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Control of *Ailanthus altissima* using cut stump and basal bark herbicide applications in an eighteenth-century fortress

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Abstract

The Cittadella di Alessandria (Italy) is a military fortification that was built in the 18th century. The site has recently been abandoned and is now colonised by weeds, including the invasive *Ailanthus altissima* weed. The aim of the study was to compare the efficacy of different herbicides (glyphosate, a mixture of aminopyralid+fluroxypyr and triclopyr+fluroxypyr), applied to cut stumps or to the basal bark of the weed. Before the cut stump application, plants were first cut at the base and then immediately sprayed. Untreated cut plants were used for comparison purposes. For the basal bark application, the lower 50 cm of the plants was sprayed with the herbicides. Two runs per study were carried out (in summer 2015 and in spring 2016). Efficacy was assessed up to 2018 by counting the resprouts and their height in the cut stump application and for the basal bark treatment by measuring the variation in the plant circumference after the treatment. The cut stump treatment carried out in summer greatly reduced the number of resprouts, compared to the spring treatment, to less than one sprout per plant when aminopyralid+fluroxypyr was used, and its efficacy lasted for about two years. The basal bark treatment was not able to control the species, but fewer circumference variations and a higher mortality were detected in plants treated with aminopyralid+fluroxypyr. Considering the high level of infestation of the site and the high risk of plant resprouting, repeated cut stump treatments with aminopyralid+fluroxypyr would be preferable to eradicate the species.

KEYWORDS

tree of heaven, glyphosate, aminopyralid, fluroxypyr, triclopyr, historic sites, plant cutting

1 INTRODUCTION

Ailanthus altissima (Mill.) Swingle (tree of haven), is an arboreous plant belonging to the Simaroubacee family. Native to China and North Vietnam, this species has by now spread to all the continents, except Antarctica. It is considered one of the most invasive plants (Weber and Gut, 2004), it can easily grow on different substrates and it tolerates air pollution, dry conditions and high concentrations of salt and heavy metals (Kowarik and Säumel, 2007; Sladonja *et al.*, 2015). It is a shade-intolerant species, thus it is difficulty for it to grow in mature forests, but it can rapidly exploit a lack of forest canopy to form dense populations. *A. altissima* can determine important ecosystemic, economic, health and social impacts (Sladonja *et al.*, 2015). The most problematic issue is related to the potential reduction of biodiversity (Motard *et al.*, 2015) in particular in protected areas (Campagnaro *et al.*, 2018). Its fast colonisation is due both to its rapid growth and highly competitive ability, and to the production of allelopathic compounds, such as the quassinoid ailanthone, which has been proven to have herbicidal activity (Demasi *et al.*, 2019). This species also infests meadows, vineyards and olive groves, and it is in particular present in urban areas and disturbed sites, such as roads, railways, field edges and fallow areas (Sladonja *et al.*, 2015; Brundu, 2017). It has been reported that *A. altissima* can cause problems to buildings and infrastructures in cities as its roots can penetrate in cracks and joints, eventually damaging roofs and walls (Caneva *et al.*, 2006; EPPO, 2019); moreover, it can have an impact on human health as some sensitive people may have allergic reactions to its pollen, and its sap can cause skin problems (Ding *et al.*, 2006). *A. altissima* is widely present in urban areas, because in the past it was often planted as an ornamental species, but it rapidly spreads and infests many areas because of its ability to produce a large quantity of seeds, that is, about one hundred thousand per year per plant, and to easily resprout (Wickert *et al.*, 2017). *A. altissima* can also infest archaeological and historic sites and cause damage, as the root system can create severe alterations to the stability and integrity of structures (Almeida *et al.*, 1994; Caneva *et al.*, 2006). In a survey conducted in historical sites in Italy, *A. altissima* was reported to be one of the most frequently found alien species in those areas (Celesti-Grapow and Blasi, 2004). *A. altissima* and other alien species usually represent only a small portion of the total species richness in archaeological sites; nevertheless, they are generally present, probably because of the degree of disturbance of these sites, as they are frequently mowed and accessed, and because many alien species are planted there for ornamental purposes (Celesti-Grapow and Blasi, 2004).

