

AperTO - Archivio Istituzionale Open Access dell'Università di Torino

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

**This is the author's manuscript**

*Original Citation:*

*Availability:*

This version is available <http://hdl.handle.net/2318/1758801> since 2020-11-16T10:52:33Z

*Published version:*

DOI:10.1111/wre.12447

*Terms of use:*

Open Access

Anyone can freely access the full text of works made available as "Open Access". Works made available under a Creative Commons license can be used according to the terms and conditions of said license. Use of all other works requires consent of the right holder (author or publisher) if not exempted from copyright protection by the applicable law.

(Article begins on next page)

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

3

4 **Silvia Fogliatto\*, Marco Milan\* and Francesco Vidotto\***

5

6 *\*Dipartimento di Scienze Agrarie Forestali e Alimentari, Università degli Studi di Torino,*  
7 *Grugliasco, Italy*

8

9

10 *Correspondence*

11 Silvia Fogliatto, Dipartimento di Scienze Agrarie Forestali e Alimentari, Università degli  
12 Studi di Torino, Largo P. Braccini 2, Grugliasco, Italy

13 E-mail: [silvia.fogliatto@unito.com](mailto:silvia.fogliatto@unito.com)

14

15 *Funding information*

16 EU EMPHASIS (Effective Management of Pests and Harmful Alien Species – Integrated  
17 Solutions) project, funded by the EU H2020 programme (Grant Agreement no. 634179)

18

19 Received 10 January 2020

20 Revised version accepted 27 July 2020

21 Subject Editor: Christian Bohren, Agroscope, Changins, Switzerland

22

23

24

25

26

27 Word count = 7692

28 **Abstract**

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

47

48 **KEYWORDS**

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

50

51

## 52 1 INTRODUCTION

53

54 *Ailanthus altissima* (Mill.) Swingle (tree of heaven), is an arboreous plant belonging to the  
55 Simaroubaceae family. Native to China and North Vietnam, this species has by now spread to  
56 all the continents, except Antarctica. It is considered one of the most invasive plants (Weber  
57 and Gut, 2004), it can easily grow on different substrates and it tolerates air pollution, dry  
58 conditions and high concentrations of salt and heavy metals (Kowarik and Säumel, 2007;  
59 Sladonja *et al.*, 2015). It is a shade-intolerant species, thus it is difficult for it to grow in  
60 mature forests, but it can rapidly exploit a lack of forest canopy to form dense populations. *A.*  
61 *altissima* can determine important ecosystemic, economic, health and social impacts  
62 (Sladonja *et al.*, 2015). The most problematic issue is related to the potential reduction of  
63 biodiversity (Motard *et al.*, 2015) in particular in protected areas (Campagnaro *et al.*, 2018).  
64 Its fast colonisation is due both to its rapid growth and highly competitive ability, and to the  
65 production of allelopathic compounds, such as the quassinoid ailanthone, which has been  
66 proven to have herbicidal activity (Demasi *et al.*, 2019). This species also infests meadows,  
67 vineyards and olive groves, and it is in particular present in urban areas and disturbed sites,  
68 such as roads, railways, field edges and fallow areas (Sladonja *et al.*, 2015; Brundu, 2017). It  
69 has been reported that *A. altissima* can cause problems to buildings and infrastructures in  
70 cities as its roots can penetrate in cracks and joints, eventually damaging roofs and walls  
71 (Caneva *et al.*, 2006; EPPO, 2019); moreover, it can have an impact on human health as some  
72 sensitive people may have allergic reactions to its pollen, and its sap can cause skin problems  
73 (Ding *et al.*, 2006). *A. altissima* is widely present in urban areas, because in the past it was  
74 often planted as an ornamental species, but it rapidly spreads and infests many areas because  
75 of its ability to produce a large quantity of seeds, that is, about one hundred thousand per year  
76 per plant, and to easily resprout (Wickert *et al.*, 2017). *A. altissima* can also infest  
77 archaeological and historic sites and cause damage, as the root system can create severe  
78 alterations to the stability and integrity of structures (Almeida *et al.*, 1994; Caneva *et al.*,  
79 2006). In a survey conducted in historical sites in Italy, *A. altissima* was reported to be one of  
80 the most frequently found alien species in those areas (Celesti-Grapow and Blasi, 2004). *A.*  
81 *altissima* and other alien species usually represent only a small portion of the total species  
82 richness in archaeological sites; nevertheless, they are generally present, probably because of  
83 the degree of disturbance of these sites, as they are frequently mowed and accessed, and  
84 because many alien species are planted there for ornamental purposes (Celesti-Grapow and  
85 Blasi, 2004).

