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ANALYSIS OF BIOLOGICAL INVASION AND USE OF DIFFERENT METHODS FOR THE CONTROL OF *Leucaena leucocephala* (Lam.) De Wit

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ABSTRACT: Biological invasions have received increasing attention from researchers worldwide and the need for prevention measures has become increasingly evident. Leucaena (*Leucaena leucocephala* (Lam.) de Wit) is a shrub species on the list of the 100 most aggressive invasive species on the planet. This work aimed to study the biological invasion and control of leucaena in the municipality of Arcos/MG in Brazil. For the evaluation of land occupation by Leucaena from 2005 to 2017, images from the Landsat 7 and 8 satellites were used. For each year evaluated, thematic maps of land occupation of the area of expansion of the exotic species were generated to quantify the total area invaded. For better visualization of the occupied areas and the different classes of vegetation in the Landsat images, maps were generated using the NDVI (Normalized Difference Vegetation Index). The average annual expansion of leucaena in the area was 0.112 ha/year. The control of the species was evaluated using an experimental design with eight treatments and three replications in randomized blocks, with the following treatments: picloram+2,4D (288 +1,080 g ha-1), triclopyr-butotyl (5 L p.c.ha-1), glyphosate (2.40 kg i.a.ha-1), isolated and the same applied later on the stump, after shallow cutting. The best control was achieved by realizing shallow cutting and applying picloram + 2,4D on the stumps, reaching an average of 80% control of the species in 360 days after treatment (DAT).

KEYWORDS: Invasive species, Herbicide, Leucaena.

ANALISE DE INVASÃO BIOLÓGICA E USO DE DIFERENTES METODOS DE CONTROLE DE *Leucaena leucocephala* (Lam.) de Wit

RESUMO: Invasões biológicas têm recebido cada vez mais atenção de pesquisadores em todo o mundo e têm

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se tornado cada vez mais evidente a necessidade de medidas de prevenção. A leucena (*Leucaena leucocephala* (Lam.) de Wit) é uma espécie arbustivo arbórea que se encontra na lista das 100 espécies invasoras mais agressivas do planeta. O objetivo deste trabalho foi estudar a invasão biológica e controle da leucena no município de Arcos/MG no Brasil. Para a realização da avaliação da ocupação do solo ocorridas pela Leucena dos anos de 2005 a 2017, foram utilizadas imagens dos satélites Landsat 7 e 8. Para cada ano avaliado foram gerados mapas temáticos de ocupação do solo da área de expansão da espécie exótica, a fim de quantificar a área total invadida, para a melhor visualização das áreas ocupadas e das diferentes classes de vegetação nas imagens Landsat, foram gerados mapas com o uso do NDVI (*Normalized Difference Vegetation Index*). A média de expansão anual da leucena na área foi de 0,112 ha/ano. O controle da espécie foi avaliado utilizando delineamento experimental com oito tratamentos e três repetições em blocos casualizados, sendo os seguintes tratamentos: picloran+2,4D (288 +1.080 g ha-1), triclopir-butotílico (5 L p.c.ha-1), glifosato (2,40 kg i.a.ha-1), isolados e os mesmos aplicado posteriormente no toco, após corte raso. O melhor controle se deu através da realização de corte raso e aplicação de picloram + 2,4D sobre os tocos, atingindo média de 80% de controle da espécie em 360 dias após o tratamento (DAT).

PALAVRAS-CHAVE: Espécies invasoras, Herbicida, Leucena.

ANÁLISIS DE INVASIÓN BIOLÓGICA Y USO DE DISTINTOS MÉTODOS PARA EL CONTROL DE *Leucaena leucocephala* (Lam.) De Wit

RESUMEN: Las invasiones biológicas han recibido una atención cada vez mayor por parte de investigadores de todo el mundo y la necesidad de medidas de prevención se ha vuelto cada vez más evidente. La leucaena (*Leucaena leucocephala* (Lam.) de Wit) es una especie de arbusto arbóreo que se encuentra en la lista de las 100 especies invasoras más agresivas del planeta. El objetivo de este trabajo fue estudiar la invasión y control biológico de Leucena en el municipio de Arcos/MG en Brasil. Para realizar la evaluación de la ocupación del suelo por parte de Leucena del 2005 al 2017 se utilizaron imágenes de los satélites Landsat 7 y 8. Con el fin de cuantificar el área total invadida, para una mejor visualización de las áreas ocupadas y las diferentes clases de vegetación en las imágenes Landsat, los mapas se generaron utilizando el NDVI (*Normalized Difference Vegetation Index*). La expansión anual promedio de leucaena en el área fue de 0.112 ha/año. El control de la especie se evaluó mediante un diseño experimental con ocho tratamientos y tres repeticiones en bloques al azar, con los siguientes tratamientos: picloran+2,4D (288 +1,080 g ha-1), triclopir-butil (5 L p.c.ha-1), glifosato (2,40 kg a.i.ha-1), aislado y aplicado posteriormente al tocón, después de la tala rasa. El mejor control se logró mediante el desbroce y la aplicación de picloram