The management of *A. altissima* requires a great deal of effort, both to avoid the spread of the species to non-infested areas and to manage it once established. Preventive methods should be adopted to hamper the dispersal of the species through seeds by limiting, for example, the movement of soil from infested areas or by giving priority to the control of large female trees to reduce seed rain (Brundu, 2017). Once the species has become established in a site, the strategies that can be adopted for its control may change according to the infested environment and may involve the use of mechanical and/or chemical means (EPPO, 2019). Previous studies found that the combination of plant cutting followed by the application of herbicides is a technique that is able to diminish the resprouting ability of the plants (Badalamenti *et al.*, 2015; EPPO, 2019).

The most common techniques used to mechanically control the weed include plant cutting and girdling, which are often ineffective if applied alone, that is, if not followed by herbicide applications, as they stimulate stump and root sprouting (DiTomaso and Kyser, 2007; Badalamenti *et al.*, 2015). Herbicide applications are generally made on stumps (cut stump application), on the bark at the basal part of the plant (basal bark application) or through injection of a product into the trunk (Meloche and Murphy, 2006). Foliar spraying can only be feasible and effective if applied to young trees or to resprouts in the active growth season (EPPO, 2019). Systemic herbicides, such as glyphosate, triclopyr and imazapyr, are those most commonly used with these techniques as they are able to translocate through the plant, thus ensuring the effectiveness of the treatment (DiTomaso and Kyser, 2007; EPPO, 2019; Fogliatto *et al.*, 2020).

However, the use of herbicides in natural environments can harm other native species, but if an invasive species has become dominant in a certain area, so that the biodiversity is compromised, it is permitted to use herbicides to eradicate the species (Gibson *et al.*, 2019). Moreover, restrictions on the use of herbicides may be in force in certain locations, such as in urban areas or in public areas frequented by people such as in archaeological and historic sites, as established by European regulations (Directive 128/2009/CE).

The aim of the present study has been to compare the efficacy and persistence of treatments based on herbicides applied to cut stumps or to the basal bark. The tested hypotheses were that the herbicide application would be better able to control *A. altissima* after cutting the plants than the herbicide application to the bark.

The study was conducted in infested areas inside the “Cittadella di Alessandria” in North West Italy, a historic military fortified citadel where the invasiveness of this species threatens the conservation and the accessibility of the site. A cut stump herbicide application was chosen as it is one of the most effective techniques to eradicate all sizes of invasive plants and it is suitable for areas that need to be freed from weeds or need to be accessed by people a short time after the treatment, as in the case of some parts of historic sites (Kochenderfer *et al.*, 2012). Basal bark was chosen as it is an easier and faster technique to apply than others, such as stem injection, on large infested clumps, like those present on bastions and in moats, and it is a method that can be applied where tree removal is not necessary and on small plants (Kochenderfer *et al.*, 2012).

2 MATERIALS AND METHODS

2.1 Description of the study area

The study was carried out from 2015 to 2018 in the historic military complex of the Cittadella di Alessandria (44,9197° N; 8,6082° E) in North-West Italy (Figure S1). The Cittadella di Alessandria is a fortress that was built during the 18th century. It covers about 74 hectares and it is constituted by several brick-made buildings and bastions, all of which are surrounded by a dry moat. The fortress continued to be used for military purposes until the Second World-War, and it was then gradually abandoned by the Italian Army. Today, after years of absence of any regular vegetation management, several parts of the fortress, including the roofs, are infested by different arboreous and herbaceous plants, and in particular by *A. altissima* (Figure S2). This species is present in different parts of the fortress and some representative and highly infested zones were chosen to evaluate different chemical control techniques of *A. altissima*.

2.2 Chemical control techniques adopted and herbicides used

Two chemical control techniques were adopted to control *A. altissima*: cut stump and basal bark herbicide applications. The former consisted in cutting the plant to the ground level and, immediately after plant felling, moistening the stump with a selected herbicide solution. Plant felling was carried out using a chainsaw. The herbicide solution was sprayed onto the cut surface (cut stump) or onto the first 50 cm of the basal part of the plant (basal bark) using a 2 litre-volume hand-pressure sprayer, and ensuring that the plant was thoroughly wet (Figure S3).

The herbicides, which were used for both techniques, were:

- G: glyphosate (Clinic® ST, SL, 360 g a.i. L^{-1}, Nufarm Italia S.r.l.);

- A+F: aminopyralid+fluroxypyr (Runway™, EO, 35.5 g a.i. L^{-1}+144.1 g a.i. L^{-1}, Dow AgroSciences Italia s.r.l.);
- T+F: triclopyr+fluroxypyr (Evade™, EC, 28.8 g a.i. L^{-1}+83.7 g a.i. L^{-1}, Dow AgroSciences Italia s.r.l.).

