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SURVIVAL TIME AND EFFECTIVENESS OF GIRDLING AND HERBICIDE METHODS IN THE CONTROL OF *Pinus elliotti*: A CASE STUDY IN SECONDARY ATLANTIC FOREST IN SOUTHEASTERN BRAZIL[1]

TEMPO DE SOBREVIVÊNCIA E EFETIVIDADE DOS MÉTODOS DE ANELAMENTO E HERBICIDA NO CONTROLE DE *Pinus elliotti*: ESTUDO DE CASO EM FLORESTA ATLÂNTICA SECUNDÁRIA NO SUDESTE DO BRASIL[1]

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ABSTRACT - *Pinus elliottii* is a notorious invasive exotic species in grassland ecosystems; however, it can also establish itself in forest ecosystems during secondary succession. In this case, killing invasive standing trees is preferable to cutting, as it reduces damage to surrounding native vegetation. This study evaluates the survival time and effectiveness of two methods for killing standing *Pinus elliottii* trees in a regenerating forest ecosystem: girdling (a 50 cm-wide ring, followed by scarification of the wound surface) and herbicide (a 30% glyphosate solution injected into three perforations in the stem). The application times of the treatments were recorded for each tree individually. Tree survival times and rates were monitored over 24 months and eight days and compared using survival analysis models and binomial test, respectively. Girdling resulted in longer survival times, but survival rates did not differ significantly between the two methods. All trees in the control group remained alive during the monitoring period. The average time per tree for girdling application was 2.3 minutes longer, resulting in a higher labor cost. However, this additional cost was less than the cost of purchasing the herbicide. Our data indicate that girdling, although resulting in longer survival time, is more effective than herbicide in killing invasive *Pinus elliottii* trees under the soil and climate conditions of this experiment.

Keywords: Adaptive management, Biological invasion, Ecological restoration, Protected areas, Silviculture.

RESUMO - *Pinus elliottii* é uma notória espécie exótica invasora de ecossistemas campestres, contudo, pode se estabelecer também em ecossistemas florestais durante a sucessão secundária. Neste caso, a morte das árvores invasoras em pé é preferível ao corte, pois reduz os danos à vegetação nativa circundante. O presente estudo avalia o tempo de sobrevivência e a efetividade de dois métodos para matar árvores em pé de *Pinus elliottii* em um ecossistema florestal em regeneração: anelamento (anel de 50 cm de largura, seguido de escarificação da superfície da ferida) e herbicida (solução de glifosato a 30% injetada em três perfurações no caule). Os tempos de aplicação dos tratamentos foram registrados para cada árvore individualmente. Os tempos e taxas de sobrevivência das árvores foram monitorados ao longo de 24 meses e oito dias e comparados com o uso de modelos de análise de sobrevivência e teste binomial, respectivamente. O anelamento resultou em tempos de sobrevivência mais longos, mas as taxas de sobrevivência não diferiram significativamente entre os dois métodos. Todas as árvores do grupo controle permaneceram vivas durante o período de monitoramento. O tempo médio por árvore para aplicação do anelamento foi 2,3 minutos maior, resultando em um maior custo de mão de obra. Porém, esse custo adicional foi menor do que o custo da compra do herbicida. Nossos dados indicam que o anelamento, embora resulte em maior tempo de sobrevivência, é mais efetivo que o herbicida para matar árvores invasoras de *Pinus elliottii*, nas condições de solo e clima deste experimento.

Palavras-chave: Manejo adaptativo, Invasão biológica, Restauração ecológica, Silvicultura, Unidades de conservação.

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1 INTRODUCTION

Over the last decades, there has been a global growing concern regarding biodiversity loss and ecosystemic service deterioration due to biological invasions (Wilcove et al. 1998, Simberloff et al. 2010, Richardson and Rejmánek 2011, Adelino et al. 2021). The genus *Pinus* engulfs some of the plant species with the highest invasive potential and comprehensive use in forest production, worldwide (Richardson and Rejmánek 2011), and in Brazil (Ziller 2000; Abreu and Durigan 2011). Environmental sustainability of *Pinus* cultivation depends upon controlling biological invasion of natural ecosystems surrounding plantations. Amongst the ten main species of *Pinus* cultivated in Brazil, *Pinus elliottii* Engelm points out with the highest biological invasion potential (Santos et al. 2021). Invasion affects natural ecosystems both in Cerrado (Zanchetta and Pinheiro 2007, Abreu and Durigan 2011), as well as in the natural regeneration of the Atlantic Forest (Carvalho et al. 2014, Ramos et al. 2019), which are two biodiversity hotspots (Myers et al. 2000).

Studies aiming at evaluating methods to kill invasive *Pinus elliottii* trees in savanna ecosystems in the Cerrado have highlighted the economic and ecological viability of felling and removing invasive individuals in case of massive invasion (Abreu 2013). However, when the situation involves scattered invasive trees, especially in regenerating forest ecosystems, felling may not be the most appropriate management strategy, due to damage resulting from trees falling onto the native surrounding vegetation (Bitencourt and Pivello 2013). Alternative methods emphasize killing standing trees, as it reduces damage to the surrounding vegetation (Lazzaro et al. 2019). Two of these methods include girdling (Dechoum and Ziller 2013, Gonçalves et al. 2015, Tavares et al. 2017), and herbicide injection into stem perforations (Abreu 2013, Lazzaro et al. 2019). However, there is not enough evaluation on the application of such methods to invasive *Pinus elliottii* trees within regenerating forest ecosystems. Effectiveness studies involving other species have evidenced that invasive trees treated either with girdling or herbicide can survive treatment (Gonçalves et al. 2015, Merceron et al. 2016, Tavares et al. 2017).

