

Evaluation of the efficiency of capsule herbicide injection for controlling invasive *Gleditsia triacanthos* L. in a riparian forest

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Abstract

A study conducted in the Department of Florida, Uruguay, assessed the injection technique for controlling the invasive tree species *Gleditsia triacanthos* L., comparing two herbicides: glyphosate and imazapyr. The study involved 90 randomly selected trees divided into three groups: glyphosate, imazapyr, and a control group. Discoloration, defoliation, and mortality were monitored from late spring 2017 to late spring 2018. Significant differences were observed in all variables at the end of the monitoring period. Trees treated with imazapyr showed a sharp increase in mortality, reaching 61.3% by spring 2018, while glyphosate-treated trees experienced a more gradual increase, with 21.1% mortality. Similarly, defoliation and discoloration were severe (>60%) in imazapyr-treated trees and moderate (26-60%) in glyphosate-treated trees. The lance injection technique showed varying effectiveness based on the herbicide used. Imazapyr demonstrated stronger effects in eliminating *G. triacanthos*, making it a potential alternative to traditional control methods. The herbicide capsule applicator used in the study minimized environmental impact by reducing agrochemical drift, thanks to its metal coating and gel solution. However, imazapyr can translocate via roots to non-target trees. No adverse effects were observed in untreated trees, and the herbicide doses used were lower compared to other methods like spraying, further reducing environmental harm. This research highlights the potential of imazapyr-based herbicides in managing *G. triacanthos* while minimizing ecological impact.

Keywords: applicator lance, glyphosate capsules, Honey locust, imazapyr capsules, riparian forest

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Evaluación de la eficacia de la inyección de herbicidas en cápsulas para controlar la invasora *Gleditsia triacanthos* L. en un bosque ribereño

Resumen

El estudio se realizó en el Departamento de Florida, Uruguay, donde se evaluó la técnica de inyección con lanza para el control de la especie arbórea invasora *Gleditsia triacanthos* L., comparando dos herbicidas: glifosato e imazapir. El estudio involucró 90 árboles seleccionados al azar divididos en tres grupos: glifosato, imazapir y un grupo de control. Se monitorearon la decoloración, la defoliación y la mortalidad desde finales de la primavera de 2017 hasta finales de la primavera de 2018. Se observaron diferencias significativas en todas las variables al final del período de monitoreo. Los



árboles tratados con imazapir mostraron un fuerte aumento en la mortalidad, alcanzando el 61,3% en la primavera de 2018, mientras que los árboles tratados con glifosato experimentaron un aumento más gradual, con una mortalidad del 21,1%. De manera similar, la defoliación y la decoloración fueron severas (>60%) en los árboles tratados con imazapir, y moderadas (26-60%) en los árboles tratados con glifosato. La técnica de inyección con lanza mostró eficacia variable según el herbicida utilizado. Imazapir demostró efectos más fuertes en la eliminación de *G. triacanthos*, lo que lo convierte en una alternativa potencial a los métodos de control tradicionales. El aplicador de cápsulas de herbicida utilizado en el estudio minimizó el impacto ambiental al reducir la deriva de agroquímicos, gracias a su recubrimiento metálico y solución de gel. Sin embargo, el imazapir puede trasladarse a través de las raíces a árboles no objetivo. No se observaron efectos adversos en árboles no tratados y las dosis de herbicida utilizadas fueron menores en comparación con otros métodos como la fumigación, lo que redujo aún más el daño ambiental. Esta investigación destaca el potencial de los herbicidas a base de imazapir en el manejo de *G. triacanthos* al mismo tiempo que se minimiza el impacto ecológico.

Palabras clave: lanza aplicadora, cápsulas de glifosato, espina de Cristo, cápsulas de imazapir, bosque ribereño

Avaliação da eficácia da injeção de herbicida em cápsulas no controle da invasora *Gleditsia triacanthos* L. em floresta ripária