All the herbicides were applied as formulated products diluted in water at 10% V/V herbicide/water.

Areas with untreated plants were used as a reference (control) for both techniques; the plants for the cut-stump technique were cut but not sprayed (Figure S4).

Three different homogenously infested zones were individuated to perform all the treatments (herbicides and the control) for each chemical control technique. The zones that received the cut stump treatments were located in the Cittadella moat, along the walls, while the basal bark treatments were carried out on infested areas on the bastions and in the moat (Figure S1). Each zone was divided into 4 separate areas of about 5-10 m in length, where the above reported treatments were applied. Each area where one of the treatments were applied was considered as a replication of a same treatment. The trials were repeated twice, in 2015 (first run) and 2016 (second run), and were used as temporal replications.

The cut stump applications were carried out both in summer, on July 20th 2015 (first run), and in spring, on May 3rd 2016 (second run) to highlight any possible differences in efficacy depending on the season in which the treatment was carried out, as already observed in our previous tests. The basal bark application was carried out on July 24th 2015 (first run) and on July 22nd 2016 (second run).

From twenty to forty *A. altissima* plants were generally present in each area. Considering that the experiments were replicated in the three zones each year, a total of 723 plants (461 in 2015 and 262 in 2016) were included in the cut stump experiment over the two years, while 411 plants were considered in the basal bark experiment (261 plants in 2015 and 150 plants in 2016). The different numbers of plants considered in the two years of the experiment were related to the different degree of infestation of *A. altissima* plants present in the different areas and zones.

2.3 Assessments

Cut stump application In each area, immediately after cutting and before applying the herbicides, a pre-treatment assessment was carried out by measuring the plant height and stump diameter of each plant, both in the first and in the second run.

The efficacy of the cut stump technique was determined by counting the number of resprouts for each treated area and dividing them by the total number of plants present at the moment of the treatment in order to calculate the average number of resprouts per plant (Figure S5; Figure S6; Figure S7). Moreover, plant height was measured from the base of the resprout to the apex of the last leaf on at least 10 resprouts per area. When fewer than 10 resprouts were present, all the plants were measured.

The assessments of the first run, treated in 2015, were conducted on September 9th 2015, May 24th 2016, July 21st 2016, June 6th 2017 and July 13th 2018 on all the treated areas. The assessments of the second run, treated in 2016, were carried out on October 12th 2016, July 7th 2017 and July 13th 2018.

Basal bark application Before the treatment, the circumference of the trunk of each plant was measured at a height of 50 cm from the ground. The measurement point was indicated with a label, stapled onto the bark, which contained an identification code in order to be able to determine the variations in circumference of each plant over time.
The assessments of the first run, treated in 2015, were conducted on May 26th 2016, August 23rd 2016, July 7th 2017 and July 13th 2018. The assessments of the second run, treated in 2016, were conducted on October 12th 2016, July 7th 2017 and July 13th 2018.

2.4 Statistical analyses

Cut stump application ANOVA and REGWF post-hoc tests ($P \leq 0.05$) were conducted separately on the average number of resprouts per plant and on the plant height to find any differences between different treatments for each run and assessment date. Prior to the analysis, the data were square root transformed to satisfy the ANOVA assumptions. The zone in which each treatment was performed was considered as a block factor.
Basal bark application The trunk circumference of each treated plant measured on the different assessment dates was expressed as the variation in circumference (%) in comparison to the measurement taken immediately before the treatment; the circumference variations of all the plants pertaining to each treatment were averaged.

Repeated measures ANOVA were conducted separately for each run to establish the effect of the applied treatments on the variations in trunk circumference over the years. The between subject factors were the different herbicide treatments and the different treated zones, while the different assessment dates (time) were considered as the within subject factor. The pre-treatment circumference was considered as the covariate. A Greenhouse-

Geisser correction was used as Mauchly's test of sphericity was always significant ($P \leq 0.05$), which means that the data did not satisfy the sphericity assumption. The interaction between treatment and assessment date was always significant ($P \leq 0.05$) for both runs. The significant interactions that were found could have led to misinterpretation of the effects of the treatment on circumference variations, thus different ANOVA analyses were conducted for each sampling date to separate the effect of the treatments at each date (Constán-Nava *et al.*, 2010). As even a small increase in circumference could have had a different impact, in terms of circumference variation, on small or large plants (with small or large circumferences before the treatment), the plants were grouped into three classes on the basis of their pre-treatment circumference: 5-10 cm class, 10-15 cm class, > 15 cm class. All the statistical analyses were conducted using IBM SPSS Statistics, version 25.