As *Pinus elliottii* stores non-structural organic compounds in its roots (Mims et al. 2018, Du et al. 2022), one might hypothesize that these reserves may help the girdled plants cope with the interruption of photoassimilates supply to the roots, due to disruption of the phloem continuum, increasing its survival time. If the ring is not too wide, this time may be enough to allow vascular reconnection between the upper and lower edges of the ring, resulting in failure of girdling to kill the plant. Agreeing with this reasoning, Durigan et al. (2020) recommend that girdling effectiveness should be evaluated for a minimum of 12 months, and that rings should be, at least, 40 cm wide. On the other hand, herbicide application aiming at controlling invasive species requires careful evaluation of the cost/benefit ratio, since herbicide type and application manner influence effectiveness (Dechoum and Ziller 2013, Gonçalves et al. 2015, Tavares et al. 2017), and damage caused by chemical compounds to local biota is worldwide recognized (e.g., Gunstone et al. 2021). In the case of *Pinus elliottii*, the limited available information supports: a) effectiveness of glyphosate injection into stem perforations undergoing bark towards marrow, and b) increasing dosages of the herbicide in relation to diameter as stems grow (Abreu 2013).

With a monitoring period of more than two years, we aimed at evaluating survival time and effectiveness of two methods: girdling with a 50 cm-wide ring (henceforth, mentioned as girdling) and glyphosate injection into stem perforations with increasing dosages as stem diameter grow (henceforth, mentioned as herbicide), to kill standing invasive trees of *Pinus elliottii* scattered in a regenerating forest ecosystem. Two hypotheses have been devised: 1) girdling leads to a longer survival period when compared to herbicide, and 2) survival rates do not significantly differ between both methods after the monitoring period is over.

2 MATERIAL AND METHODS

2.1 Study area

Our study was carried out at *Paranapanema Ecological Station* (PEcS), a 640-ha legally Protected Area located in the Paranapanema municipality, southwestern Sao Paulo State, Brazil (23º32’02’’S and 48º45’29’’W, with an altitude of 630 m above sea level). Regional climate is Cfa, with dry winters and monthly average temperatures of 22º C (maximum) and 18º C (minimum) (Ventura et al. 1965). The main soil class found at PEcS is Oxisols (Soil Survey Staff 1999; Novais et al. 2010). The natural vegetation can be classified as Seasonal Semideciduous Forest (a subtype of Atlantic Forest) in different successional stages (Cielo-Filho et al. 2017).

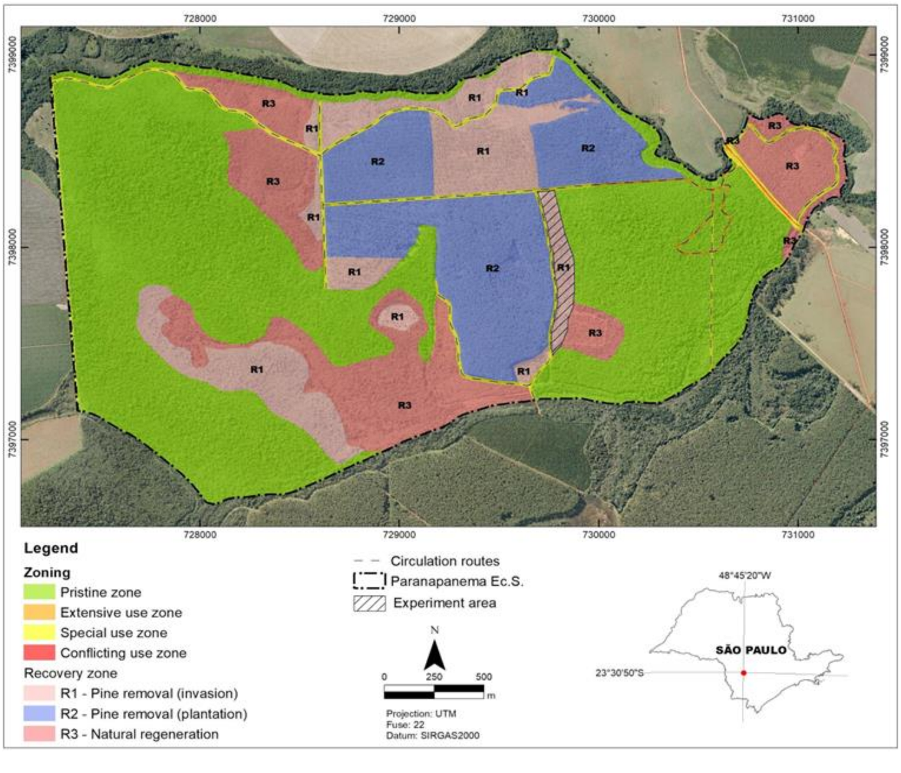
Originally, the area that PEcS occupies nowadays belonged to the neighboring Paranapanema State Forest (PSF), where *Pinus elliottii* stands were planted in the 1960s. PSF is a category of protected area in the Brazilian environment legislation that allows implementing experimental plantations of economically significant forest species. That means the exotic species had been cultivated in part of the current territory belonging to the PEcS until 2020, when *Pinus elliottii* stands were clear-cut based on the Management Plan recommendations for the protect area (São Paulo 2018). The different PEcS management zones included in the Management Plan were defined, and the area corresponding to the old *Pinus elliottii* stands was mapped as a Recovery Zone R2 (Figure 1). In addition to clear-cutting the pine stands in R2, the Management Plan mandated the elimination of scattered individuals of *Pinus elliottii* existing in Recovery Zone R1 (Figure 1), which includes our experimental area, and where the land cover is currently characterized by secondary forests that have been invaded by the exotic species during the natural regeneration process (São Paulo 2018). According to reports from employees, the experimental area was previously used as a firebreak and storage area for machinery, implements, and timber; and there were no pine trees or native arboreal vegetation in that area before it was abandoned in the 1990s. This situation characterizes *Pinus elliottii* as an invasive species in the regenerating forest ecosystem studied.