+ 2.4D en los tocones, alcanzándose un promedio del 80% de control de la especie a los 360 días después del tratamiento (DDT).

PALABRAS CLAVES: Especies invasoras, Herbicida, Leucena.

INTRODUCTION

In Brazil, about 40% of the introductions of invasive alien species are due to ornamental plant use and pet breeding. The attention of researchers and environmental managers to these species is relatively recent, considering the large number of invasives already established in the national territory since the beginning of the European colonization process in the 16th century (LEÃO et al., 2011; SAMPAIO; SCHMIDT, 2013).

Leucaena (*Leucaena leucocephala* (Lam.) de Wit) is a shrubby tree species native to Mexico and Central America that has been planted in many tropical countries, including southwest Asia, Africa, and South America, as a source of shade for other commercial crops, between plantation corridors (windbreak or green belt) and for timber production. However, characteristics such as rapid growth, production of seeds in large quantities, ability to reproduce sexually and asexually (successive regrowth after cutting), pioneer succession, and tolerance to diverse environments make the species considered invasive in many parts of the world (OKIGBO, 1984; NOBLE, 1989; BLOSSEY; NÖTZOLD, 1995).

This species can reach three meters in height in the first year and has an excellent capacity for regeneration. The outstanding highlight of the species lies in its multiplicity of uses: as a timber, fodder, and soil-improving plant, especially when intercropped with other crops (DRUMOND, 1992).

Several countries have used the species for timber production, plant recomposition, green manure, and pasture shading. International organizations strongly promoted its cultivation due to its usefulness as a source of fodder and firewood, called a miracle tree in the early years of its global cultivation (GISP, 2005). It was also used in Brazil for forest recomposition in the Santa Helena Biological Refuge

(RBSH), located in southern Brazil, west of Paraná (1990). In Tarumã (SP) in the early 1980s, a Leucaena plantation was carried out in a rocky outcrop area through direct seeding 200 m away from the riparian zone and forest fragment.

Leucaena has become established in more than 120 tropical, subtropical and warm-temperate countries worldwide following deliberate introductions for agroforestry systems and other purposes. Once established, Leucaena is difficult to control and eradicate as it regrows vigorously after cutting. Cut stumps must be treated with diesel or other chemicals for eradication, and even then, the soil seed bank can remain viable for at least 10-20 years after seed dispersal (OLCKERS, 2011; HUGHES, 2010).

Although little information exists on the specific control of leucaena, various mechanical and chemical techniques developed for other woody invasives may be relevant. If proven feasible, mechanical control may become an appropriate option for treating dense leucaena infestations using equipment such as excavators with blade attachments, plows with blades, or tractors and machinery equipped with destructive equipment (VITELLIE PITT, 2006; FOLKERS, 2010).

Seedling and regrowth control can be accomplished with herbicides using a few techniques. However, unlike other woody weed species, control with herbicides in Leucaena is more efficient in controlling younger plants, preferably by foliar spraying (CAMPBELL et al., 2019).

Given the panorama of great invasive potential combined with the difficulties in controlling the invasion after the establishment of the species, the present work evaluated the characteristics of the invasion by Leucena in an area degraded by mining activities and tested different forms for its control.