Resumo

Um estudo realizado no Departamento da Flórida, Uruguai, avaliou a técnica de injeção de lança para o controle da espécie arbórea invasora *Gleditsia triacanthos* L., comparando dois herbicidas: glifosato e imazapir. O estudo envolveu 90 árvores selecionadas aleatoriamente, divididas em três grupos: glifosato, imazapir e um grupo controle. A descoloração, desfolha e mortalidade foram monitoradas desde o final da primavera de 2017 até o final da primavera de 2018. Diferenças significativas foram observadas em todas as variáveis no final do período de monitoramento. As árvores tratadas com imazapir apresentaram um aumento acentuado na mortalidade, atingindo 61,3% na primavera de 2018, enquanto as árvores tratadas com glifosato registraram um aumento mais gradual, com 21,1% de mortalidade. Da mesma forma, a desfolha e a descoloração foram severas (>60%) nas árvores tratadas com imazapir e moderadas (26-60%) nas árvores tratadas com glifosato. A técnica de injeção com lança apresentou eficácia variável de acordo com o herbicida utilizado. O Imazapyr demonstrou efeitos mais fortes na eliminação da *G. triacanthos*, tornando-o uma alternativa potencial aos métodos tradicionais de controle. O aplicador de cápsulas do herbicida utilizado no estudo minimizou o impacto ambiental ao reduzir a deriva de agroquímicos, graças ao seu revestimento metálico e solução de gel. No entanto, o imazapyr pode ser translocado através das raízes para árvores não alvas. Não foram observados efeitos adversos em árvores não tratadas e as doses de herbicidas utilizadas foram mais baixas em comparação com outros métodos como a pulverização, reduzindo ainda mais os danos ambientais. Esta pesquisa destaca o potencial dos herbicidas à base de imazapir no manejo de *G. triacanthos*, minimizando ao mesmo tempo o impacto ecológico.

Palavras-chave: lança aplicadora, cápsulas de glifosato, acácia de espinhos, cápsulas de imazapir, mata ribeirinha

1. Introduction

Invasive alien species (IAS) have generated numerous environmental, economic, and social problems worldwide and their dispersal has accelerated due to globalization⁽¹⁾. IAS strongly modify natural communities, affecting the provision of fundamental ecosystem services to human livelihoods and development⁽²⁾. This resulted in their detection and management in natural habitats as one of the main global conservation problems⁽³⁾. A great deal of effort has been invested to mitigate the damage caused by these species. Numerous studies have analyzed the economic impact of invasive management and control, estimating annual losses of billions of dollars⁽⁴⁾⁽⁵⁾. The European Union has funded almost 300 projects aimed at addressing the problem of IAS, with a total investment exceeding €132 million⁽⁶⁾. A study in Chile found that seven IAS produce losses of USD 87 million per year, and this could increase to USD 2 billion by 2027 if no action is taken⁽⁷⁾. In the eastern hills of Uruguay, a state company invested more than USD 500 per hectare every 4 months to eliminate dense

areas of *Ulex europaeus* L. that hinder circulation on roads and paths, access to power lines, and infrastructure maintenance⁽⁸⁾.

Invasive tree species represent a significant threat to forests, as they compete with native species for resources such as sunlight, water, and soil nutrients⁽⁹⁾⁽¹⁰⁾. Their rapid growth and ease of propagation allow them to colonize large areas, displacing native species and disrupting the ecological balance⁽¹¹⁾. These invasive trees can also modify forest structure, decrease biological diversity, and negatively affect native plant and animal species that depend on a specific environment⁽¹²⁾. To counteract these impacts, control efforts have been implemented that include eradication of invasive species, restoration of natural habitats, and public communication about the risks associated with these species. These measures are crucial to protect the integrity of the forests and preserve biodiversity in the long term.

In the Río de la Plata grasslands (RPG) region, IAS represent a serious problem and are one of the main environmental challenges⁽¹³⁾⁽¹⁴⁾⁽¹⁵⁾. In this sense, the countries of the region have developed national⁽¹⁶⁾ and regional⁽¹⁷⁾ strategies for the control of IAS. In the case of Uruguay, the reference entity on the subject is the Invasive Alien Species Committee (CEEI in Spanish), responsible for the development and implementation of the National Management Plan for Invasive Alien Species⁽¹⁸⁾. Currently, 42 invasive species have been identified in the country, 17 of which are invasive plants⁽¹⁸⁾. In this regard, four species: *Eragrostis plana* Nees, *Ligustrum lucidum* W. T. Aiton, *Gleditsia triacanthos* L., and *Ulex europaeus* have been recognized as the most problematic invasive plants at the national level⁽⁸⁾. The CEEI also created a Response Protocol for Biological Invasions of Invasive Alien Species in 2018⁽¹⁹⁾.

In the case of forest ecosystems, biological invasions are identified as the main cause of native forest degradation in Uruguay⁽²⁰⁾⁽²¹⁾. The main woody species invading native forests are: *Ligustrum lucidum*, *Ligustrum sinense*, *Gleditsia triacanthos*, *Populus alba*, *Fraxinus pennsylvanica*, *Morus alba*, and *Rubus ulmifolius*⁽²¹⁾. These species compete for resources with native species, alter the hydrological cycle, reduce biodiversity, change nutrient cycles, increase the risk of forest fires, and alter the structure and composition of the forest⁽²²⁾. These impacts are negative for the health of forest ecosystems⁽²²⁾.