The experimental area is located in the easternmost portion of R1 and adjacent to an area in the R2 where a *Pinus elliottii* stand was planted in the 1960s and clear-cut in 2020 (Figure 1). Local landscape is smoothly undulate, with well-drained Oxisols and Montane Semideciduous Forest vegetation (São Paulo 2018). It is a forest ecosystem undergoing a secondary succession process initiated in the 1990s. Currently, the forest canopy comprises native trees with an average height of 10 m, and scattered *Pinus elliottii* invasive adult trees.

Soil and phyto-physiognomic conditions, as well as disturbance events and invasive species density, are relatively uniform throughout the experimental area. Invasive *Pinus elliottti* trees vary in size (most of them with diameter at breast height >15 cm, often bigger than 30 cm) and derive from an ancient invasive process that occurred over decades, along with the secondary succession process of the native forest. Due to such characteristics, and to preserve the surrounding native vegetation, killing standing pine trees is required to control invasion.

Figure 1. Map of management zones at PEcS (São Paulo, 2018), indicating the experimental area location, comprising part of the easternmost portion of the Recovery Zone R1 (hatched area). The Recovery Zone R2 comprises the area formerly occupied with *Pinus elliottii* stands clear-cut from 2019 to 2020.

Figura 1. Mapa do zoneamento da Estação Ecológica de Paranapanema – EEcP, indicando a localização da área do experimento, na porção mais oriental da Zona de Recuperação R1 (área hachurada). A Zona de Recuperação R2 corresponde à área anteriormente ocupada por povoamentos de *Pinus elliottii* removidos entre 2019 e 2020.



2.2 Experimental design

To evaluate the effectiveness of girdling and herbicide methods aiming at controlling invasive *Pinus elliottii* trees within the regenerating forest ecosystem, a sample of ninety-six trees was used, comprising individuals with DBH (diameter at breast height) equal to or higher than 15 cm; only pine trees in good phytosanitary status were considered. The ninety-six invasive trees were distributed into three spatially separated groups; each group contained thirty-two trees and corresponded to one of the three treatments (girdling, herbicide, and control). The shortest distance between two groups was approximately 200 m. The application of different treatments in spatially isolated groups considered the relative uniformity of the experimental area, and aimed at excluding the hypothesis that the herbicide effects influence other treatments, as we know that glyphosate may be exuded through the target plant roots towards surrounding non-target individuals (Yamada and Castro 2007). We randomly assigned a treatment to each of the three groups. Numbered aluminum tags were fixed on the invasive ninety-six trees and their DBH (Diameter at Breast Height) was measured. In each group, the invasive trees were selected so that there were eight individuals in each of four DBH classes (Table 1).

Table 1. DBH classes of the trees considered in our study, perforation depth on stems (Perf. depth), and respective volume of the herbicide solution injected (Vol. sol.).

Tabela 1. Classes de Diâmetro à Altura do Peito (DBH) das árvores consideradas no presente estudo, profundidade da perfuração nos caules (Perf. depth), e respectivo volume de solução injetado (Vol. sol.).

DBH class	Perf. depth (cm)	Vol. sol. (30%) per tree (mL)	Vol. sol. (30%) per perforation (mL)
≥ 15 cm - 20 cm	15	21	7
> 20 cm - 25 cm	17	27	9
> 25 cm - 30 cm	20	36	12
> 30 cm	22	42	14

2.3 Treatment application

Treatments were applied in a hot and rainy season, a period of intense vegetative activity from November 22 to 24, 2021 (girdling), and from December 01 to 02, 2021 (herbicide). Machetes were used for girdling with the removal of inner and outer bark layers (periderm, phloem and vascular cambium) in a 50-cm wide ring located at 70 cm above ground level (Figure 2a). Machetes were also used for scarification of the wound surface after bark removal. This operation consisted of scraping the whole wood surface where bark was removed with vertical machete movements so that residues of the vascular cambium, which could restore vascular tissues, would be eliminated (Figures 2b-c).