3 RESULTS

3.1 Cut stump application

The pre-treatment assessment carried out after plant cutting and before the herbicide application in the first run (2015) highlighted an average trunk diameter of about 4 cm, while the plant height ranged from 3.4 m to 3.8 m (data not shown).

In the pre-treatment assessment of the second run (2016), the plant size was slightly more variable than in the previous year; a trunk diameter that ranged between 4 cm and 6 cm and a plant height that ranged between 3.3 m and 5.2 m were recorded (data not shown).

In all the assessments carried out on plants cut and treated in 2015, the lowest number of resprouts was recorded in the case of the A+F application, with values that were always lower than one resprout per plant (Table 1). The highest number of resprouts was observed in the case of the control, where the plants were only cut, even though the number was never significantly different from the other treatments, except for the A+F. Nevertheless, in the last assessment (July 2018), carried out 3 years after the treatment, non-significant differences were found among all the tested treatments.

In the same assessments, the smallest values of the average height of the resprouts were found for the treatment with A+F, which was always statistically different from the other treatments, except for the last assessment. The tallest resprouts were observed in the control, with values ranging from about 58 cm, a few months after the treatment, to more than 430 cm after three years. The treatments with G and T+F led to resprouts with an intermediate height between the control and the A+F application.

Thus, the first run highlighted that the most effective treatment was plant cutting followed by the application of A+F, as it resulted in the shortest and least numerous resprouts, even though the differences from the other treatments were only moderate after three years. The least effective treatment was the control in which *A. altissima* plants were only cut.

Table 1 near here

In the treatment carried out in 2016, the number of resprouts per plant was only significantly different between treatments for the first assessment, on July 2016, with the A+F application being the most effective, as it showed the lowest number of resprouts (less than one per plant) (Table 2). In the same assessment, no significant differences were recorded for all the other treatments, including the control.

The resprout height varied significantly between treatments in all the assessments (Figure S5; Figure S6; Figure S7). The A+F and G applications resulted in the shortest resprouts for all the years. The T+F treatment resulted in intermediate height resprouts; for example, in the first assessment, this treatment led to resprouts of about 65 cm in height, that is, being between the roughly 36 cm recorded for the treatments with A+F and the 146 cm recorded in the control. In the assessments carried out in July 2016 and June 2017, the plants that were only cut (control) led to the tallest resprouts. In the last assessment, the lowest height was recorded for the A+F treatment, with G not being statistically different from all the other treatments. In this run, the differences in plant diameter and height found in the pre-treatment assessment did not have any influence on the number of resprouts, as the control plants were only different from the A+F treatment in the first assessment and were similar to the others in the following years. The height of the control plants was higher in the pre-treatment assessment and maintained the highest values in the first two assessments after the treatment; a similar trend was observed in the first run (Table 1; Table 2).

Table 2 near here

The two runs of the cut stump treatments confirmed the higher efficacy of A+F, especially in the case of the summer application in the first run. In the second run, G showed a similar efficacy to that of A+F, while its efficacy was slightly lower in the first run. The technique of only cutting the *A. altissima* plants was not effective in reducing its ability to resprout. Even though the number of resprouts in the control was similar to that of the other treatments, they were significantly taller than the treatment with A+F, with values reaching more than 490 cm in the last assessment.

3.2 Basal bark application

Approximately 30 plants in each area received different herbicide treatments in 2015 (first run) and their average trunk circumferences, measured before the treatment, ranged from about 11 cm to 14 cm (data not shown). In the second run, carried out in July 2016, about 15 plants that had a similar trunk circumference were present in each area, with values that ranged from about 15 cm to about 18 cm (data not shown).

3.2.1 First run

The repeated measure ANOVA showed an effect of the assessment (time) on the circumference variation (RM Anova: assessment, $F_{2.3,556.0}$=135.8, P=0.00). Interactions between the assessment and zone (RM Anova: assessment*zone interaction, $F_{2.3,556.0}$=26.6, P=0.00) and between the assessment and treatment were found (RM Anova: assessment*treatment interaction, $F_{6.9,556.0}$=7.2, P=0.00), thus showing that the circumference variation over time differed in relation to the treatment.

