

Article

Chemical Control of the Invasive Tree *Ailanthus altissima*

Jordi Soler * and Jordi Izquierdo 

Department of Agri-Food Engineering and Biotechnology, Universitat Politècnica de Catalunya, 08860 Castelldefels, Spain; jordi.izquierdo@upc.edu

* Correspondence: soleraaa@hotmail.com; Tel.: +34-689460905

Abstract: Many natural areas are colonised by the invasive species *Ailanthus altissima* (Mill.) Swingle. Its presence in natural ecosystems damages the ecological richness while competing with native flora. *A. altissima* is one of the most widespread weed species in natural areas of temperate regions such as conservation parks, archaeological sites and communication corridors. Not many active ingredients are available to control this weed since the most popular, glyphosate, has been banned by many municipalities. To test the efficacy of alternative herbicides, naturally occurring populations in Collserola Conservation Park in Barcelona (Catalonia, Spain) were treated with different herbicides using three different techniques. Aclonifen, metribuzin, flazasulfuron, metsulfuron-methyl, fluroxypyr, isoxaflutole + thiencazone-methyl and triclopyr mixed with 2,4-D, fluroxypyr, aminopyralid and clopyralid were applied by stem injection, cut stump injection or basal bark techniques to trees of about 5 cm diameter. Cut stump and stem injection both gave almost total control of the trees while basal bark showed more varied results depending on the herbicide. The best control was achieved when flazasulfuron or triclopyr were present as active ingredients and poorer control was observed when using metsulfuron-methyl or isoxaflutole + thiencazone-methyl. Aclonifen showed no damage to the trees. Metribuzin worked better if the cut stump injection technique was used. These results showed that several alternatives are available to the use of glyphosate, which has been banned for some uses due to environmental concerns.

Keywords: *Ailanthus altissima*; herbicide; weed control; invasive plant; fluroxypyr; triclopyr; flazasulfuron; metsulfuron-methyl; 2,4-D; aminopyralid; clopyralid; isoxaflutole; thiencazone-methyl; aclonifen; metribuzin



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1. Introduction

Ailanthus altissima (Mill.) Swingle is an invasive plant that occurs in many countries [1]. It is a dioecious species that belongs to the family Simaroubaceae and has both sexual and vegetative reproduction. Female trees can produce a large quantity of samara fruit-type each year [2], which can be transported by wind. Additionally, it has high capacity for root sprouting [3], with a well-developed root system that, once it is established, is very difficult to remove. Apparently separated individuals can be connected along 27 m by the same root system [4]. Its competitive behaviour is enhanced by the presence of quassinoid ailanthone, an allelopathic compound with herbicidal activity [5]. Considering these biological traits, a correct strategy should be well defined before carrying out an eradication plan to avoid further spread of the tree.

A. altissima is frequently found near transportation corridors [6] and in disturbed and urban areas [7,8]. Management of this weed is achieved using different methods such as biological, mechanical, chemical, or combinations of these [9–11]. Biological control can be achieved by injecting into the trunk a dilution of *Verticillium nonalfalfae* (Inderb., H.W. Platt, R.M. Bostock, R.M. Davis and K.V. Subbarao) [12] or with the Coleoptera *Eucryptorrhynchus brandti* (Harold) and *E. chinensis* (Olivier), which in China feed from natural populations of *A. altissima* trees [13] and in the United States have shown high specificity to *A. altissima* [14,15] and were capable of transmitting *V. nonalfalfae* in laboratory

conditions [16]. Mechanical control can be carried out by cutting or pulling out the tree and its roots [10,17,18], but cutting or disturbing the area promotes vegetative regrowth [19–21]. Mechanical control is frequently followed by chemical control with herbicide [10,20].

Chemical control by means of herbicides has been demonstrated to be more effective than mechanical control alone [11,17,22–24]. Chemical control can be carried out by means of different techniques such as stem injection, basal bark, cut stump or foliar spray. Stem injection is performed by either drilling the trunk and filling each hole with the herbicide [22], injecting solid capsules containing the herbicide into the trunk [25] or by hack-and-squirt, which consists in applying the herbicide into cuts made with a hacksaw [23]. Basal bark consists in applying herbicides over the lower part of the trunk [20]. Cut stump consists in either spraying, using a paintbrush or drilling the stump [11,20,26]. Foliar spray means spraying the herbicide over the aerial part [27].

The herbicides used in chemical control must have high phloem translocation to reach the root system and avoid plant root sprouting. Active ingredients such as glyphosate, 2,4-D, fluroxypyr, triclopyr, clopyralid, metsulfuron-methyl or flazasulfuron are systemic herbicides with phloem translocation [28,29]. Glyphosate has been widely used against *A. altissima* with mortalities ranging from 90% to total control [22,23,30]. Metsulfuron-methyl [31], aminopyralid + fluroxypyr [20] and triclopyr alone [17,23] or combined with fluroxypyr [20] are also used to control natural populations although they apparently do not allow total control. Injection of triclopyr capsules showed low mortality rates [32], but basal bark applications with an oil produced total mortality [17,24]. Nowadays, in certain locations triclopyr alone and glyphosate are restricted and some new commercial mixtures containing triclopyr seem not to produce complete mortality [20]. New trials are required to evaluate the efficacy of triclopyr mixtures. Fluroxypyr alone has been found to be effective for another root-suckering plant, *Harungana madagascariensis* Poiré [33], but no data are available for *A. altissima*. Moreover, in the literature no data were found for new active ingredients such as metribuzin, acifluorfen, isoxaflutole + thienacarbazone-methyl mixture, fluroxypyr or flazasulfuron.