The herbicide method was applied with the use of a combination and adaptation of procedures adopted by Abreu (2013) and Lazzaro et al. (2019). A glyphosate 30% solution in clean water dyed with violet gentian was used to increase operational safety and help to detect any splashing or leaking. Glyphosate is a systemic, non-selective herbicide of the chemical glycine group that acts due to the blocking of Enol-Pyruvyl-Shikimate-Phosphate Synthase, reducing photosynthetic efficiency and aromatic amino acids production within meristematic tissues, thus leading to growth interruption, tissue degradation and plant death (Oliveira Jr. et al. 2011).

The product used was the Original®Roundup Plus - N-(phosphonomethyl) glycine di-ammonium salt: 577.0 g/L (57.7% w/v); N-(phosphonomethyl) glycine acid equivalent: 480.0 g/L (48.0% m/v); other ingredients: 678.0 g/L (67.8% w/v). The solution was previously prepared using a beaker and taken to field inside an enteral nutrition flask (Figure 2d).

A 20-mL syringe was used to inject the herbicide into stem perforations made with a 12 V, 1.5 Ah portable electric drill at the height of 1 m above ground level (Figures 2e-g). Perforations (diameter = 1 cm) were made in variable depths so that we could accommodate the solution volume for each diameter class (Table 1), and followed the up-down direction through bark into marrow to store the solution. Three equidistant perforations of 120 degrees were made around the circumference in all treated trees. In each perforation, the solution volume varied according to the tree diameter, so injected volume was progressively larger as diameter increased (Table 1).

Perforations were sealed with 1.5-cm wooden billets, inserted under pressure with a hammer, after injections (Figure 2h). We previously evaluated perforation sealing using water dyed with violet gentian, without herbicide, and we concluded that sealing was efficient as it avoided any splashing and leaking of the injected liquid, even though the resin tends to flow quickly into the hole after perforation. Because of such a feature observed in *Pinus elliottti* wood, both herbicide injection and hole closure were conducted immediately after trees were perforated.

Our experiment was authorized by Núcleo de Acompanhamento de Projetos do Instituto de Pesquisas Ambientais (Project Monitoring Center at Environmental Research Institute) and by the PEcS managing board - Fundação Florestal (Forestry Foundation). Procedures involving herbicide are provided for in the PEcS Management Plan (São Paulo 2018) and in the Normative Instruction Number 7 from IBAMA (July 02, 2012). All procedures involved the use of personal protective equipment and respected the herbicide manufacturer's specifications.

2.4 Data collection

After applications, we monitored the ninety-six trees for a period of 24 months and eight days in ten evaluations, in different dates. All of the trees were checked in each evaluation, in the same day. Through visual inspection of both tree stem and crown (with aid of 10x50 binoculars), symptoms regularly used to diagnose damage in plants caused by mechanical and chemical nonliving factors were monitored (Green et al. 2011). Thus, we analyzed the loss of natural pigmentation of the needles and necrosis of the vascular tissue, both evidenced by brownish appearance. The progression of symptoms were classified as follows: (0) absence of browning of the needles; (1) browning of the needles in less than 50% of crown; (2) browning of the needles in more than 50%, but less than 100%, of crown; (3) browning of the needles in the whole crown; and, (4) plant death due to necrosis in the vascular system, as verified with bark incision (Figures 2i-j). Incisions to verify necrosis in the vascular system were carried out only after trees had been classified as class 3.

Naming symptoms progression classes 1 and 2 is relatively complex and involves a subjectivity component while going through visual inspection, without precise measurement tools, as transition between classes is gradual. On the other hand, naming classes 0, 3 and 4 (death event) is much simpler and more objective, as it derives from verifying absence of browning of the needles, browning of the needles in the whole crown and necrosis in the vascular system, respectively, conditions that are easy to detect visually. Due to subjectivity, interpersonal variation may occur specially while naming classes 1 and 2. In order to avoid them, the same person (the first author) monitored and classified all the trees.

The span of months between evaluations was adjusted throughout the monitoring period after we realized that changes on monitored attributes became slower as time passed by. Thus, we adjusted the interval of initial evaluations (one-two months) to four-five months during the final period. The experiment was terminated after a period of monitoring for 24 months and eight days, and symptoms progression and time to death data were used to assess treatments

In our study, we considered the effectiveness of each method as a combination of efficacy (death plant) and efficiency (death plant with the least possible effort). The effort required to apply each method was expressed in terms of the additional cost inherent in each: the girdling method has an additional cost due to the longer application time required (see Results); the additional cost of the herbicide method is due to the need to purchase the product. We measured the average application time of each treatment, timing treatment applications for each tree, individually, for both methods. We calculated the additional cost for the most time-demanding method (girdling - see Results) by multiplying the number of additional hours required for the application of the method over the rural worker’s labor hour cost. The additional cost of the herbicide method was considered to be the cost of acquiring the product. In both calculations, we considered the management effort for 1 ha of a forest ecosystem invaded with Pinus elliottii, being 178 adult trees per hectare (Ramos et al. 2019). These calculations included wage costs and herbicide prices in Brazil, quoted in commercial dollars on Nov. 25, 2025, i.e., R$ 1.00 = USD 5.38 (https://www.bcb.gov.br). Values were obtained on the internet, checked in the same day, and reported in Results.

Figure 2. Procedures adopted in the experiment: (a-c) tree submitted to girdling; (d) material used to prepare the herbicide solution; (e) perforation; (f) and (g) injection; (h) perforation closure; (i) and (j) death verification in trees submitted to girdling and herbicide, respectively. In 2i dead regenerative tissue originated from the upper edge of the ring can be observed.