The ANOVA analyses carried out to highlight the effects of the different treatments on plant circumference at each date and for each circumference class showed that, in the majority of cases, the basal bark treatment with A+F was the most effective, as the plant circumference increased less than in the other treatments (Figure 1). The only exceptions were the absence of significant differences between treatments in the first two assessments (May and August 2016) in the smaller plants (5-10 cm class).

The lowest efficacy was generally observed for the G application, as the plants showed the greatest increase in the trunk circumference, which was even higher than the control plants, compared to the other treatments. Only in small plants (5-10 cm class) in the July 2017 and 2018 assessments, did the application of T+F lead to the worst results and show an increase in the circumference of almost 25% and about 30%, respectively, compared to their circumference before the treatment. All the herbicides were generally able to limit plant growth in a similar way on small plants, except for T+F, which showed less efficacy.

The T+F mixture showed an intermediate efficacy between A+F and G for the other circumference classes, and permitted a similar plant growth to that of the untreated control plants to be achieved.

Figure 1 near here

In the last assessment, smaller plants (5-10 cm) were in general controlled slightly better than the other plant classes. However, very few plants died as a consequence of the basal bark treatments, with plant mortality values that were always lower than 20% (data not shown).

3.2.2 Second run

The repeated measure ANOVA analyses showed the absence of the effect of the assessment (time) on the circumference variation (RM Anova: assessment, $F_{1.4,155.2}$=0.8, P=0.40), as well as the absence of an interaction between the zone and assessment (RM Anova: assessment*zone interaction, $F_{1.4,155.2}$=2.2, P=0.13). However, the interaction between the assessment and treatment was significant (RM Anova: assessment*treatment interaction, $F_{4.1,556.0}$=10.3, P=0.00) and the comparisons of the treatments were therefore again carried out separately for each assessment.

The assessment carried out a few months after the treatment (October 2016 assessment) showed an absence of significance, in terms of circumference variation between the used herbicides and the control, as all the plants were able to grow similarly (Figure 2). In the following assessment, that is, in July 2017, a year after the treatment, A+F showed the lowest circumference growth, with maximum increase values of 4%. In the smallest plants (5-10 cm class), the control plants grew more than all the treated plants, while both the control plants and the plants treated with G showed a marked increase in circumference for the largest plants (> 15 cm class). No difference in growth was observed for the intermediate class of plants (10-15 cm circumference) among all the compared treatments.

In the final assessment, in July 2018, the G and T+F-treated plants grew similarly and were not different from the control plants, except for smaller plants in which G slightly limited the circumference growth. Again in the last assessment, the treatment with A+F gave the best growth reduction and the variation in the treated plants was negative in the smallest circumference class, compared to the pre-treatment, as half of the plants died.

In general, the basal bark treatments carried out in 2016 were more effective than those performed in the first run (July 2015), as demonstrated by a generally more limited circumference growth. A slightly higher number of dead plants was observed in the last assessment of the second run than in the first one, with the highest mortality (about 50% of the treated plants) and the presence of treatment symptoms (deformed buds and leaves, dead branches and bark detachments) on plants treated with A+F (data not shown; Figure S8).

Figure 2 near here

4 DISCUSSION

In this study, two chemical methods that are commonly applied to control invasive weed species, that is, cut stump followed by a herbicide treatment and a basal bark herbicide application, were tested to control the growth of *A. altissima* in a historical site. Herbicide application has been indicated as one of the most effective methods to control invasive species, even though some risks of harming the native vegetation exist (Wagner *et al.*, 2017).

In the present study, not only have different control methods been tested, but also different herbicides. Glyphosate was chosen as it is the most common means of managing weeds, including invasive species, in non-agricultural areas (Weidenhamer and Callaway, 2010; Badalamenti *et al.*, 2015; Fogliatto *et al.*, 2020). However, being a non-selective herbicide, it can also damage other arboreous and herbaceous plants that are near the treated areas, and it can lead to a more difficult vegetation recovery after the treatment, which is not desirable in historical sites visited by people, as in the case of the Cittadella di Alessandria (Slopek and Lamb, 2017; Wagner *et al.*, 2017). For this reason, another two selective herbicides (T+F and A+F) were included in the study. The T+F mixture incorporated two herbicides that had previously been used to control exotic species, and their effects had been demonstrated to last for up to two seasons after the treatments; moreover, they degrade rapidly in the environment and are selective for grass species (Gibson *et al.*, 2019). The A+F mixture has similar characteristics, as these herbicides have successfully been used to eliminate several other difficult weeds, such as kudzu (*Pueraria montana*) (Weaver *et al.*, 2016).