As *A. altissima* is a typical weed of non-cropped land, potential environmental impacts should be considered when controlling natural populations with herbicides. For example, it has been reported that after basal bark applications of aminopyralid and triclopyr against *Prunus padus* L., soil residues of those herbicides originating from root exudates of treated plants were found potentially threatening the natural surrounding flora [34]. Similarly, triclopyr residues were detected in soil near treated areas after basal bark applications [35]. Glyphosate applications injected to *Leucaena leucocephala* (L.) L. Rico left soil residues that were considered to come from either root exudates or dead falling leaves [36]. Due to the potential existence of root exudates and the persistence of the active ingredients in the soil, the rate of degradation in the soil should be considered to avoid damage to the environment or to non-target plants [34].

As mentioned above, the restrictions on the use of active ingredients such as triclopyr and glyphosate by some municipalities [37] mean there is a demand to find new, effective herbicides. New candidates must have a high phloem transport rate and be applied with the most effective technique. Moreover, the high sprout capacity of *A. altissima* requires a long-term follow-up of the results of the application, since some studies found that one year later plant density did not decrease [10] or even increased [11,20] compared to the starting point. This study has been able to check the mortality of the trees between two and up to five years after treatment (from 2019 to 2024).

The objectives of this study were, therefore, to (i) find the best season for the treatment (autumn or summer), (ii) find the most efficient herbicide application technique (stem injection, cut stump injection or basal bark) and (iii) perform long-term follow-up testing of the efficacy of different herbicides.

2. Materials and Methods

2.1. Study Site

The study was carried out from 2019 to 2022 in the southern slope of the Conservation Park of Serra de Collserola in Catalonia in Northeastern Spain (Figure 1a), northwest of Barcelona (Figure 1b). The park covers 8300 hectares, with *A. altissima* being present in 40 hectares. This metropolitan park has detected several invasive species, such as *Arundo donax* L., *Agave americana* L., *Opuntia ficus-indica* (L.) Miller, *Pennisetum villosum* Johnston, *Robinia pseudoacacia* Poiret and *A. altissima*, the latter being highly spread. Its proximity to the city of Barcelona favours biological invasions of species escaped from private gardens, and transport corridors that cross this forest favour their dispersion. A factor that facilitates the spread of *A. altissima* is the continuous maintenance of roads and railways by mechanical weed control, which promotes resprouting. The area has Mediterranean climate conditions with a mean annual temperature of 15.5 °C and an average of 553 mm of precipitation during the past nine years [38]. Climate conditions during the study are shown in Figure 2.

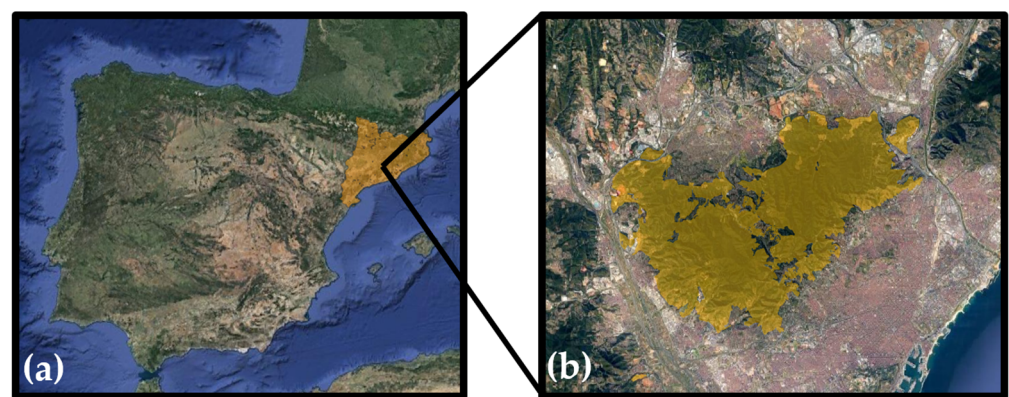


Figure 1. Location of Catalonia (a) and the Conservation Park of Serra de Collserola (b), where the experimental plots were set up.

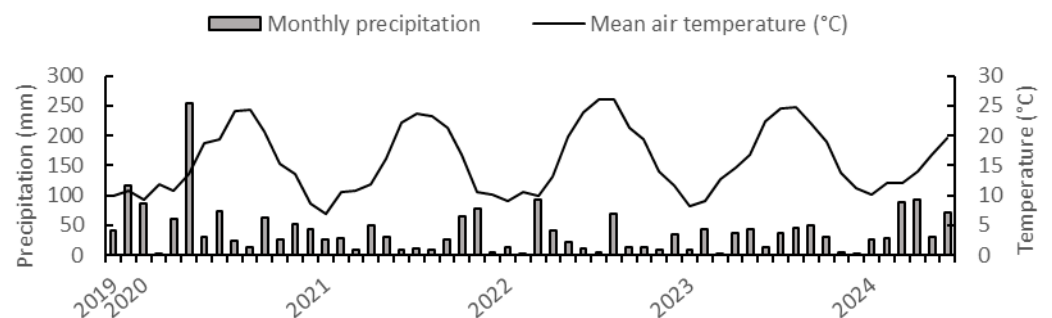


Figure 2. Monthly mean air temperature and precipitation during the experiments, from November 2019 until June 2024, in Barcelona (Catalonia, Spain).