Figura 2. Procedimentos adotados no experimento: (a-c) árvore submetida ao anelamento; (d) material utilizado para preparar a solução herbicida; (e) perfuração; (f) e (g) injeção; (h) fechamento da perfuração; (i) e (j) verificação da morte em árvores submetidas ao anelamento e ao herbicida, respectivamente. Em 2i, pode-se observar tecido regenerativo morto originado da borda superior do anel.

2.5 Data analysis

After the monitoring period, we evaluated symptoms progression data on a descriptive basis for a better understanding of how trees responded to girdling and herbicide. Then, we analyzed mortality data (time to death of each tree) by using three survival analysis models: Cox, Exponential, and Weibull (Crawley 2013). By doing it, we aimed at comparing survival times of both killing methods considering a possible interaction between treatment effects and tree diameters. The null hypothesis that survival times do not differ between both methods was tested. Analyses were done according to Crawley (2013).

Initially, a data bank called time-to-death data (Supplementary Table S1) including the amount of sixty-four trees submitted to girdling and herbicide was built with the following vectors: nmdeath - number of months elapsed till plant death, treatment (G for girdling, and H for herbicide), and dbh - tree diameter at breast height (cm). Some trees survived after the monitoring period in both treatments (seven survived girdling, and three survived herbicide). Those trees were also considered in the survival analyses and were censored with an additional vector (status) in the data bank: 1 for uncensored trees (death observed until the monitoring period), and 0 for censored trees (living at the end of the monitoring period). Survival data were represented with Kaplan-Meier curves.

Then, without considering assumptions on error distribution, we adjusted the non-parametric model of Cox proportional risks. Significant results for both treatment effect and interaction between treatment and diameter were found with this first analysis. Thus, two other parametric models based on two types of error distribution, exponential and Weitbull, were evaluated. These two models differ regarding risk of death assumed: a constant risk is assumed in the exponential model, but risk may increase or decrease along the monitoring period in the Weitbull model. We compared both parametric models using Anova, and the most complex model, Weitbull, was adopted due to the existence of significant difference found in the explanatory power. Based upon this model, we predicted the average survival times of trees for both killing treatments, i.e., the necessary span of time required for killing all trees with each method. Moreover, we also calculated the actual average times until death by considering the survival times of trees that died until the monitoring period.

With a binomial test (Crawley 2013), we compared the ratio of dead trees with girdling and herbicide at the end of the monitoring period. The null hypothesis, that the ratio of dead trees does not differ between both methods, was assessed. Besides these analyses, we also measured the time in minutes, per tree, required for application of girdling and herbicide methods (Supplementary Table S2), and compared the average application times between methods with the *t* test. Analyses were performed with the R language version 4.3.3 (R Core Team 2024) and the Survival package (Therneau 2024), at 0.05 significance level, codes available in Supplementary Table S3.

3 Results

Trees of the control treatment did not change throughout the monitoring period, except one individual, which was placed in class 2 during the eighth observation because of crown breakage, remaining so until the experiment was over (Table 2). Excepting that single tree (whose crown broke due to a windstorm), all other trees of the control were placed in symptom progression class 0, i.e., they remained in the same vegetative status observed when monitoring began. These results allowed us to rule out the hypothesis that mortality could have occurred due to other causes unrelated to girdling or the herbicide; therefore, these methods were compared exclusively with each other.

In both treatments, most trees were found dead as monitoring ended, and no tree was placed in symptom progression class 0 at the end of the period (Tables 3 and 4). However, it does not imply that identical effects were found in both methods. When we compare tables 3 and 4, we notice that girdling led to a longer survival time; trees submitted to herbicide died quicker. Such assumption is supported with the analysis of Kaplan-Meier survival curves; the curves show that the survival rate decreases more rapidly with the herbicide treatment, especially at the beginning of the monitoring period (Figure 3).

Table 2. Classification of trees (control treatment) throughout the monitoring period with ten evaluations in different dates. Assigning symptom progression class 4 indicates the death of the tree. See the text for definitions of the other classes.

Tabela 2. Classificação das árvores (tratamento controle) ao longo do período de monitoramento, com dez avaliações em datas diferentes. A atribuição da classe de progressão de sintomas 4 indica a morte da árvore. Consulte o texto para obter as definições das outras classes.

Date	Months	Class 0	Class 1	Class 2	Class 3	Class 4	Total
Dec. 27, 2021	1.17	32	0	0	0	0	32
Jan. 31, 2022	2.20	32	0	0	0	0	32
Mar. 15, 2022	3.63	32	0	0	0	0	32
May 27, 2022	6.07	32	0	0	0	0	32
Jul. 21, 2022	7.90	32	0	0	0	0	32
Oct. 05, 2022	10.43	32	0	0	0	0	32
Jan. 04, 2023	13.47	32	0	0	0	0	32
Mar. 28, 2023	16.23	31	0	1	0	0	32
Aug. 21, 2023	21.10	31	0	1	0	0	32
Jan. 03, 2024	25.60	31	0	1	0	0	32

Table 3. Classification of trees (girdling treatment) throughout the monitoring period with ten evaluations in different dates. Assigning symptom progression class 4 indicates the death of the tree. See the text for definitions of the other classes.