The study, which has confirmed our initial hypotheses, has demonstrated that the cut-stump treatment was effective in controlling *A. altissima*; a higher efficacy was in fact obtained when the A+F mixture was applied. In the treatment carried out in 2015 (run 1) and monitored for 3 years, the A+F treatment was able to limit the number of resprouts to fewer than one per plant, and its effect lasted for two years after the treatment. In 2016 (run 2), this treatment was again the most effective, even though non-significant differences with the other herbicides, in terms of number of resprouts, were detected after the first assessment. The higher efficacy observed in the first run than in the second one, for the A+F treatment, can be attributed to the different periods in which the herbicide treatments were carried out: the first run in summer and the second run in spring. This behaviour is confirmed by the fact that woody species are generally better controlled when plants translocate the reserves to the roots, for winter storage, and not to the shoots as occurs in spring (DiTomaso and Kyser, 2007; Badalamenti *et al.*, 2015; Enloe *et al.*, 2018). Moreover, the carbohydrates in the roots are at their lowest level mid-summer and the plants, once cut, cannot rely on these reserves to produce new shoot as they generally do, thus their regenerative capacity is at its lowest in this period (Kays and Canham, 1991).

After three years, the effect of the herbicide treatments was almost similar to that of only cutting the plants, thus suggesting that a single treatment is often not sufficient to completely eradicate the species, especially in the case of dense infestations. The need for multiple cut stump treatments to increase plant control and to hamper resprout production has already been observed for this and other species, such as *Betula populifolia* (grey birch) (Kays and Canham, 1991; Constán-Nava *et al.*, 2010). Thus, our study suggests that, in order to completely eradicate *A. altissima,* it is necessary to repeat summer treatments with effective herbicides every two years at least.

The use of T+F and G after cutting showed an intermediate level of efficacy, in terms of number of resprouts and height. Contrasting results are reported in the literature about the effectiveness of G when used in the cut stump technique, as some studies found a high efficacy on small plants while the application of G to stumps was ineffective in others; however, in general, in agreement with the present study, G is able to partially limit *A. altissima* growth, especially on small plants, and if used at a high concentration, even though other herbicides can give better results (Meloche and Murphy, 2006; Constán-Nava *et al.*, 2010; Badalamenti *et al.*, 2015).

Cutting the *A. altissima* plants without treating the stump with herbicides, a technique used as the control in this experiment, resulted in numerous and tall resprouts in both runs. Several previous studies found similar results, and not even annual cutting repeated for 5 years permitted the density and height of *A. altissima* to be limited (Meloche and Murphy, 2006; Constán-Nava *et al.*, 2010). In this study, the basal bark treatments showed a lower efficacy than cutting followed by a herbicide application to the stump. Previous studies generally found a quite high effectiveness in controlling many tree species, but only if the herbicides were applied together with an oil carrier that helps the herbicide to penetrate the bark (Burch and Zedaker, 2003; Bowker and Stringer, 2011). In the present study, the applied herbicides were diluted in water, as was also done for the cut stump, to obtain a better comparison of the techniques. The absence of an oil carrier and the use of highly diluted products (formulated product at 10%) could be two of the reasons for the low efficacy found in our study.

As already observed for cut stump, the herbicide that showed the highest efficacy in both runs and for all plant sizes was A+F. In a previous study, aminopyralid provided high efficacy for both cut stump and basal bark techniques when applied to control invasive weeds (Harmoney, 2016), a result that has partially been confirmed by our findings. Numerous studies that included basal bark treatments to control invasive plants used triclopyr, as it has been demonstrated to be effective in controlling sprouts, while its use after cut stump has often resulted in high percentages of resprouts (Burch and Zedaker, 2003; DiTomaso and Kyser, 2007; Harmoney, 2016).