The target species of this study, *Gleditsia triacanthos*, is a deciduous Fabaceae tree, native to North America⁽²³⁾ and considered one of the main arboreal invaders in Argentina and Uruguay⁽²⁴⁾⁽²⁵⁾⁽²⁶⁾. The impacts of this species on native forests have been reported in multiple investigations⁽²⁰⁾⁽²⁷⁾⁽²⁸⁾⁽²⁹⁾⁽³⁰⁾⁽³¹⁾. These include the displacement of native species, generating loss biodiversity on a local scale⁽²⁹⁾. After *G. triacanthos* becomes the dominant species, major changes occur in ecosystem processes⁽³²⁾. Different studies have shown that the litter generated by this species produces changes in the decomposition rate, affecting nutrient recycling into the soil⁽³²⁾⁽³³⁾. In addition, by replacing native species in riparian areas, *G. triacanthos* affects geomorphological processes due to its columnar growth, which results in a lower capacity to protect against water erosion compared to native species⁽³⁴⁾. *G. triacanthos* not only invades the forest areas but also the surrounding natural grasslands, causing the loss of areas of high potential for livestock and agriculture⁽²¹⁾.

The main methods for controlling *G. triacanthos* L. in the RPG region are mechanical removal of trees from the ground and chemical control with herbicide⁽³⁴⁾⁽³⁵⁾. In terms of chemical control in the region, different active ingredients have been used, such as triclopyr, isopropylamine salt, and picloram⁽¹⁹⁾⁽³⁰⁾⁽³⁴⁾⁽³⁶⁾, applied on the stem by banding or drilling. In terms of chemical dosing, it has been determined that hack and squirt treatment presents high mortality of individuals but generates higher environmental impact by herbicide drift. Herbicide drift affects native plants, contaminates surface water and affects soil microorganisms⁽²¹⁾. On the other hand, a study on the control of *G. triacanthos* carried out in the National Park of Esteros de Farrapos e Islas del Río Uruguay found that trunk perforation and the application of herbicides by injection is a control technique efficient and low herbicide drift⁽³⁰⁾. In a study carried out in Entre Ríos (Argentina), different herbicides (isopropyl-

amine salt, aminopyralid/triclopyr, fluroxypyr-methyl and metsulfuron-methyl) were applied using two methods: (1) brushing on the stump immediately after felling and (2) drilling the trunk and applying herbicides by injection⁽³⁷⁾. In adult trees, drilling the trunk seems to be more suitable, as it showed high mortality, low time investment and low environmental impact due to the absence of herbicide drift⁽³⁷⁾.

An alternative herbicide application technology to those being used in the RPG region (e.g., felling, girdling, trunk drilling and herbicide application or stem-brushing) is the injection of an herbicide capsule with an applicator lance. In this case, the operator uses the lance to inject the capsule into the cambium layer of the tree; and once injected, the herbicide travels from the point of application to the roots and aerial structures, causing gradual wilting and browning of the foliage, eventually resulting in the death of the individual⁽³⁸⁾. In a trial in the United States, imazapyr capsules were injected by lance into the *Ailanthus altissima* tree, achieving total mortality of the treated individuals⁽³⁹⁾. In another study, the comparative efficacy of five individual tree treatments for stems mortality and prevent sprouting was evaluated⁽⁴⁰⁾. The treatments were glyphosate (active ingredients: isopropylamine salt) capsule application by lance; garlon (active ingredients: triclopyr) application at the base of the trunk with drill; cut and brush with tordon (active ingredients: picloram); cut and brush with glyphosate, and trunk piercing with tordon injection⁽⁴⁰⁾. In this case, the greater efficiency in the application of glyphosate capsules by lance was highlighted. This treatment showed a regrowth rate of 30% of all trees at one year of application and 5% regrowth at 2 years⁽⁴⁰⁾. Finally, in a study in Australia, imazapyr and glyphosate capsules were applied by lance to two woody species: *Annona glabra* and *Cascabela thevetia*, the treatment with imazapyr capsules being more effective⁽⁴¹⁾.

Despite the numerous documented records of the use of the lance for herbicide capsule application in different species and world regions, including its use for the control of IAS, there is no history of its use in *G. triacanthos*, a highly invasive tree in the southern hemisphere. Given that the lance has not been used in the RPG region and that *G. triacanthos* is one of the main invaders in the region, the aim of this study is to evaluate the efficiency of two herbicides, glyphosate and imazapyr, injected into the trunk using a lance, to control adult trees of *G. triacanthos* in a high conservation value area.