2.2. Experimental Design and Sampling

The area with the experimental plots was located at around 270 m above sea level. Each plot comprised an area of approximately 2 m × 2 m with 10 trees of 5 cm diameter measured at 1 m above ground level. Herbicide treatments were applied at different times: November 2019, June and September 2020 and October 2021 and 2022. Each treatment had three replicates, and plots were separated at least 10 m to avoid cross-mortality. The herbicides, techniques and doses are shown in Table 1. The trial in 2020 aimed to determine if autumn applications were better than summer ones to control the tree, as suggested by the literature [20,39]. Based on the results obtained, all the subsequent experiments were conducted in autumn.

Table 1. Summary of the treatments: the application technique, the season and year, the active ingredients of the herbicide with the commercial name and brand in brackets, the mode of action according to HRAC *, the rate of dilution of the herbicide in water v/v herbicide/water and the amount of active ingredient applied per tree (AI). * HRAC: Herbicide Resistance Action Committee. Manufacturers: Corteva, Sevilla, Spain; UPL, Liege, Belgium; Ascenza, Paterna, Spain; Nufarm, Barcelona, Spain; Bayer, Lyon, France.

Technique	Season	Year	Active Ingredient (Commercial Name, Manufacturer); Mode of Action *	Rate v/v (%)	AI (mL or g) tree ⁻¹
Basal bark	autumn	2021	Clopyralid 60 g L ⁻¹ + triclopyr 240 g L ⁻¹ (Silvanet, Corteva); 4	80	0.5 + 1.9
			Fluroxypyr 200 g L ⁻¹ (Starane 20, Corteva); 4	75	1.5
			Triclopyr 240 g L ⁻¹ + aminopyralid 30 g L ⁻¹ (Tordon Star, Corteva); 4 + 4	60	1.4 + 0.2
		2022	2.4-D 93 g L ⁻¹ + triclopyr 103.6 g L ⁻¹ (Genozone zx, UPL); 4 + 4	75	0.7 + 0.78
			Flazasulfuron 250 g kg ⁻¹ (Register 25, Ascenza); 2	56	1.40
			Isoxaflutole 225 g L ⁻¹ + thiencazone-methyl 90 g L ⁻¹ (Adengo, Bayer); 27 + 2	75	1.69 + 0.68
			Metsulfuron-methyl 200 g kg ⁻¹ (Racing, Nufarm); 2	10	0.2
Cut stump injection	autumn	2021	Clopyralid 60 g L ⁻¹ + triclopyr 240 g L ⁻¹ (Silvanet, Corteva); 4 + 4	80	0.5 + 1.9
			Fluroxypyr 200 g L ⁻¹ (Starane 20, Corteva); 4	75	1.5
			Metribuzin 600 g L ⁻¹ (Sencor Liquid, Bayer); 5	80	4.8
			Triclopyr 240 g L ⁻¹ + aminopyralid 30 g L ⁻¹ (Tordon Star, Corteva); 4 + 4	60	1.4 + 0.2
		2022	2.4-D 93 g L ⁻¹ + triclopyr 103.6 g L ⁻¹ (Genozone zx, UPL); 4 + 4	75	0.7 + 0.78
			Flazasulfuron 250 g kg ⁻¹ (Register 25, Ascenza); 2	56	1.40
			Isoxaflutole 225 g L ⁻¹ + thiencazone-methyl 90 g L ⁻¹ (Adengo, Bayer); 27 + 2	75	1.69 + 0.68
			Metsulfuron-methyl 200 g kg ⁻¹ (Racing, Nufarm); 2	10	0.2
		2019	Aclonifen 600 g L ⁻¹ (Challenge, Bayer); 32	40	2.3
			Metribuzin 600 g L ⁻¹ (Sencor Liquid, Bayer); 5	40	2.3
			Triclopyr 90 g L ⁻¹ + fluroxypyr 30 g L ⁻¹ (Garlon GS, Corteva); 4 + 4	1	0.009 + 0.003
				10	0.09 + 0.03
		2020	Fluroxypyr 200 g L ⁻¹ (Starane 20, Corteva); 4	50	0.7
				75	1.3
Stem injection	autumn	2021	2.4-D 93 g L ⁻¹ + triclopyr 103.6 g L ⁻¹ (Genozone zx, UPL); 4 + 4	75	0.7 + 0.78
			Clopyralid 60 g L ⁻¹ + triclopyr 240 g L ⁻¹ (Silvanet, Corteva); 4 + 4	80	0.5 + 1.9
			Fluroxypyr 200 g L ⁻¹ (Starane 20, Corteva); 4	75	1.5
			Metribuzin 600 g L ⁻¹ (Sencor Liquid, Bayer); 5	80	3.2
			Triclopyr 240 g L ⁻¹ + aminopyralid 30 g L ⁻¹ (Tordon Star, Corteva); 4 + 4	60	1.3 + 0.2
			Triclopyr 90 g L ⁻¹ + fluroxypyr 30 g L ⁻¹ (Garlon GS, Corteva); 4 + 4	60	0.54 + 0.18
		2022	2.4-D 93 g L ⁻¹ + triclopyr 103.6 g L ⁻¹ (Genozone zx, UPL); 4 + 4	75	0.7 + 0.78
			Flazasulfuron 250 g kg ⁻¹ (Register 25, Ascenza); 2	56	1.40
			Isoxaflutole 225 g L ⁻¹ + thiencazone-methyl 90 g L ⁻¹ (Adengo, Bayer); 27 + 2	75	1.69 + 0.68
			Metsulfuron-methyl 200 g kg ⁻¹ (Racing, Nufarm); 2	10	0.2
			Metribuzin 600 g L ⁻¹ (Sencor Liquid, Bayer); 5	80	4.8
			Triclopyr 90 g L ⁻¹ + fluroxypyr 30 g L ⁻¹ (Garlon GS, Corteva); 4 + 4	60	0.4 + 0.1
	summer	2020	Triclopyr 240 g L ⁻¹ + aminopyralid 30 g L ⁻¹ (Tordon Star, Corteva); 4 + 4	60	1.3 + 0.2
				80	1.7 + 0.2