MATERIAL AND METHODS

STUDY AREA

The study was carried out in a mining-industrial complex located in the municipality of Arcos/MG, Brazil, geographic coordinates 20º 18' 58.63" S, 45º 35' 4.39"

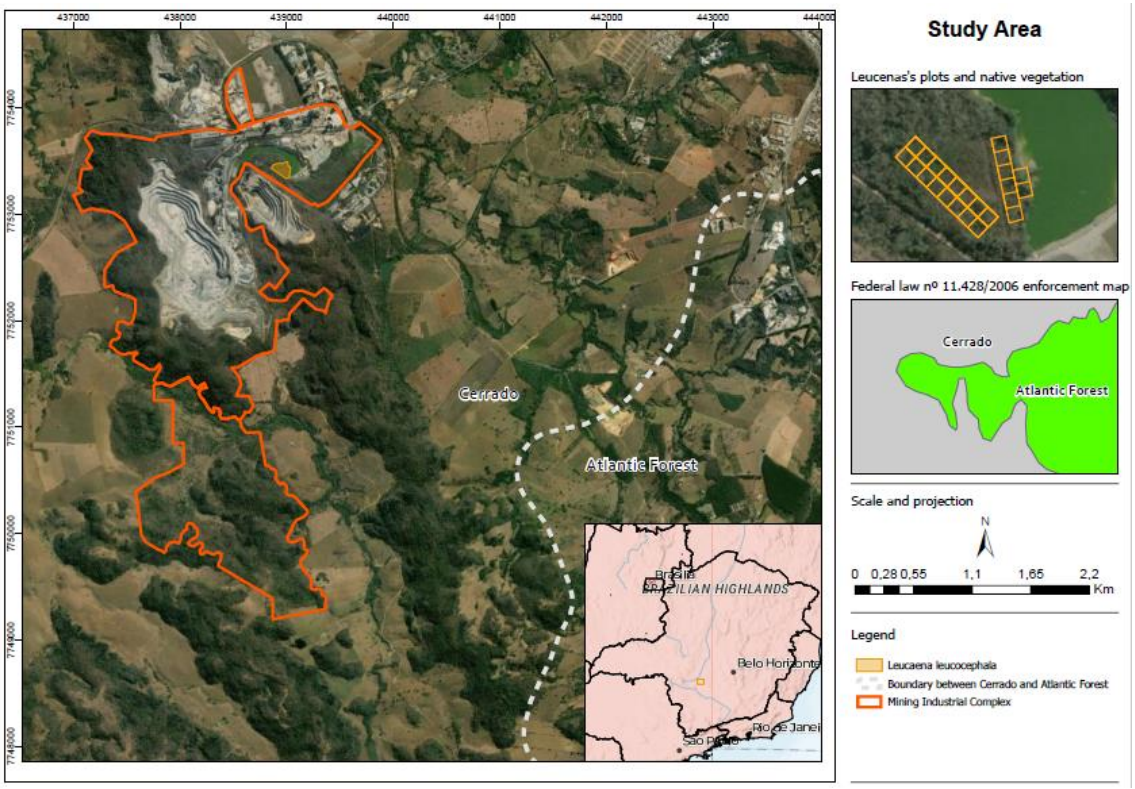
W and altitude of 710 m, where there are leucena massifs in areas destined for environmental conservation - Private Natural Heritage Reserve - RPPN. According to those responsible for the enterprise, leucena seedlings were planted in the 1980s to recover the area degraded by mining activities, having approximately 6.70 hectares. However, the species spread in open and degraded areas, becoming invasive. The area was always the same company, which never had a planting of other species. This area was used as a plot for the different management.

The study area is in Brazil's Cerrado and Atlantic Forest Biomes transition region. According to the Brazilian Institute of Geography and Statistics- IBGE, this area is entirely within the boundaries of the Cerrado biome. However, when evaluating the limits of application of Federal Law No. 11,428, of September 22, 2006, which provides for the use and protection of native vegetation of the Atlantic Forest Biome, regulated by Federal Decree No. 6,660, of November 21, 2008, the site is within the limits of the Atlantic Forest (IBGE, 2019) (Figure 1).

The regional climate is characterized by seasonality, with rains in summer and dry winter, classified as a humid subtropical climate of Koppen's Cwa type. In the municipality of Arcos, the average temperature is 20.7 °C. The warmest months of the year are January to March, with an average temperature of 23.4 °C, while June and July have the lowest average temperature, comprising 16.4 °C.

According to studies by Meguro et al. (2007), the area has annual rainfall characteristics between 1,000 and 1,500 mm. Besides being the wettest, the quarter from December to February has the highest water surplus and the most active surface runoff.

Figure 1 - Study area emphasizing the proximity to the border of the Cerrado and Atlantic Forest Biomes and the map of application of federal law nº. 11,428/2006.



Source: Prepared by the authors (2022).