2. Materials and Methods

2.1 Area of Study

The study was conducted in the riparian forest of the Arroyo del Potrero, a stream in the Arteaga estate, located in Parada Arteaga, Florida Department, Uruguay (33°44'00.29" S; 55°30'33.18" W) (**Figure 1**). Managed by a forestry company and categorized as a high conservation value (HCV) area according to the Forest Stewardship Council (FSC) certification scheme, the Arteaga estate has 7085 hectares. The experiment conducted in this study spans an area of 1.5 hectares, where the riparian forest shows a high degree of invasion by *G. triacanthos*. According to the soil chart of Uruguay, the work is located within the San Gabriel - Guaycuru soil unit⁽⁴³⁾, where the predominant soil is Lithic Hapludoll⁽⁴²⁾. The texture of the soils of the San Gabriel - Guaycuru soil unit is loamy with the presence of gravel, and the average pH of the horizons corresponds to 5.9⁽⁴³⁾.

Under the Köppen-Geiger climate classification Uruguay is in Cfa region, which has a humid subtropical climate, characterized by warm summers and precipitation distributed throughout the year⁽⁴⁴⁾. In the study area, the mean annual temperature is 19.2 °C and the annual accumulated precipitation is 1262.5 mm⁽⁴⁵⁾.

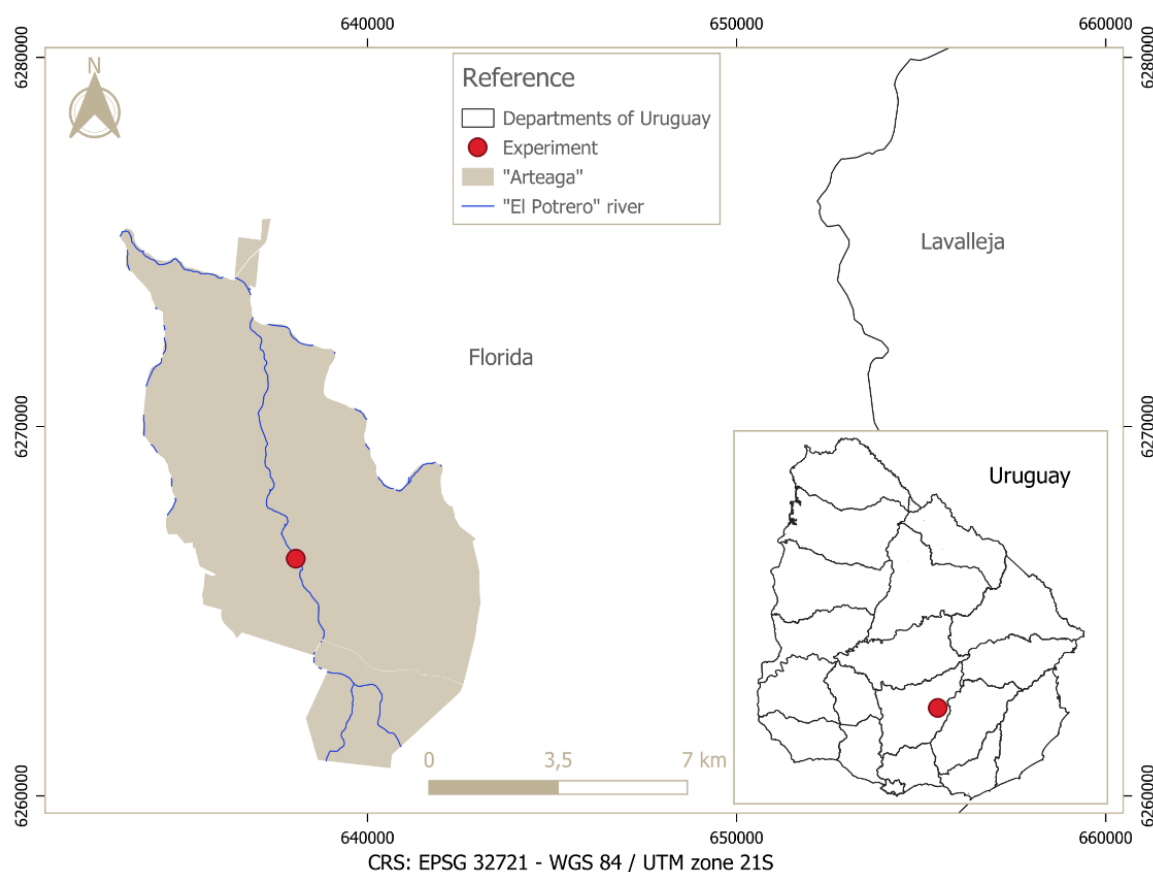


Figure 1. Map of the study site location near El Potrero river in Florida Department, Uruguay

2.2 Experimental Design

An experiment to evaluate the effectiveness of two herbicides (imazapyr and glyphosate) in the control of adults of *G. triacanthos*, using a lance herbicide capsules applicator, was installed at the end of 2017. Since there was no background about the use of this equipment in Uruguay, an authorization process was needed. An authorization was obtained from the General Directorate for Agricultural Services (DGSA in Spanish) of Uruguay for its use with capsules containing 0.15 g of glyphosate or imazapyr, both at 83.5% of concentration.