The techniques used to apply the herbicides were stem injection, cut stump injection and basal bark applications. Stem injection consisted in making four holes in the stem of the plant at 4 cm above ground level with an 8 mm drill bit and injecting 2.5 mL per hole of the herbicide solution with a syringe. Cut stump injection consisted in cutting the plant at 4 cm above ground level and immediately after plant felling injecting 2.5 mL per hole of herbicide solution with a syringe in four holes made in the stump with an 8 mm drill bit. Basal bark consisted in painting with the herbicide solution the first 100 cm of the basal part

of the plant with a paintbrush. Control trees for each technique had the same procedures as described above applied, but water was applied instead of the herbicide solution.

Efficacy of the treatments was determined by monitoring three times per year the state of the tree (dead or alive), in spring (April), summer (June) and autumn (September), starting the observations the next season after the treatment and all ending in June 2024. A tree with a green bud in the stem or in the stump was considered alive.

2.3. Statistical Analyses

All statistical analyses were conducted using the SAS software, version 9.4 (SAS Institute Inc., Cary, NC, USA).

2.3.1. Season Efficacy

To determine the best season to carry out the treatments, in 2020, three herbicides (metribuzin, triclopyr + fluroxypyr and triclopyr + aminopyralid) were applied at the same rate with the same technique, stem injection, in summer (June) and autumn (October). These herbicides were selected to obtain data about their efficacy on *A. altissima*, because not much information is available regarding their use in *A. altissima* control. The mortality rate of the seasons was analysed using a generalized linear model (GLM) with a Tukey test to find significant differences ($p \leq 0.05$). Since the results showed that autumn treatments gave the best efficacy, all subsequent experiments were made that season.

2.3.2. Application Technique Efficacy

For the study of the most efficient technique, several herbicides were applied at the same rate using the three techniques in autumn: stem injection, cut stump injection and basal bark, simultaneously. The efficacy of the technique was analysed by conducting a GLM test. GLM allows working with values including 0 and 100% and considers the differences in data size contained in each group. To define significant differences, a Tukey test was conducted as a mean separation procedure ($p \leq 0.05$). Metribuzin was tested only with stem injection and cut stump injection techniques (see Table 1).

2.3.3. Herbicide Efficacy and Technique

The efficacy of the herbicides was evaluated in all experiments and analysed by conducting a GLM test on the mortality rate observed for autumn treatments considering the technique. To define significant differences, a Tukey test was carried out ($p \leq 0.05$). In a preliminary analysis of all data, year and repetitions were shown not to be significant, so these data were pooled in all analyses. Stem injection treatments in autumn 2019 and fluroxypyr (200 gL^{-1}) in autumn 2020 applied at 50% were not included in the GLM analyses because no other techniques were tested and no comparison between techniques was possible. However, the observed efficacies of those herbicides are reported in the Section 3.

3. Results

3.1. Best Season for Treatments

Significantly higher mortality ($p = 0.028$) was achieved in autumn (mean = 93.3%, SD = 16.6) compared to summer (mean = 75.3%, SD = 33.2), no matter the herbicide applied (Figure 3a). Triclopyr mixtures produced complete mortality in both seasons (mean = 100, SD = 0) although the mixture with fluroxypyr applied in summer did not reach 100% (mean = 91.6%, SD = 14.4). Metribuzin showed less efficacy than triclopyr mixtures, with efficacy being higher in autumn (mean = 80%, SD = 26.4) than in summer (mean = 34.3%, SD = 19.1). All herbicides showed significantly better efficacy than the untreated plots ($p \leq 0.05$) (Figure 3b). According to these results and the literature [20,30,40], all subsequent experiments and analyses were conducted with herbicides applied in autumn.