The region has a well-defined rainy season between November and January and a pronounced dry season between May and August. In 2019, when Leucaena was controlled by cutting and applying herbicides, the accumulated total rainfall was 1263 mm (INPE, 2020 - data station A565 Bambuí/MG). The study area's soil is alkaline, which presents high fertility characteristics.

SPATIAL ASSESSMENT OF THE INVASIVE SPECIES DISTRIBUTION

In order to evaluate the land occupation occurred by Leucena and its expansion between the years 2005 and 2017, images from the Landsat 7 satellites, equipped with an ETM + sensor (Enhanced Thematic Mapper), and Landsat 8, equipped with an optical sensor, the OLI (Operational et al.) and the thermal infrared sensor, the TIRS (Thermal et al.) were used. The sensors were chosen because they have bands that cover the main features of the spectral curve of plants, with sufficiently comprehensive

scenes, in addition to having radiometric quality suitable for the study and being economically viable.

This study used images with 30 m resolution for the Landsat 7 ETM+ satellite and 30m (15m) for the Landsat 8 OLI+TRS satellite, according to Table 1.

Table 1. Bands, spectral resolution and dates of the passage of multitemporal images of the study area's Landsat-7 ETM and 8 Olli+trs.

bands	Spectral resolution (µm)	Passage dates
3	0,63 - 0,69	09 de july de 2005
4	0,76 – 0,90	07 de july de 2010
5	1,55 – 1,75	07 de july de 2010
4	0,64 - 0,67	03 de agust de 2014

Source: Prepared by the authors (2022).

For each year evaluated, thematic maps of land use and occupation of the area of expansion of the exotic species were generated to quantify the total area invaded. All image processing was performed using the ESRI ArcGIS Desktop application version 10.4, and the images were obtained through the Earth Observing System (EOS) software. The images' supervised classification was carried out to differentiate the areas covered by leucena from other types of land cover.

The method used for this classification was the minimum distance method, which assigns each unknown pixel to the class whose mean is closest to it. To better visualize the occupied areas and the different vegetation classes in the Landsat images, maps were generated using the NDVI (Normalized Difference Vegetation Index). The NDVI is calculated by the difference between the Near Infrared and Red bands, normalized by the sum of the same bands. The model used was proposed by Rouse et al. (1973).

EVALUATION OF MANAGEMENT TO CONTROL THE INVASIVE SPECIES

In the same area where leucena was introduced in the 1980s, the research on the management of the species was developed using the experimental design of

randomized blocks with eight treatments and three replications. The experimental units consisted of plots 15 m wide by 15 m long (225 m2) totaling 1.40 hectares, each containing at least ten leucena plants.

The treatments tested consisted of the herbicides alone and shallow cutting on the plants, applied in post-emergence modalities. The application was carried out with the use of a backpack pump on the trunks and stumps, shortly after the execution of the shallow cutting of the leucena trees, according to each of the proposed treatments: 1 - Witness, 2- picloram + 2,4D (288 +1,080 g.ha-1) directly on the trunk of the trees, 3 - triclopyr-butotyl directly on the trunk of the trees (5 L p.c. ha-1), 4 - glyphosate directly on the tree trunk (2.40 kg i.a..ha-1), 5 - Clear-cutting without tree removal, 6 - Clear-cutting without tree removal and application of picloram on the stumps (4%), 7 - Clear-cutting without tree removal and application of Triclopyr-butyl on the stumps (5 L p. c./ha) and 8 - Clear-cutting without tree removal and application of triclopyr-butotyl on the stumps (5 L p. c./ha). c./ha) Moreover, 8 - Clear-cutting without tree removal and glyphosate application on the stumps (2.40 kg i.a.ha-1).

Evaluations were carried out at 30, 60, 180 and 360 days after treatment (DAT) of the following parameters: percentage of control of the exotic species, through visual evaluation using a percentage scale, where 0 (zero) means the absence of symptoms (plant epinasty, decrease in leaf area and leaf curling, growth stoppage) and 100 %, death of all plants; the number of regenerating individuals of the exotic species (considered regenerating shoots occurred); measurement of height from the ground to the highest part of the plant (meters) and measurement of stem circumference at breast height. Data were submitted for analysis of variance and means were compared by Tukey's test at 5% probability.