A total of 90 trees were randomly selected to establish the different treatments. The trees have a mean CBH (circumference at breast height) of 27.04 cm (SD 14.20 cm). The distance between trees was variable, and no minimum distance was established between them. Herbicide capsules were applied by lance at the base of the trunk to all the treated trees (**Figure 2**). The total dose of active ingredient applied to each tree was calculated according to the size of the individuals, injecting a capsule every 0.15 m of the CBH (1: 0 - 0.15 m; 2: >0.15 - 0.30 m; 3: >0.30 - 0.45 m, and 4: >0.45 m). The dosing criteria, defined by the authors of the study, followed the precautionary principle by reducing the doses by one-third compared to the manufacturer's recommendation (E-Z-Ject lance, Odom Processing Engineering Consulting, Inc., Waynesboro, Mississippi), as the experiment took place in a high conservation value (HCV) area and the equipment had not been previously used in the country. Of the total trees, 30 were injected with imazapyr capsules, 30 with glyphosate capsules, and 30 untreated trees were used as controls. All trees were numbered, and each treatment was indicated with a different color. Due to the loss of some labels during the monitoring period of some trees treated with glyphosate, the number of trees monitored during the whole period for this treatment was 19, so the total number of trees monitored until the end of the study was 79.



Figure 2. Application of herbicide capsules to *Gleditsia triacanthos* L. trees. The photograph highlights, in red, glyphosate capsules applied to the stem of a *G. triacanthos* L. individual

The study was installed in November of 2017, late spring of southern hemisphere, coinciding with the growing season and time of greatest metabolic activity and sensitivity of the species⁽⁴⁶⁾. Monthly evaluations were made throughout the year until December 2018, except for the cold months (May to September), when the species has very low metabolic activity and no leaves. During the monitoring visits, each tree underwent a visual crown assessment adapted from the Servicio de Sanidad Forestal y Equilibrios Biológicos of Spain⁽⁴⁷⁾, quantifying: i) percent discoloration (0: null [0-10] %, 1: slight [11-25] %, 2: moderate [26-60] %, 3: severe [>60] %, 4: [100] % dry); ii) percent defoliation (0: null [0-10] %, 1: slight [11-25] %, 2: moderate [26-60] %, 3: severe [>61] %, 4: [100] % dry). In the case of mortality evaluation, dead trees were those corresponding to category 4 of defoliation (i.e.: dry trees). The visual evaluation was performed by the same field operator, ensuring consistency in the results obtained.

2.3 Statistical Analysis

Based on the field data collected, a database was constructed for statistical analysis, with the data organized into three groups according to the treatment applied: imazapyr, glyphosate, and control.

Due to the non-normality of the data non-parametric methods were used. Kruskal-Wallis tests were applied to compare discoloration and defoliation among treatments and evaluations dates. Since mortality is a binomial variable, Fisher's exact test was applied. All the analyses were performed for each date individually using PMCMRplus library available in R language.

The post-hoc analysis used in discoloration, defoliation and mortality cases was Conover's test with Bonferroni correction, available in the `kwAllPairsConoverTest` function of the PMCMRplus library. The temporal evaluation by variable was carried out through Friedman tests with `friedman.test` function of R, because each determination per individual on the different observation dates constituted repeated measurements over time. In these analyses, the control group was not included, since it did not show mortality, defoliation, or evident discoloration throughout the study. In all cases, a $p < 0.05$ was considered significant. All analyses were carried out using R (version 4.3.0) (R Foundation for Statistical Computing, Vienna, Austria) and RStudio interface (version 2023.06.0) (RStudio PBC, Boston, MA, USA).

3. Results

One year after herbicide application, significant differences in the mortality rates of *G. triacanthos* trees were observed among treatments (**Table 1**). At the end of the study, trees injected with imazapyr capsules presented the highest percentage of mortality compared to glyphosate treatment and control trees, where no mortality was recorded (**Table 1**). In the case of average discoloration and defoliation, there were also significant differences among the treatments. At the end of the study, imazapyr-treated trees showed a severe discoloration and defoliation (>60%), glyphosate-treated trees showed a moderate defoliation and discoloration, while control trees showed no signs of discoloration nor defoliation (**Table 1**).