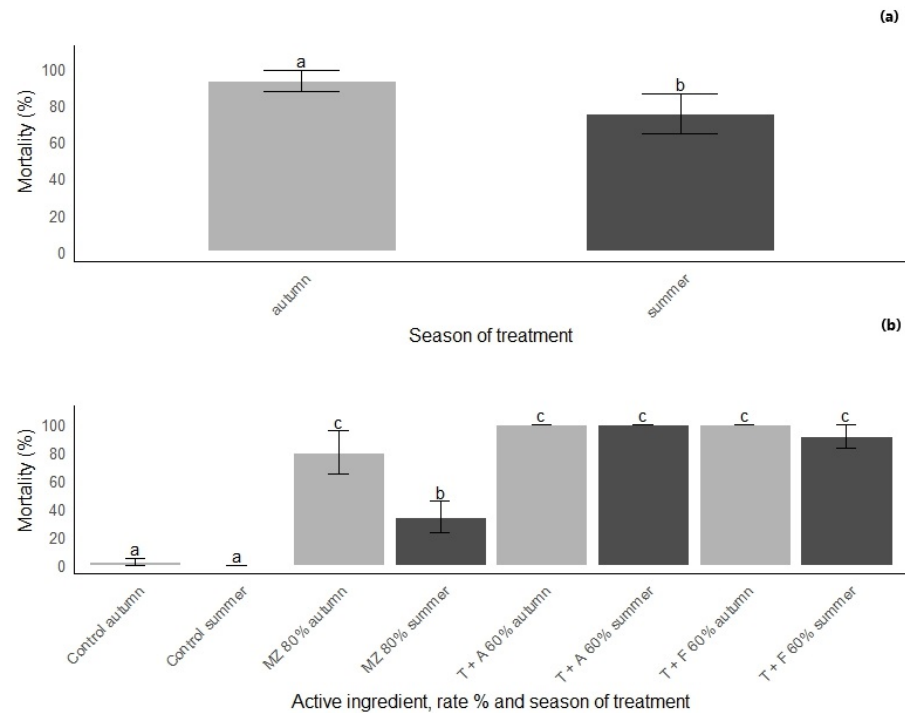


Figure 3. (a) Mortality (%) pooled by active ingredient and season at last count (June 2024), (b) Mortality separated by active ingredient and season, of *A. altissima* trees of 5 cm diameter treated in autumn (October 2021) and summer (June 2020) by stem injection with metribuzin (MZ), triclopyr + aminopyralid (T + A), and triclopyr + fluroxypyr (T + F). Values with the same letters are not significantly different according to the Tukey test ($p \leq 0.05$). Bars represent the standard errors of the means.

3.2. Application Technique

When comparing the efficacies of each technique using the same herbicide and rate, significant differences were observed between techniques ($p < 0.05$) and among the herbicides used ($p < 0.05$). Cut stump reached the best efficacy (mean = 100%, SD = 0) although it did not differ significantly from stem injection (mean = 95.4%, SD = 11.4). Basal bark showed the poorest efficacy (mean = 79%, SD = 35.6) (Figure 4). Interaction of herbicide and technique was significant ($p > 0.01$), mainly due to the absence of mortality produced by isoxaflutole + thienicarbazone-methyl applied with basal bark (mean = 0, SD = 0) (Figure 5).

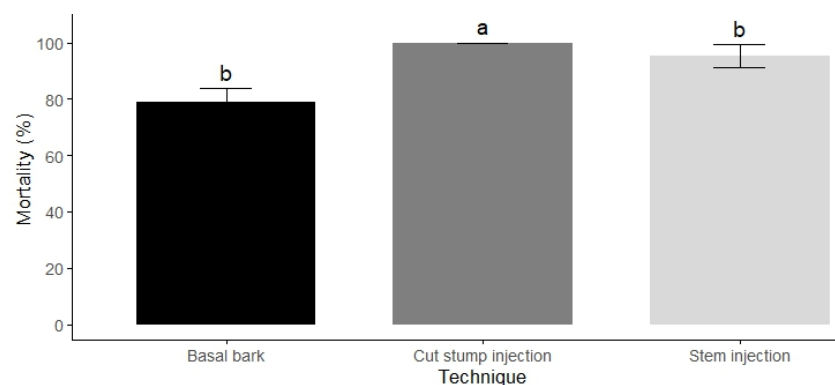


Figure 4. Mortality (%) at last count (June 2024) of *A. altissima* trees of 5 cm diameter according to the technique applied (basal bark, cut stump injection and stem injection) and treated during autumn (October) 2021 and 2022. Values with the same letters are not significantly different according to the Tukey test at $p \leq 0.05$. Bars represent the standard errors of the means.