RESULTS AND DISCUSSION

BIOLOGICAL INVASION

The area used to recover the mining activity through planting leucena presented the invasive and competitive potential of leucaena plants. The growth of the leucaena population occupied an area of abandoned brachiaria pasture, making it impossible to regenerate other species and enrich the understory. Lima (1996) describes the use of these species in the recovery of degraded areas occupying extensive continuous areas of monocultures, metaphorically denominated by the media as "green deserts", for not allowing growth of species in the understory, and can lead to suppression of plant species, microorganism and birds. The area where the species was planted is approximately 2 km from the invaded area. After starting development in 2005, there was an expansion of 0.40 ha between 2005 and 2010, 0.42 ha between 2010 and 2014, and 0.53 ha between 2014 and 2017. The average annual expansion of leucaena in the area was 0.112 ha/year (Table 2).

Table 2. Data on the expansion of land occupation by leucaena.

Expansion of land occupation				
Period (years)	Area perimeter (ha)	Period (years)	Increase (ha)	Increase (%)
2005 – 2010	0,40	5	0,40	29%
2010 – 2014	0,82	4	0,42	31%
2014 – 2017	1,35	3	0,53	40%
	total invaded area:		1,35ha	100%

Source: Prepared by the authors (2022).

Possibly, the species was introduced in the invaded area by seeds from the recovery area due to the high seed production capacity of this species. Although dispersal is predominantly autochorial, there are reports that the species is zoochoric

and that its seeds can be dispersed by birds and ants, allowing transportation beyond the limit of its canopy (BAKER et al., 1965, 1974).

LEUCAENA CONTROL

Triclopyr and glyphosate products (treatments 3 and 4) with direct application on the trunk of leucena trees were ineffective. In contrast, treatment 2 using picloran + 2,4D showed an average of 65% and 63% control at 30 and 60 days, respectively. However, plant recovery occurred, with 80% regeneration at 360 DAT (Table 3).

In field evaluation, the treatment with direct application of picloram + 2,4D on the trunk (treatment 2) started with slow sprouting in only some of the branches, and the other branches of the Leucaena evaluated were visually dry. Compared to the vigor of the trees in the control and clear-cut areas, this result denoted a delay in the sprouting of the species tested with direct application.

Table 3. Percentage of control of the different treatments on different days after treatment (DAT).

Treatments	Pecentage of control			
	30 DAT	60 DAT	180 DAT	360 DAT
1 – Control	0,0 %	0,0 %	0,0 %	00,0 %
2 –Picloram + 2,4D	65%	63%	30 %	20 %
3 - Triclopyr	0,0%	0,0 %	0,0 %	0,0 %
4 – Glyphosate	0,0%	0,0 %	0,0 %	0,0 %
5 – Clear-cut	50%	10%	0,0 %	0,0 %
6 – Cut + picloram + 2,4D	90%	80%	80%	80%
7 – Cut + triclopyr	70%	50%	0,0 %	0,0 %
8 – Cut + glyphosate	30%	20%	0,0 %	0,0 %

DAT: days after treatment
Source: Prepared by the authors (2022).

Regarding the application of the herbicides on the base of the stem, after cutting the leucena plants, the product triclopyr (treatment 7) showed better performance compared to direct application on stumps (treatment 8), reaching average control of 70 and 50% at 30 and 60 DAT. The application of glyphosate directly on the stumps resulted in 20% control. Plant recovery also occurred for the treatments with triclopyr and glyphosate, reaching 100% recovery (treatments 3, 4, 7 and 8 - Table 3).