Tabela 3. Classificação das árvores (tratamento de anelamento) ao longo do período de monitoramento, com dez avaliações em datas diferentes. A atribuição da classe de progressão de sintomas 4 indica a morte da árvore. Consulte o texto para obter as definições das outras classes.

Data	Months	Class 0	Class 1	Class 2	Class 3	Class 4	Total
Dec. 27, 2021	1.17	32	0	0	0	0	32
Jan. 31, 2022	2.20	32	0	0	0	0	32
Mar. 15, 2022	3.63	7	25	0	0	0	32
May 27, 2022	6.07	7	25	0	0	0	32
Jul. 21, 2022	7.90	1	30	1	0	0	32
Oct. 05, 2022	10.43	1	26	5	0	0	32
Jan. 04, 2023	13.47	0	9	6	3	14	32
Mar. 28, 2023	16.23	0	8	4	2	18	32
Aug. 21, 2023	21.10	0	5	2	0	25	32
Jan. 03, 2024	25.60	0	5	2	0	25	32

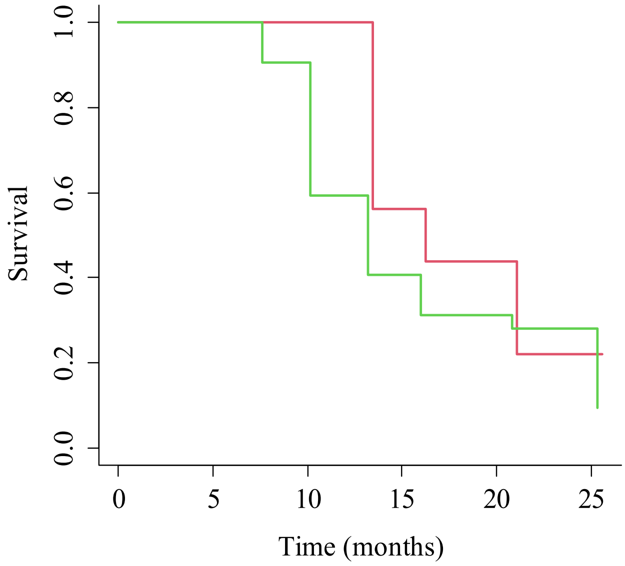
Table 4. Classification of trees (herbicide treatment) throughout the monitoring period with ten evaluations in different dates. Assigning symptom progression class 4 indicates the death of the tree. See the text for definitions of the other classes.

Tabela 4. Classificação das árvores (tratamento herbicida) ao longo do período de monitoramento, com dez avaliações em datas diferentes. A atribuição da classe de progressão de sintomas 4 indica a morte da árvore. Consulte o texto para obter as definições das outras classes.

Data	Months	Class 0	Class 1	Class 2	Class 3	Class 4	Total
Dec. 27, 2021	0.90	0	32	0	0	0	32
Jan. 31, 2022	1.93	0	8	18	6	0	32
Mar. 15, 2022	3.37	0	7	17	8	0	32
May 27, 2022	5.80	0	4	11	17	0	32
Jul. 21, 2022	7.63	0	4	8	17	3	32
Oct. 05, 2022	10.17	0	2	8	9	13	32
Jan. 04, 2023	13.20	0	2	4	7	19	32
Mar. 28, 2023	15.97	0	1	3	6	22	32
Aug. 21, 2023	20.83	0	1	1	7	23	32
Jan. 03, 2024	20.33	0	0	1	2	29	32

Figure 3. Kaplan-Meier Survival curves: red line for girdling, and green line for herbicide.

Figura 3. Curvas de sobrevivência de Kaplan-Meier: linha vermelha para anelamento e linha verde para herbicida.



In the next paragraphs, we present the results of statistical inference derived from comparisons between girdling and herbicide. The survival analysis using Cox proportional risk model has rejected the null hypothesis that survival times did not differ between girdling and herbicide (z = 2.71; *p-value* = 0.0067). That analysis also revealed the interaction between treatment and diameter (z = -2.23; *p-value* = 0.0257). Similar results were found with the survival analysis using Weitbull model (z = -2.79; *p-value* = 0.0052, for comparison between survival times, and z = 2.51; *p-value* = 0.0122, for interaction).

The survival analysis using the exponential model has neither revealed significant difference between survival times of both methods (z = -1.60; *p-value* = 0.11), nor has confirmed interaction (z = 1.36; *p-value* = 0.17). There was a statistically significant difference between Weitbull and exponential models in terms of explanatory power (p < 3.2e-14). Thus, our choice was a more complex model (Weitbull), which considers a variable risk of death and is, in our case, decreasing as time elapses, as shown with the scale parameter value lower than 1 (0.356).

Average times observed until death were 16.05 months for girdling, and 14.64 for herbicide. Average times forecasted with Weitbull were 21.12 months for girdling, and 18.46 months for herbicide. The survival time of trees submitted to girdling was higher than that of trees submitted to herbicide. However, the binomial test did not reject the null hypothesis that there is no difference in the ratio of dead trees between both methods at the end of monitoring (chi-squared test = 1.0667; gl = 1; *p-value* = 0.3017). Thus, the mortality rate obtained with girdling (78%) did not significantly differ from the mortality rate obtained with herbicide (91%).