Table 1. Average discoloration and defoliation with its standard error (SE), and percentage mortality, at the last evaluation date (Dec. 2018), are shown for each treatment group

Treatment	Discoloration*	Defoliation*	Mortality (%)
Imazapyr (n=30)	3.3 (± 0.1) a	3.3 (± 0.2) a	61.3 a
Glyphosate(n=19)	2.1 (± 0.3) b	2.0 (± 0.3) b	21.1 b
Control (n=30)	0.0 (± 0.0) c	0.0 (± 0.0) c	0.0 c

* The defoliation and discoloration categories are as follows: 0: null [0-10] %, 1: slight [11-25] %, 2: moderate [26-60] %, 3: severe [>60] %, 4: [100] % dry.

$\pm$ Different letters indicate significant differences among treatments ($p < 0.05$).

The temporal evolution of *G. triacanthos* tree mortality by treatment is shown in **Figure 3**. Tree mortality showed significant differences in both herbicide treatments, imazapyr ($p < 0.001$) and glyphosate ($p = 0.023$). In the imazapyr treatment, mortality showed a stable initial phase of low mortality, around 6%, until the fourth evaluation date, with a sharp increase from the spring months (October, November and December) (**Figure 3**). For trees treated with glyphosate, the behavior was different, with a progressive increase in mortality until the third and fourth evaluation date (15.8%), and then remained without significant variations until the end of the study (**Figure 3**).

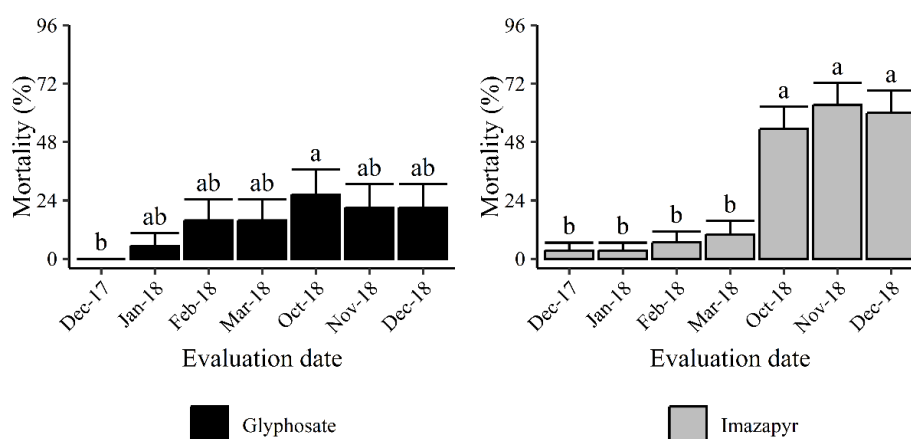


Figure 3. Cumulative percentage mortality observed for individuals treated with imazapyr (n = 30) and glyphosate (n = 19)

Different letters indicate significant differences between evaluation dates for each treatment, as determined by Conover ($p < 0.05$). Vertical bars represent standard error.

In the case of defoliation and discoloration, significant differences were also detected between the different evaluation dates, both for the group of trees treated with imazapyr ($p < 0.001$) and those treated with glyphosate ($p < 0.001$) (Figure 4). In imazapyr-treated trees, the temporal pattern of discoloration and defoliation was like that of mortality, showing a sustained increase during the first five dates, and from spring onwards it stabilized around a final value ($>60\%$) (Figure 4). The discoloration and defoliation pattern of glyphosate-treated trees also mirrored the mortality temporal trend (Figure 4).