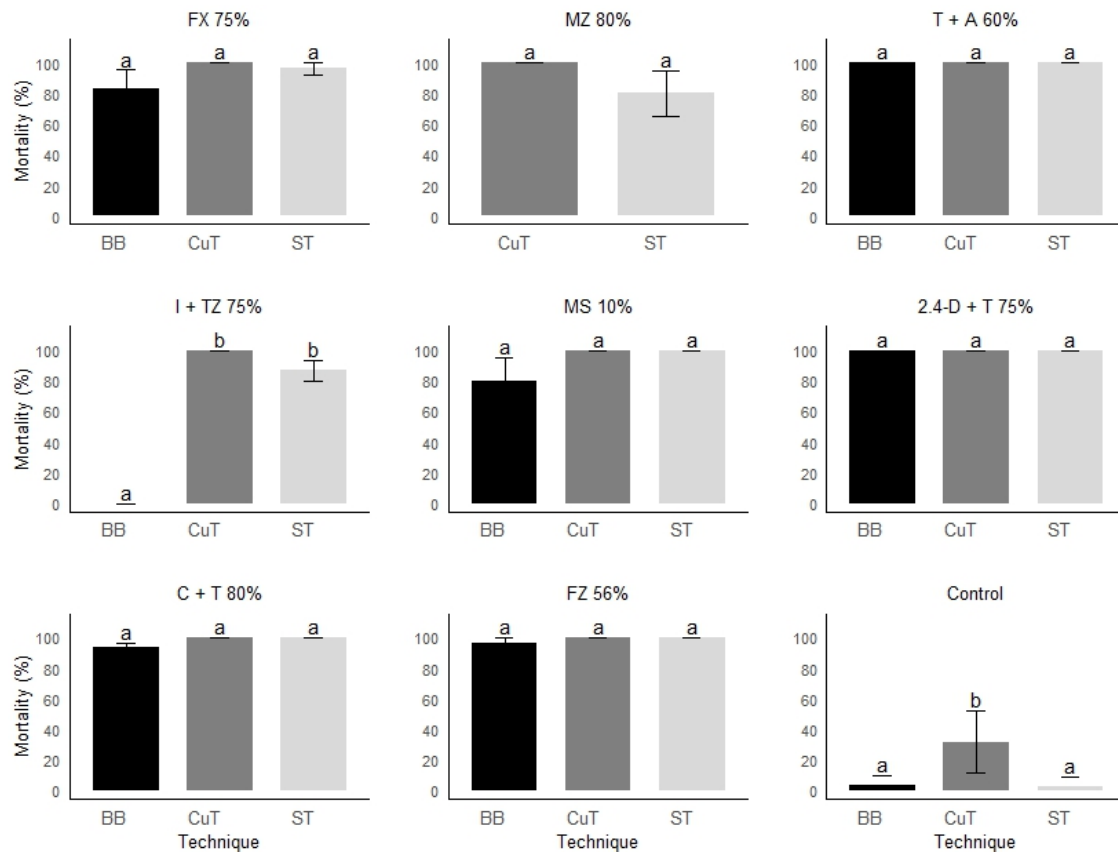


Figure 5. Mortality (%) at last count (June 2024) of *A. altissima* trees of 5 cm diameter treated with different techniques (BB—basal bark; CuT—cut stump injection; ST—stem injection) in autumn (October) 2021 and 2022. Values with the same letters are not significantly different according to the Tukey test at $p \leq 0.05$. Bars represent the standard errors of the means. FX (fluroxypyr 200 g L⁻¹); MZ (metribuzin 600 g L⁻¹); T + A (triclopyr 240 g L⁻¹ + aminopyralid 30 g L⁻¹); I + TZ (isoxaflutole 225 g L⁻¹ + thiencazuron-methyl 90 g L⁻¹); MS (metsulfuron-methyl 200 g kg⁻¹); 2.4-D + T (2.4-D 93 g L⁻¹ + triclopyr 103.6 g L⁻¹); C + T (clopyralid 60 g L⁻¹ + triclopyr 240 g L⁻¹); FZ (flazasulfuron 250 g kg⁻¹).

All herbicides showed similar high efficacy (near 100% mortality) when applied with the cut stump injection or stem injection techniques (Figure 5). Basal bark showed the poorest results. It must be highlighted that the metribuzin stem injection produced a high mortality despite its xylem translocation (mean = 80%, SD = 26.4). Some mortality in control trees was observed (mean = 31.6%, SD = 21.2) with cut stump injection likely due to the cut, with no resprouts afterwards.

3.3. Herbicide Efficacy and Technique

The results on the efficacy of the different herbicides and application techniques are shown in Table 2. Significant differences were observed between herbicides ($p < 0.05$) and herbicides by technique ($p < 0.05$), as explained above.

2.4-D + Triclopyr at a proportion of 75% and triclopyr + aminopyralid at a proportion of 60% were the best herbicides, producing total mortality independent of the technique used (mean = 100%, SD = 0). Clopyralid + triclopyr at a proportion of 80% achieved total mortality with cut stump and stem injection and high global mortality pooling all techniques (mean = 97.7%, SD = 4.4). Fluroxypyr diluted at 75% caused complete mortality of the plants with cut stump injection, and although stem injection and basal did not reach 100% efficacy (98 and 83.3% mortality respectively), their results did not differ significantly from those of cut stump injection. Flazasulfuron, with no significant differences among techniques, produced total mortality with both cut stump injection and stem injection.

Isoxaflutole + thien carbazon-methyl at a proportion of 75% achieved total mortality for cut stump injection, followed by stem injection; however, no damage was observed in the trees to which the basal bark technique was applied. This herbicide also had the poorest efficacy of all herbicides (mean = 62.2% and SD = 47.3, pooling the three techniques). Metsulfuron-methyl at a proportion of 10% produced total mortality for cut stump injection and stem injection, although the mortality was lower when applied with basal bark, but with a high mean mortality for the three techniques (mean = 93.3%, SD = 16.5), the differences were not significant. Metribuzin at 80% was only applied with cut stump (mean mortality = 100%) and stem injection (mean = 80%, SD = 26.4) and in both techniques the mortality was high as well as the pooled mortality of the herbicides (mean = 90%, SD = 13.2).

Table 2. *A. altissima* mortality (%) by different herbicides and techniques at the last count (June 2024) for treatments carried out during autumn (September 2020 and October 2021 and 2022). Values with the same letters are not significantly different at $p \leq 0.05$. Rate: dilution of the herbicide in water v/v herbicide/water. SD: standard deviation.