In summary, results from our analyses have corroborated the hypotheses of the present study, i.e., girdling has led to longer survival times in comparison with herbicide, and the mortality rate did not differ significantly between both methods at the end of monitoring. In other words, both methods did not differ in terms of efficacy, although tree killing is faster with herbicide.

Regarding the efficiency of both methods, it is important to consider the application times of each treatment. The average application time per tree for girdling (9.8 minutes, DP = 3.44) was longer than that for herbicide (7.5 minutes, DP = 1.46). The difference between average times was statistically significant (t = -3.5507, df = 41.822, *p-value* = 0.00097).

In terms of application time, girdling was 2.3 minutes slower (average time per tree) than the herbicide. That means over 6.8 hours of additional working hours would be necessary to girdle trees within a 1-ha area, considering the plant density of invasive *Pinus elliottii* (178 individuals per hectare) as reported by Ramos et al. (2019), and assuming that these individuals would be homogeneously distributed into the four diameter classes considered in the present study. If we consider the average salary of R$ 2,091.00 gained by Brazilian rural workers in November, 2025 added with 68.18% of social and labor taxes (https://www.salario.com.br/profissao/trabalhador-rural-cbo-623110), the final monthly cost of a rural worker is (2,091 * 1.6818) / 5,38 = USD 653.65. Thus, by taking into consideration 176 working hours per month (44 hours weekly), the additional cost to girdle trees, per hectare, is (653.65 / 176) x 6.8 = USD 25.26.

Based on R$ 136.00 (USD 25.28) per liter of Roundup Original®Mais in November, 2025 (https://www.roundup-original-bayer.com.br/herbicidas), on the average herbicide volume per tree of (1,008 x 0.3) / 32 = 9.45 mL (based on Table 1), and on the average density of *Pinus elliottii* mentioned before, the additional cost per hectare for herbicide is 0.00945 x 178 x 25.28 = USD 42.52.

4 Discussion

The biological attributes of *Pinus elliottii* responsible for its invasive potential include continuous, massive seed dispersal with a high germination rate (Bechara et al. 2013). At PEcS, fragments of the Semideciduous Seasonal Forest were flanked by experimental plantations of *Pinus elliottii* for decades, so continuous and massive seed dispersal occurred. That propagule pressure was responsible for establishing scattered individuals of the invasive species within regenerating forest ecosystems surrounding the experimental plantations, as reported to other forest physiognomies, including the Riparian Forest (Ramos et al. 2019), and the Mixed Ombrophilous Forest (Zenni and Simberloff 2013). These conditions require constant monitoring and the adoption of control methods for the invasive species, especially within areas that focus on native biodiversity conservation and natural resources.

In order to contribute to the practice of invasion control, we have evaluated the effectiveness of girdling and herbicide with experiments aimed at killing scattered standing *Pinus elliottii* trees in a regenerating forest ecosystem. Girdling had been employed at PEcS tentatively in 2015, with the use of up to 30-cm wide rings and no wound surface scarification. However, that management failed in more than 50% of trees, and bark recovery was observed on the wound surface of surviving plants (Roque Cielo-Filho, personal observation). Merceron et al. (2016) reported that bark recovery, with subsequent re-establishment of phloem continuum and sap transportation onto roots, is the main cause of survival to girdling with 30-cm wide rings in the invasive species *Acer negundo* L., in forest ecosystems. Girdled trees that survived the treatment would not yet have exhausted non-structural carbohydrate reserves (NCR) in their roots, which were responsible for keeping root tissue cells alive until the bark recovered (Merceron et al. 2016).

Probably NCR in the roots may also be responsible for the survival of the seven *Pinus elliottii* girdled trees observed at the end of the monitoring period in our study. Although we do not know whether *Pinus elliottii* may store enough NCR inside roots to survive for long periods after the interruption of phloem continuum, one knows that those root reserves play a key role for the species metabolism. Thus, Mims et al. (2018) and Du et al. (2022) have reported the storage of NCR in both fine and coarse roots of adult *Pinus elliottii* trees, as well as depletion of such reserves during the growth season (summer) and their recovery during the physiological dormancy season (winter). This seasonal pattern of use and recovery of NCR in roots suggests that applying girdling at the end of the growth period, after reserve depletion, could have accelerated the death of plants in our study.

It is noteworthy that bark recovery was not observed in the seven girdled trees that survived until the monitoring period was over, although regenerative tissues similar to the column-like structures involved in bark recovery after girdling in *Pinus canariensis* reported by Chano et al. (2015) were observed on the wound surface in the present study. As occurred with *Pinus canariensis*, in our study these tissues originated from the upper edge of the rings and developed toward the lower edge (Figure 2i). However, we also realized that the ring width (50 cm) has hindered the reconnection of upper and lower ring edges and then, the re-establishment of phloem continuum in these girdled trees.

Besides a wide ring, we speculate that careful scarification procedure applied to the wound surface also helped to avoid bark recovery. Cambium zone generally comprises a thin (< 100 μm thick) cylinder of multi-layered meristematic cells (Wilczek-Ponce et al. 2021), so it is hard to tell whether cambial tissue is totally removed during girdling. The scarification procedure after girdling may have minimized the permanence of vascular cambium fragments on the wound surface, which otherwise could contribute to the re-establishment of the phloem continuum.