Plant size also has an influence on the efficacy of the basal bark treatment, as we observed that large plants (circumference class >15 cm) grew more than the smaller ones, and the technique was less effective on larger plants (Figure 1 and Figure 2). In previous studies, basal bark was found to be more effective on small plants with a diameter of between 2 and 5 cm or on young plants with thin or immature barks (Nelson *et al.*, 2006; Oneto *et al.*, 2010).

The two tested techniques, cut stump and basal bark herbicide applications, can be applied in different situations; cut stump is in fact more easily performed on large trees or on plants with a thicker bark, and in the case of highly infested areas, where it is better to cut the plants before devitalisation (DiTomaso and Kyser, 2007). On the other hand, the basal bark technique is more suitable for small plants in less dense infestations, where the basal part of the plant is easily accessible, or in areas where tree removal is not necessary, such as in forests, and where falling plants do not create any risks for people (Oneto *et al.*, 2010). In historical sites frequented by people, such as the Cittadella di Alessandria, the cut stump technique may be more appropriate to eradicate invasive trees both because of its higher efficacy and the necessity of completely eliminating vegetation to make the site more accessible to the public. However, the use of herbicides in areas accessed by people is not permitted or is strictly limited by European and Italian laws. Nevertheless, some derogations have been introduced for areas in which invasive plants are present, for example, when no other effective alternative means are available and/or the infestation may affect biodiversity; in these cases, herbicides may be applied, while taking appropriate risk management measures (Directive 2009/128/CE, Article 12; Decreto Interministeriale 22 January 2014), such as closing the treated areas to the public during the treatment and for a certain time afterwards. Moreover, the use of control techniques that need a small amount of herbicides to be effective, such as those tested in the study, are particularly suitable for areas frequented by the public. Both the basal bark and cut stump techniques have low risks of off-site movement, as these products are applied to specific parts of the plants (Oneto *et al.*, 2010).

In conclusion, the experiments carried out in this historical site can be considered as a case study for the eradication of invasive plants in other similar locations worthy of conservation. Further studies are needed to establish the correct time interval between applications that would permit the infestation to be eliminated in the shortest time. Moreover, long-term monitoring and follow-up treatments should be included in the maintenance programmes of infested areas to prevent any further spread of infestations and damage to valuable areas that deserve to be preserved.

As an alternative to herbicides, a promising more sustainable control technique for this invasive species could be the use of biological control agents, such *Verticillium nonalfalfae*, a highly efficient fungus that has been demonstrated to be able to control *A. altissima* (Harris *et al.*, 2013; Kasson *et al.*, 2014; Maschek and Halmschlager, 2017; Pisuttu *et al.*, 2020). This method could be a valid alternative in particular in environmental fragile areas and in areas frequented by people where herbicide applications are restricted or prohibited. However, more studies are needed to evaluate the effects of *V. nonalfalfae* on non-target species and to better understand whether such methods can be extensively used to control invasive trees and thus limit the use of herbicides.

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No conflict of interest has been declared.

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Supporting Information

FIGURE S1 Map of the Cittadella di Alessandria with the zones considered in the study in which the cut stump and basal bark herbicide application were tested on *A. altissima*.

FIGURE S2 *A. altissima* infestation present in the dry moat and on the bastions of the Cittadella before the study in 2015.

FIGURE S3 Basal bark treatment with glyphosate on *A. altissima* plants.

FIGURE S4 Details of an area in which the cut stump technique was implemented in 2015. A control area (only cut plants) with *A. altissima* resprouts about a month after the treatment is visible in the foreground.

FIGURE S5 Resprouts of *A. altissima* a month after the cut stump and glyphosate application in 2016.

FIGURE S6 Resprouts of *A. altissima* from root buds after a cut stump treatment with glyphosate.

FIGURE S7 Resprouts of *A. altissima* from root buds after a cut stump treatment with triclopyr+fluroxypyr.

FIGURE S8 Symptoms (deformed buds and leaves) of the basal bark treatment with aminopyralid+fluroxypyr on *A. altissima* plants a year after the application.

Figure legends

FIGURE 1 Variation of the plant circumferences (%) in the different assessments after the basal bark herbicide application performed in July 2015 on plants pertaining to three classes of plant circumference (5-10 cm, 10-15 cm and >15 cm). Values with the same letters are not significantly different, according to the REGWF test ($P \leq 0.05$). Comparisons between treatments were made separately for each assessment and circumference class. The compared values have letters with the same font: italics for the compared values for the 5-10 cm circumference class, regular for the compared values for the 10-15 cm circumference class, and bold for the compared values for the 10-15 cm circumference class. The absence of letters means the comparison was not significant. Bars represent the standard errors of the means.