Herbicide Active Ingredient	Rate (%)	Technique	Mortality (%)	SD
2,4-D + Triclopyr	75	Basal bark	100 ^a	0
		Stem injection	100 ^a	0
		Cut stump injection	100 ^a	0
Clopyralid + Triclopyr	80	Basal bark	93.3 ^a	5.7
		Stem injection	100 ^a	0
		Cut stump injection	100 ^a	0
Fluroxypyr	75	Basal bark	83.3 ^a	20.8
		Stem injection	98 ^a	4.4
		Cut stump injection	100 ^a	0
Flazasulfuron	56	Basal bark	96.6 ^a	5.7
		Stem injection	100 ^a	0
		Cut stump injection	100 ^a	0
Isoxaflutole + Thien carbazon-methyl	75	Basal bark	0 ^a	0
		Stem injection	86.6 ^b	11.5
		Cut stump injection	100 ^b	0
Metsulfuron-methyl	10	Basal bark	80 ^a	26.4
		Stem injection	100 ^a	0
		Cut stump injection	100 ^a	0
Metribuzin	80	Stem injection	80 ^a	26.4
		Cut stump injection	100 ^a	0
Triclopyr + Aminopyralid	60	Basal bark	100 ^a	0
		Stem injection	100 ^a	0
		Cut stump injection	100 ^a	0
Control (water)	100	Basal bark	3.3 ^a	5.7
		Stem injection	2.5 ^a	4.3
		Cut stump injection	31.6 ^b	25.2

In untreated plots, mortality of trees was low, as expected. Only cut stump injection caused mortality among the plants (mean = 31.6%, SD = 25.2). Cutting trees seems to damage them, although an increase in sprouting is expected.

Certain herbicides were applied at different rates than those explained so far. A summary of these herbicides and the main findings is reported below since their results may provide relevant information for practitioners on the efficacy at different application rates. Triclopyr 90 gL⁻¹ + fluroxypyr 30 gL⁻¹ was applied using stem injection during November 2019 in proportions of 1% and 10%. The lower proportions did not damage the trees and only some mortality was observed at 10% (mean = 74%, SD = 25.1). Metribuzin 600 gL⁻¹ was applied diluted at 40% in November 2019 and the mortality observed five

years after the treatment was weaker (mean = 73%, SD = 30.3) than the results described above for the 80% treatment. Fluroxypyr 200 gL⁻¹ at 50% was applied in September 2020 and the mortality observed four years after treatment was complete (mean = 100%, SD = 0) as it was for the 75% treatment. Triclopyr 240 gL⁻¹ + aminopyralid 30 gL⁻¹ was applied at 80% by stem injection in June 2020 and mortality was complete (mean = 100%, SD = 0) four years after the treatment as it was for the 60% reported above. Moreover, aclonifen 600 gL⁻¹ at 40% was applied with stem injection in autumn 2019 but no damage to the trees was observed (mean = 0%, SD = 0). This active ingredient has little mobility inside plants [41] and it was discarded in the next evaluations.

4. Discussion

4.1. Best Season for Treatment Efficacy

The efficacy of the treatments was higher when the herbicide was applied in autumn, with complete control in many cases, than in summer. This higher efficacy in autumn is confirmed by the fact that woody species are generally better controlled when plants translocate the reserves to the root, for winter storage, and not to the shoots, as occurs in spring or summer [39]. Similar findings were obtained for treatments against *A. altissima* in September [17] and October [23], while no or very poor mortality was found when treatments were carried out in summer [22]. However, the dose may play an important role in the efficacy because other authors achieved almost total control in summer by the cut stump technique [31].

4.2. Efficacy of the Applied Techniques

Application of the herbicides by cut stump injection in trees of about 5 cm diameter showed the best efficiency, as other authors found [10,20]. Cut stump treatments remove the aerial part of the plant, so the herbicide remains near the root system, which is particularly important in this weed due to its high resprouting capacity. Some studies have applied the herbicide by spraying on the stump, also with good results [23].

The lowest efficacy was observed in the basal bark applications, which can be attributed to the lack of penetration of the herbicide through the bark. The herbicide solution was applied without oil or surfactants that help the herbicide to penetrate the bark [17,24]. Basal bark applications are expected to have lower effectiveness the thicker the bark of the plant is. Several studies found that basal bark treatments had almost total mortality for trees with diameters up to 10 cm [24,25], while for bigger trees (11–18 cm diameter) the mortality decreased to 50% [20]. Consequently, this technique would be most appropriate for plants with small diameters, less than 5 cm, because their bark will be thinner, and the herbicide will penetrate more easily. Furthermore, in thinner plants it will be more difficult to make holes to inject the herbicide. For medium diameters (10 cm maximum) the three techniques may work well, and the selection will be determined by the means available.

Comparing treatments of other species with bigger diameters, the cut stump treatment had a few resprouts for the root-suckering tree *Fraxinus angustifolia* Vahl (19 cm basal diameter) depending on the active ingredient [42]. Cut stump and basal bark treatments were effective, showing high mortality for the resprouting tree *Triadica sebifera* (L.) Small LC. [40] with a 13.7 cm basal diameter.

Another factor to be considered when controlling weeds in natural environments is the cost of control. As treatments must be selective to avoid offside movements that can damage non-target plants, foliar spraying is not feasible. This means that the treatments must be applied tree by tree and manually, which is very time-consuming and expensive. It has been estimated that injection or basal bark treatments against *A. altissima* can be 15 times more expensive than foliar applications [43] and strategies that include up to three consecutive applications can be effective but very expensive [44]. Despite the major costs, selective treatments such as stem injection and basal bark are techniques that preserve non-target plants [45].

Note that regardless of the chosen technique, long-term monitoring of the results of the application is desirable because of the capacity of the species to sprout [6,11,20].