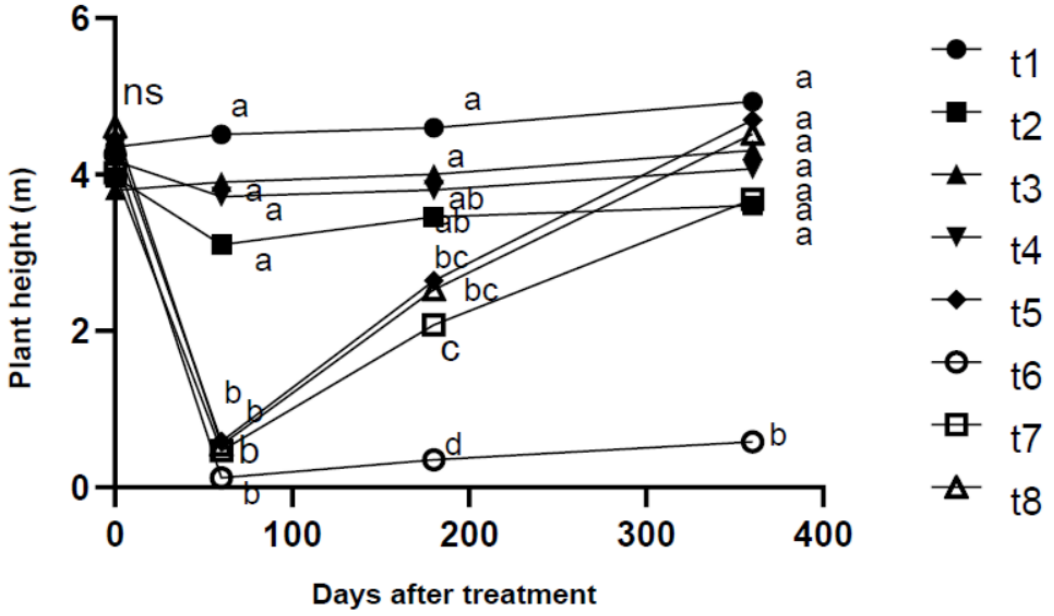
The shallow cutting of leucaenas followed by applying picloran+2,4D (treatment 6) was the most efficient treatment in the control of Leucena among those tested, reaching 90 and 80% control at 30 and 60 DAT, respectively. Although triclopyr and picloran+2,4D have the exact mechanism of action considered auxin mimetics (Vidal,1997), their use showed different results. This can be explained by the presence of two substances, which are 2,4-D (2,4-Dichlorophenoxyacetic acid) and picloram (4-amino 3,5,6 trichloro-2-pyridine carboxylic acid). These products are latifolicides, and 2,4-D has a short to medium persistence in soils and may, according to Silva et al. (2007), cause intoxication in sensitive species such as soybeans, beans, cotton, and other eucotyledons. Picloram has a high residual period and may cause environmental contamination by leaching into deeper soil profile layers, reaching water courses (SANTOS et al., 2007).

Picloram, often associated with 2,4-D, is used in direct applications to the trunk immediately after the plant is cut to control broadleaf weeds of arboreal, shrubby, or sub-shrubby size in pasture areas (FRANCESCHI et al., 2017).

The effects of treatments on height and circumference in Leucaena plants were observed. It was possible to observe that at 60 days, there is a difference between treatments. All treatments using the cut differ from those that only applied herbicides and witnesses. In the evaluation at 360 DAT for plant height, there is no difference between the treatments and the control, except for the treatment with cutting and use of picloram+2,4D (Treatment 6 - Figure 2). The same occurred in the evaluation of plant circumference with rapid recovery in the use of herbicides already at 60 DAT, also

recovering the plants that underwent the treatment of cutting and use of triclopyr and glyphosate at 360 DAT (Treatments 7 and 8 - Figure 3).

Figure 2: Leucaena height (m) measured on different days after the physicochemical treatments.