FIGURE 2 Variation of plant circumference (%) in the different assessments after the basal bark herbicide application performed in July 2016 on plants pertaining to three classes of plant circumference (5-10 cm, 10-15 cm and >15 cm). Values with the same letters are not significant different, according to the REGWF test ($P \leq 0.05$). Comparisons between treatments were made separately for each assessment and circumference class. The compared values have letters with the same font: italics for the compared values for the 5-10 cm circumference class, regular for the compared values for the 10-15 cm circumference class, and bold for the compared values for the 10-15 cm circumference class. Bars represent the standard errors of the means.

TABLE 1 Average number of resprouts per plant and average resprout height (m) (± SE) measured in the different assessments for each cut stump treatment after the first run of the trial (mid July 2015).

Assessments	treatment	Assessment dates				
		September 2015	May 2016	July 2016	June 2017	July 2018
average	control (cut only)	4.7 ± 1.88 b	2.1 ± 1.21 b	3.9 ± 0.46 b	2.4 ± 0.52 b	1.8 ± 0.28 ns
number of	G	2.1 ± 0.15 ab	0.7 ± 0.34 b	2.4 ± 0.50 ab	2.6 ± 0.84 b	1.3 ± 0.57
resprouts/plant	T+F	1.4 ± 0.22 b	1.0 ± 0.36 b	1.6 ± 0.54 ab	1.2 ± 0.58 ab	0.9 ± 0.06
± SE	A+F	0.1 ± 1.88 a	0.0 ±0.00 a	0.7 ± 0.09 a	0.4 ± 0.19 a	0.7 ± 0.21
	control (cut only)	58.5 ± 3.00 c	125.8 ± 7.92 c	180.4 ± 19.66 d	296.0 ± 31.71 c	431.0 ± 34.49 b
Average height	G	45.0 ± 4.05 b	83.7 ± 8.09 b	59.7 ± 5.27 b	183.7 ± 19.87 b	352.0 ± 25.23 ab
(m) ± SE	T+F	43.9 ± 2.85 b	106.2 ± 6.34 c	85.6 ± 4.81 c	157.5 ± 11.53 b	330.0 ± 42. 52 ab
	A+F	13.8 ± 8.51 a	0.0 ± 0.00 a	29.8 ± 3.46 a	87.8 ± 12.73 a	283.3 ± 33.04 a

Analyses were conducted separately on a number of resprouts per plant and resprout height values between treatments for each assessment date. Values in each column with the same letters are not significantly different, according to the REGWF test; ns: non-significant ($P \leq 0.05$); DF: the average number of resprouts/plant=2; DF: the average height varies as a function of the number of resprouts per treated area in each assessment.

TABLE 2 Average number of resprouts per plant and average resprout height (cm) (± SE) measured for each cut stump treatment in the different assessments after the second run of the trial (early May 2016).

Assessments	Treatment	Assessment dates		
		July 2016	July 2017	July 2018
Average number of resprouts/plant	control	2.6 ± 0.04 b	2.6 ± 0.22 ns	2.5 ± 0.31 ns
	G	2.2 ± 0.61 b	1.5 ± 0.18	1.8 ± 0.24
	T+F	4.3 ± 0.14 b	2.0 ± 0.48	1.9 ± 0.13
	A+F	0.5 ± 0.11 a	1.8 ± 0.97	1.8 ± 0.15
Average height (cm)	control	145.7 ± 16.47 c	306.7 ± 19.00 c	491.7 ± 66.35 b
	G	35.9 ± 2.58 a	110.5 ± 10.57 a	372.8 ± 27.00 ab
	T+F	64.4 ± 4.92 b	162.6 ± 16.92 b	305.5 ± 42.66 b
	A+F	36.1 ± 3.11 a	116.3 ± 9.69 a	265.0 ± 17.38 a

Analyses were conducted separately on the number of resprouts per plant and resprout height values between treatments for each assessment date. The values in each column with the same letters are not significantly different, according to the REGWF test; ns: non-significant ($P \leq 0.05$); DF: average number of resprouts/plant=2; DF: average height varies as a function of the number of resprouts per each treated area in each assessment.