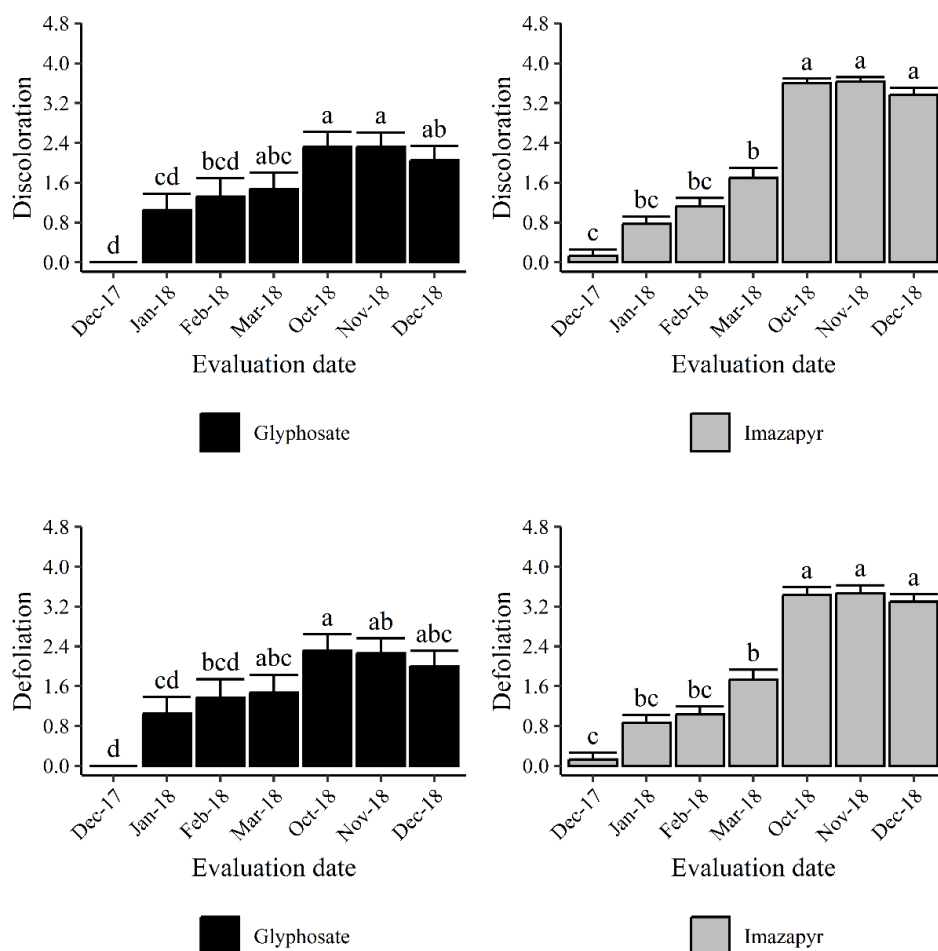


Figure 4. Mean defoliation and discoloration observed for individuals treated with imazapyr ($n = 30$) and glyphosate ($n = 19$)

Different letters indicate significant differences between evaluation dates for each treatment individually by Conover ($p < 0.05$). Vertical bars indicate standard error.

4. Discussion

The herbicide capsules injection by lance proved to be an effective technique to control *Gleditsia triacanthos* in a riparian forest. Imazapyr was more effective than glyphosate in controlling *G. triacanthos*. In the trees treated with imazapyr mortality was three times higher than those treated with glyphosate. This result is consistent with previous studies in which imazapyr achieved higher mortality of invasive woody plants than other herbicides⁽³⁹⁾, including glyphosate⁽⁴¹⁾. In contrast, studies on *Ailanthus altissima* found a similar response among injection of imazapyr, glyphosate and triclopyr capsules, which could indicate a species-specific response to herbicides⁽⁴⁸⁾.

Several studies, where the manufacturer's recommended (E-Z-Ject lance, Odom Processing Engineering Consulting, Inc., Waynesboro, Mississippi) dosage was applied, indicate that the injection of glyphosate capsules is not effective for controlling various tree genera such as *Populus*, *Betula*, and *Ilex*, due to the low mortality rates observed⁽⁴⁹⁾⁽⁵⁰⁾. The results of the present study align with these findings, as the glyphosate-treated trees exhibited a low mortality rate, slightly exceeding 20%. However, a previous study in a riparian forest of a protected area in Uruguay recorded a 66% mortality of *G. triacanthos* adults treated with glyphosate⁽³⁰⁾. It should be noted that in that study the technique was different from the present study, applying herbicide by drilling the trunk with a drill and subsequent application of 50% glyphosate⁽³⁰⁾.

The injection of herbicide capsules using a lance generates less environmental impact than other IAS control methods used in the RPG region. The metal capsules safely store the herbicide in a gel solution, releasing it gradually and in contact with the vascular tissue of the plant, avoiding herbicide drift⁽³⁹⁾⁽⁴¹⁾. In addition, the lance control method uses a lower herbicide dose per tree than other IAS control methods, such as herbicide cutting and stump painting, stem banding and spraying, or stem drilling and herbicide application⁽³⁴⁾⁽³⁶⁾. On the other hand, the loss of herbicide through capsules injected into the trees was negligible. However, some studies have indicated that herbicide injected via capsules can secondarily be transmitted to non-target species through connections in the root system⁽³⁹⁾⁽⁴⁸⁾. Here, the treatments were carried out in a forest stand dominated by *G. triacanthos* with the presence of a few native trees. Throughout a monitoring period of 12 months, no damage (i.e. discoloration, defoliation, mortality) was observed in any of the native trees adjacent to the *G. triacanthos* herbicide treated individuals. Additionally, no damage was observed in *G. triacanthos* trees that were not treated, even when located in proximity to treated trees.

