to herbicides in soil had also showed later good performance as phytoremediators, indicating greater potential for further clomazone phytoremediation studies.

CONCLUSION

Clomazone adversely affected to a greater or lesser degree most of the forest species tested in relation to growth. However, *Inga marginata*, *Caesalpinia ferrea* and *Schinopsis brasiliensis* stood for tolerating the herbicide effects, remaining in good conditions. Thus, they might present potential for phytoremediation in recovery programs of areas contaminated by this herbicide.

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RESUMO: Entre os herbicidas com elevado impacto ambiental em sítios não alvo no Brasil, o clomazone tem se destacado. A alta solubilidade desse herbicida em água pode resultar em lixiviação, ocasionando contaminação de mananciais de água subterrânea e cursos d'água, com possível degradação de matas ciliares. Esta circunstância pode ser mitigada por meio de processos de fitorremediação. Este trabalho objetivou identificar espécies arbóreas tolerantes ao clomazone visando utilizá-las em programas de biorremediação. Foram avaliadas doze espécies florestais: *Inga marginata* Willd, *Handroanthus serratifolius* (A.H. Gentry) S.Grose, *Jacaranda puberula* Chan, *Cedrela fissilis* Vell, *Calophyllum brasiliense* Cambess, *Psidium myrsinoides* Berg, *Tibouchina granulosa* Cogn, *Caesalpinia ferrea* Mart ex. Tul, *Caesalpinia pluviosa* DC, *Terminalia argentea* Mart & Zucc, *Schinopsis brasiliensis* Eng e *Schizolobium parahyba* (Vell) Blake. Foi utilizado o delineamento em blocos ao acaso com quatro repetições. Foram feitas 3 aplicações do herbicida clomazone com intervalos de 20 dias (aos 60, 80 e 100 dias após o plantio), cada aplicação foi correspondente a metade da dose comercial de 2.0 L ha⁻¹. Foram avaliados a altura da planta, o diâmetro do caule, o número de folhas, a área foliar e o acúmulo de biomassa seca. Constatou-se que todas as espécies sobreviveram à aplicação de clomazone, contudo *I. marginata*, *C. ferrea* e *S. brasiliensis* apresentaram maior tolerância ao herbicida, demonstrando potencial para o uso em programas de fitorremediação de áreas contaminadas pelo clomazone.

PALAVRAS-CHAVE: Biorremediação. Herbicida. Lixiviação.

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