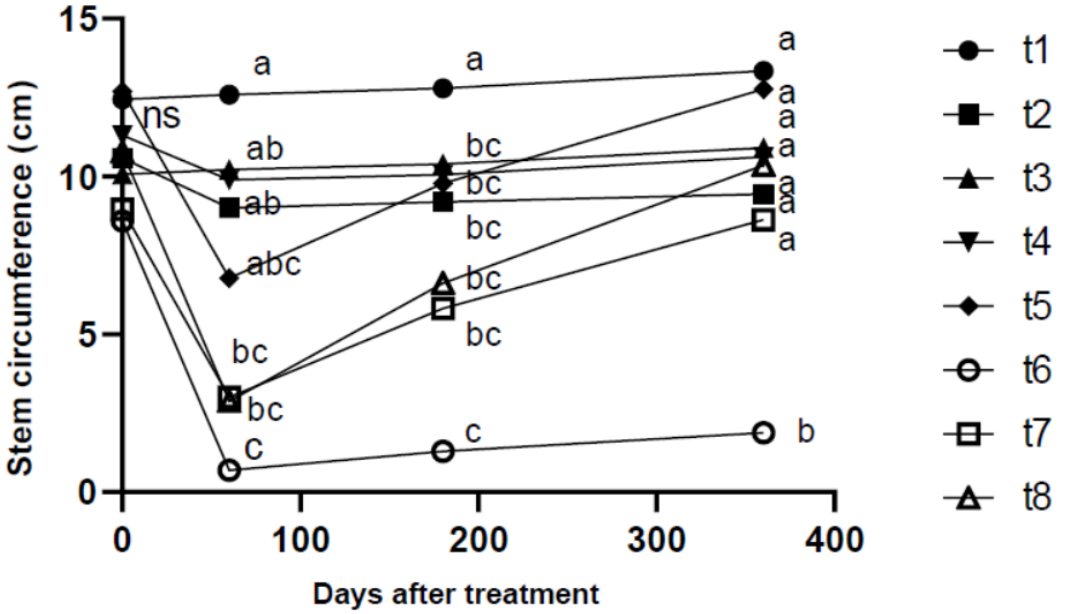


t1 – Control, t2 –Picloram + 2,4D, t3- Triclopyr, t4 – Glyphosate, t5 – Clear-cut, t6 – Cut + picloram + 2,4D, t7 – Cut + triclopyr t8 – Cut + glyphosateMeans with the same letter do not differ by Tukey's test (P> 0.05).Ns= not significant. Source: prepared by the author (2022).

L. leucocephala has a high capacity for vegetative reproduction. In research developed in Taiwan, Peng, Wang, and Kuo (2019) found that after the leucena cuts, there were numerous sprouts on the stumps, with more than ten new sprouts after the previous ones were cut. This sprouting represents a situation of difficult control of this species. Even with continuous cutting operations, the number and length of shoots did not decrease, indicating the vitality of *L. leucocephala*.

In this work, the authors reported that they ringed the plants and injected glyphosate; although this treatment caused defoliation in all treated trees, the shoots still grew at the lower edge of the girdled area. In summary, girdling with or without glyphosate application was inefficient in controlling *L. leucocephala* trees.

Figure 3 - Measurement of the circumference of the Leucaena in the trunk at breast height (cm) on different days after the physicochemical treatments.



t1 – Control, t2 –Picloram + 2,4D, t3- Triclopyr, t4 – Glyphosate, t5 – Clear-cut, t6 – Cut + picloram + 2,4D, t7 – Cut + triclopyr t8 – Cut + glyphosateMeans with the same letter do not differ by Tukey's test (P> 0.05).Ns= not significant. Source: prepared by the author (2022).

The plots that underwent shallow cutting without the application of herbicides (treatment 5) showed 100% sprouting and germinative development of the trunks, in agreement with the works that indicate that leucena has asexual reproduction capacity and successive regrowth capacity after cutting (BAKER et al., 1965).

Caldeira and Castro (2012) found similar results for picloram applications with and without mechanical cracks directed to the stump of *Tectona grandis* L.f. plants. They observed satisfactory control results, even in treatments where no physical damage had been done to the stump of the plants.

Mendes et al. (2016) conducted similar research to control the yellow thistle (*Tecoma stans* (L.) Juss.) in pasture areas, applying herbicides (picloram, triclopyr, and others) on the trunk of the plants that were cut. All herbicide treatments resulted in control levels above 95%. In this study, cutting resulted in more efficient treatments than the treatment that used only the mowing of the aerial part. This result was due

to the ability of the plants to regrow, which is also observed in leucena, where only the use of cutting the plants was not efficient for its control.

Experiments using chemical control efficiently controlled leucaena at the seedling stage (HAWTON et al., 1990). However, using herbicides at the adult stage does not achieve the same results (WANG; HUNG, 2005).

An experiment was conducted to control L. leucocephala through ecological restoration in a coastal forest of the Hengchun Peninsula in Taiwan. After cutting down the trees, 17 native species, including 11 fast-growing species, were immediately planted. After two years of restoration ecology, the dominance of L. leucocephala was inhibited, and the diversity of native tree species in this forest continued to increase (CHEN et al., 2011).

Walton (2003) suggested using a blade plow to remove the root system of L. leucocephala and prevent its roots from resprouting. In South Africa, *L. leucocephala* was wholly removed using this method.

CONCLUSION

Comparing the application of the three usual commercial herbicides (picloram +2,4D, triclopyr and glyphosate) for the control of leucena (L. leucocephala) with direct applications on the trunk and after performing shallow cutting of the stem, it is concluded that the best result for eradication of the species is the application of picloram +2,4D on the stumps (after cutting) reaching an average of 80% effectiveness in control. Even in direct applications, without cutting, picloram +2,4D was more effective than triclopyr and glyphosate, achieving reasonable control results up to 60DAT. This herbicide has persistence in the soil. The positive environmental aspect is the longer control time of germination and/or emergence of Leucaena.

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