The absence of response to herbicide application in the first evaluation dates could be explained by the fact that the product is slowly released from the capsule solution into the cells of the vascular system, causing the vascular system to be progressively affected⁽³⁸⁾⁽³⁹⁾. However, in those trees treated with imazapyr capsules, there was a significant increase in mortality in the spring following the start of the experiment. This increase is explained by the fact that this herbicide is rapidly transported through the plant, both via xylem and phloem, accumulating in the meristematic regions, and consequently affecting sprouting⁽⁵¹⁾. Likewise, the herbicide is transported to subterranean storage organs, causing branch and root death⁽⁵¹⁾⁽⁵²⁾. In deciduous species, such as *G. triacanthos*, the mobilization of photoassimilates towards the roots occurs in late autumn, transporting the herbicide and causing the death of root tissues⁽³⁸⁾⁽⁵³⁾. This effect becomes evident in the budding months during spring, which explains the sharp increase in mortality observed in this work.

Gleditsia triacanthos is a species with a great invasion capacity given its characteristics of high fruiting, dispersal, germination, clonal reproduction, and rapid growth⁽⁵⁴⁾⁽⁵⁵⁾⁽⁵⁶⁾. In this vein, it is also recognized that *G. triacanthos* has a high rate of resprout and compensates for damage, particularly when control is carried out without the use of herbicides or in insufficient herbicides doses⁽³⁴⁾⁽⁵⁷⁾. The high survival of glyphosate-treated trees (79%) and the moderate survival of imazapyr-treated trees (40%) can be a product of the applied dose, which was one-third lower than the recommendation of the manufacturer of the E-Z-Ject lance, because the study takes place in a HCV area. It is necessary to increase the number of studies that analyze dose-response curves to ensure the efficiency of control of this invasive species in different sites with different herbicides⁽³⁴⁾. In addition, dead individuals should be monitored for at least two years to confirm that they do not resprout, attending the background in this matter⁽³⁴⁾.

When asked about the costs of IAS management and control, the company that manages the HCV area reports that the cost of *G. triacanthos* control with traditional methods (mechanical, drilling and banding) varies within a range of 35 USD to 575 USD per hectare (conversation with Ivan Grela; unreferenced). The cost per hectare of application with a lance falls within this range (93 USD). However, while there would be no net cost reduction, the speed of application of the lance is greater, covering larger areas. In addition, the active ingredi-

ent is captured in the metal capsule, reducing the probability of content loss, as opposed to drilling, where the hole remains open. On the other hand, the drill for trunk drilling is subject to the autonomy of the batteries and the lance only needs the impact provided by the force of the operator.

In this work, we evaluated the efficiency of two herbicides, injected by capsules with a lance, to control *G. triacanthos* individuals during the first year after application. Trees injected with capsules of both imazapyr and glyphosate significantly increase tree discoloration, defoliation and mortality. The results found here constitute the first record of the use of herbicide capsule application by lance on this IAS. Future studies should focus on extending the monitoring period of treated trees for at least two years and replicating it on invaded forests in other locations. This could be useful for obtaining more robust conclusions about the effect of herbicides on tree mortality, taking into account the possibility of resprouting of *G. triacanthos*. In addition, the effectiveness of mortality of trees could be evaluated according to different doses, in order to adjust a dose that maximizes the mortality of *G. triacanthos* trees.

5. Conclusions

The injection of imazapyr capsules with lance in *G. triacanthos* trees generated a significant increase in the mortality of treated individuals, which makes this technique a new alternative for the control of this invasive species. The imazapyr capsules not only generated a higher mortality of individuals than the glyphosate capsules, but also greater defoliation and discoloration of the foliage of *G. triacanthos* trees were observed. Based on the results of this work, glyphosate capsules are not recommended for the control of *G. triacanthos*, because low mortality of individuals was observed.

The study also confirmed that the lance application method is a viable and efficient alternative to traditional herbicide application methods. It minimizes environmental risks by reducing herbicide drift and allows for precise dosing. Given that no adverse effects were observed on adjacent native trees during the 12-month monitoring period, the method appears to be environmentally safe for use in high conservation value areas. However, the results also indicate that optimal herbicide dosing needs to be further evaluated, particularly in regions with varying ecological conditions. Future studies should focus on extending the monitoring period to assess potential long-term effects, including the risk of resprouting, and refine herbicide application strategies for enhanced control of *G. triacanthos* and other IAS across different landscapes.

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Transparency of Data

Available data: The entire data set that supports the results of this study was published in the article itself.

Author contribution statement

	Rizzo I	Baietto A	Hirigoyen A	Toranza C
Conceptualization				
Formal analysis				
Investigation				
Writing – original draft				

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