In addition to bark recovery, another cause of survival to girdling, as reported in literature, is regrowth observed on the stem region below the ring. This survival strategy after girdling has been recorded for the invasive Angiosperm trees *Neltuma juliflora* (Sw.) Raf. (Gonçalves et al. 2015) and *Acer negundo* (Merceron et al. 2016). The ability to regrow after damage is, in general, less frequent amongst Gymnosperms in comparison to Angiosperms, particularly in mature trees of the genus *Pinus,* although *Pinus elliottii* is one of the few species capable of regrowing at the basal portion after fire disturbance (Burrows 2021). We did not, however, observe regrowing of girdled individuals in our experiment. The capacity to regrow after damage and to survive after girdling depends on the species; thus, girdling should be evaluated for each invasive tree species, individually (Merceron et al. 2016).

As in girdling, herbicide application procedures were crucial for our results. Other studies have assessed glyphosate injection into stem perforations with progressively higher doses of the herbicide as plant dimensions increase (Abreu 2013, Lazzaro et al. 2019); that procedure, such as ours, has proved to be appropriate. Thus, a positive correlation between time until death/censorship and DBH of trees submitted to herbicide has been observed ($r = 0.3543$; GL = 30; *p-value* = 0.0466). That correlation has resulted in a significant interaction between treatment and DBH in survival analyses. Bigger trees, then, survive for longer periods, even with higher doses of the herbicide. If dosage had not been increased for larger trees, the method efficacy would have probably been lower.

It should be noted that the correlation between time to death/censorship and BDH was not observed for girdled trees ($r = -0.0751$; GL = 30; *p-value* = 0.6827). Such results point out differences in survival mechanisms for both girdling and herbicide methods. As we have already discussed, survival to girdling is probably related to utilization of NCR in roots. The quantity of root reserves necessary for an adult girdled tree to survive for a certain period might be proportional to its size; thus, large, and small trees may have the same survival potential to girdling. On the other hand, survival to herbicide depends upon the plant size (Vila-Aiub et al. 2018). As size increases, the ratio *active ingredient:biomass unit* decreases; this process is called 'size-driven herbicide dilution' and states that higher quantities of glyphosate are necessary to obtain the same effect on larger plants, in comparison to smaller ones (Vila-Aiub et al. 2018).

In general, studies on invasive plant control have reported a higher level of efficacy achieved with chemical than with manual methods (e.g.: Dechoum and Ziller 2013, Gonçalves et al. 2015, Knapp et al. 2023). According to Merceron et al. (2016), though, girdling may be an effective method if rings are re-made on trees with bark recovery. Besides that, these authors have mentioned that girdling is an ecological friendly method as it allows controlling invasion in more sensitive environments, such as riparian forests. Our results have matched those found by Merceron et al. (2016) and suggest that girdling can have the same efficacy as herbicide. Our hypothesis has been reinforced, that is, girdling results in a longer survival time compared to that of the herbicide, although mortality rates at the end of monitoring did not differ significantly between both methods. Furthermore, unlike what was reported by Merceron et al. (2016), we did not verify bark recovery on girdled trees and, thus, rings were not re-made. Besides species-specific reasons, that probably occurred due to a larger ring (50 cm) and wound surface scarification.

Girdling required a longer average application time, per tree. It does not mean, however, that girdling is less efficient than the herbicide. When comparing additional application costs, one can see that the additional cost per hectare resulting from the necessary time to girdling (USD 25.26) was lower than the additional cost per hectare to apply glyphosate considering its input cost (USD 42.52). If we consider that neither method differed significantly in efficacy, we can conclude that, if other costs involved (besides those evaluated in our study) do not differ significantly between both methods, girdling is more efficient and, thus, more effective than herbicide.

5 Conclusions

When comparing the killing methods of standing *Pinus elliottii* invasive trees, girdling was more effective than the herbicide; efficacy was not different between both, but efficiency was higher with girdling. Nevertheless, girdling requires longer periods to kill invasive trees, thus we need more time to evaluate its effectiveness. We could not observe bark recovery on girdled trees with the adoption of 50-cm wide rings and wound surface scarification procedures. Based on available literature information, and on the above referred observations prior to the current study, we suggest that such procedures were essential to obtain success with girdling.

Although we did not evaluate the effect of seasonality on girdling, literature information suggest that this method might be more effective if applied at the end of summer (growth season) when NCR are at the lowest inside roots. We conclude that, under the soil and climate conditions analyzed, girdling is preferable to herbicide as it is more effective and may prevent environments from being chemically contaminated. However, when we consider the fact that environmental variables may interfere in girdling performance (Gonçalves et al. 2015, Merceron et al. 2016), we emphasize that girdling should be evaluated for other invasive *Pinus elliottii* populations under diverse soil and climate contexts before its application can be widely recommended. Our study has generated information that impacts the practice of controlling *Pinus elliottii* within natural areas where the species is invasive, and our data may contribute to guide management strategies, taking into account various ecological, logistic, and financial aspects involved.

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7 Credit authorship contribution statement

Roque Cielo-Filho: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Resources, Validation, Writing - original draft, Writing - review & editing. Maria Teresa Zugliani Toniato: Data curation, Investigation, Validation, Roles/Writing - review & editing. Edgar Fernando de Luca: Investigation, Resources, Validation, Writing - review & editing.

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