4.3. Best Herbicide

Flazasulfuron and mixtures containing triclopyr showed the best efficacy in controlling the tree, no matter the technique used. Flazasulfuron produced total mortality in cut stump injection and stem injection and also good results applied by the basal bark technique, which indicates that it can be a good alternative to glyphosate regardless of the technique, at least against the plants of 5 cm that were evaluated. This is the first report of flazasulfuron's use as a herbicide against *A. altissima* and further studies are recommended to evaluate its efficacy on trees of different diameters and rates.

We used triclopyr in ester form and we found that triclopyr with 2,4-D, clopyralid or aminopyralid produced total mortality with cut stump injection, and a mortality rate higher than 90% for basal bark treatments in autumn; however, another study found that with triclopyr (salt form) + fluroxypyr or aminopyralid + fluroxypyr diluted at 10% v/v herbicide/water and similar diameters for cut stump treatments, almost total control was achieved three years after treatment, while mortality decreased for basal bark treatments with bigger diameters (11–18 cm) [20]. Triclopyr ester form penetrates better and is more toxic for plants than salt forms, which cannot easily penetrate the plant cuticle and are recommended to be applied over cut stumps [9]. The effectiveness of herbicides can be dependent on the temperature. We applied triclopyr + fluroxypyr in summer and autumn, achieving total mortality in autumn (October 2021), with a mean monthly temperature of 16.75 °C, and a mortality rate of 91.6% in summer (June 2020) with a mean monthly temperature of 19.37 °C [38]; furthermore, ester forms of triclopyr are found to be more effective at cooler temperatures because they are highly volatile [9]. The high efficacy of triclopyr found in our work and by the abovementioned studies could be explained by its demonstrated translocation ability. Studies of foliar applications of triclopyr in species such as *Parthenocissus quinquefolia* (L.) Planchon [46], *Prosopis juliflora* (Swartz) Rafinesque seedlings [47] and 15-month-old plants of *Rhododendron maximum* L. [48] found this active ingredient in the roots.

Fluroxypyr showed high efficacy although it decreased when applied by basal bark. In contrast to our findings, treatments over the resprouting tree *Triadica sebifera* found that fluroxypyr had better results than triclopyr with basal bark treatments [40], and total control was achieved by using either fluroxypyr or triclopyr over *Celtis Sinensis* Persoon trees with basal bark treatments [49]. There are some reports saying that fluroxypyr translocation can be affected by weather conditions. For example, ref. [50] found that fluroxypyr translocation was favoured at 18 °C and decreased at 24 °C, and ref. [51] found no difference of the absorption of fluroxypyr for the same temperatures; however, ref. [52] found that the wetter the soil is, the higher the mortality for *Kochia scoparia* (Linnaeus) Scott. In our experiments, we did not find a significantly different efficacy when it was applied by stem injection in two separate years, September 2020 (mean mortality = 96%, SD = 4.2) and October 2021 (mean = 98%, SD = 4.4), despite weather conditions differing during the month after each treatment (mean air temperature was 14.4 °C in September 2020 and 12.9 °C in October 2021; accumulated pluviometry was 19.2 L/m² in September 2020 and 87.8 L/m² in October 2021).

Contrary to other studies, we found that metsulfuron-methyl applied with both cut stump and stem injection produced complete mortality in treated trees, while a lower mortality (96%) was reported for cut stump treatments [31], probably explained because those trees had bigger diameters (up to 36 cm), the application was in summer and not autumn, and the rate was 10.6 g ai/L instead of our 20g ai/L. However, these results confirm the importance of the season and the dose applied to the final outcome.

The basal bark application of isoxaflutole + thienencarbazone-methyl produced no mortality of trees. This is one of the herbicides in which the application technique matters.

For this herbicide, only cut stump injections should be considered since it seems that its active ingredients do not have capacity to penetrate through the bark of the stems.

Doses applied in our experiments were chosen according to previous studies. The triclopyr rates were based on other triclopyr (48%) works [17,23,24]. For metsulfuron-methyl, we relied on the positive results found in the study of [31], and for the other herbicides, rates were adapted to apply to each tree a similar amount of active ingredient to that of triclopyr. Probably for the herbicides in which the applied rates reached complete efficacy, lowering rates would be feasible, particularly if an oil carrier were added. Consequently, further studies are required to find the lowest efficient rate of these herbicides considering the size of the trees.

Although environmental effects of herbicides were not evaluated, no damage was observed in the surrounding plants. By injecting the herbicide into the plant, potential hazards such as drift losses are avoided. Flazasulfuron seems to not affect soil biochemical properties [53]. However, further studies looking for exudates of these herbicides in the soil and its toxicity in surrounding vegetation should be carried out, according to the reports of several studies [34,54].

5. Conclusions

Autumn was the best season to control *A. altissima* with herbicides because during this period of the year the descendent movement of the sap in the plant facilitates its translocation to the root system, completely killing the individual.

Cut stump injection was the best technique for the evaluated herbicides, with the mixtures of triclopyr with 2,4-D and aminopyralid confirmed as the best herbicides to control the weed, no matter the technique used. Several other herbicides showed complete control with cut stump and/or stem injection while basal bark failed with some active ingredients. Although these herbicides demonstrated complete control over *A. altissima*, the addition of oil or surfactant into the solution may improve its efficacy, allowing lower doses. Further studies are encouraged testing lower doses and efficacy on trees of different diameter to find the optimal parameters for herbicide applications and develop sustainable management programs for natural ecosystems. There are alternatives to glyphosate available to control *A. altissima*.

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