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

96           The most common techniques used to mechanically control the weed include plant  
97 cutting and girdling, which are often ineffective if applied alone, that is, if not followed by  
98 herbicide applications, as they stimulate stump and root sprouting (DiTomaso and Kyser,  
99 2007; Badalamenti *et al.*, 2015). Herbicide applications are generally made on stumps (cut  
100 stump application), on the bark at the basal part of the plant (basal bark application) or  
101 through injection of a product into the trunk (Meloche and Murphy, 2006). Foliar spraying  
102 can only be feasible and effective if applied to young trees or to resprouts in the active  
103 growth season (EPPO, 2019). Systemic herbicides, such as glyphosate, triclopyr and  
104 imazapyr, are those most commonly used with these techniques as they are able to translocate  
105 through the plant, thus ensuring the effectiveness of the treatment (DiTomaso and Kyser,  
106 2007; EPPO, 2019; Fogliatto *et al.*, 2020).

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

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

117           The study was conducted in infested areas inside the “Cittadella di Alessandria” in  
118 North West Italy, a historic military fortified citadel where the invasiveness of this species  
119 threatens the conservation and the accessibility of the site. A cut stump herbicide application

120 was chosen as it is one of the most effective techniques to eradicate all sizes of invasive  
121 plants and it is suitable for areas that need to be freed from weeds or need to be accessed by  
122 people a short time after the treatment, as in the case of some parts of historic sites  
123 (Kochenderfer *et al.*, 2012). Basal bark was chosen as it is an easier and faster technique to  
124 apply than others, such as stem injection, on large infested clumps, like those present on  
125 bastions and in moats, and it is a method that can be applied where tree removal is not  
126 necessary and on small plants (Kochenderfer *et al.*, 2012).

127

## 128 **2 MATERIALS AND METHODS**

129

### 130 **2.1 Description of the study area**

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

142

### 143 **2.2 Chemical control techniques adopted and herbicides used**

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

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

152 - G: glyphosate (Clinic<sup>®</sup> ST, SL, 360 g a.i. L<sup>-1</sup>, Nufarm Italia S.r.l.);

153 - A+F: aminopyralid+fluroxypyr (Runway™, EO, 35.5 g a.i. L<sup>-1</sup>+144.1 g a.i. L<sup>-1</sup>, Dow  
154 AgroSciences Italia s.r.l.);  
155 - T+F: triclopyr+fluroxypyr (Evade™, EC, 28.8 g a.i. L<sup>-1</sup>+83.7 g a.i. L<sup>-1</sup>, Dow AgroSciences  
156 Italia s.r.l.).

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

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

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

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

174 From twenty to forty *A. altissima* plants were generally present in each area. Considering that  
175 the experiments were replicated in the three zones each year, a total of 723 plants (461 in  
176 2015 and 262 in 2016) were included in the cut stump experiment over the two years, while  
177 411 plants were considered in the basal bark experiment (261 plants in 2015 and 150 plants in  
178 2016). The different numbers of plants considered in the two years of the experiment were  
179 related to the different degree of infestation of *A. altissima* plants present in the different  
180 areas and zones.

181

### 182 **2.3 Assessments**

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

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

192 The assessments of the first run, treated in 2015, were conducted on September 9<sup>th</sup>  
193 2015, May 24<sup>th</sup> 2016, July 21<sup>st</sup> 2016, June 6<sup>th</sup> 2017 and July 13<sup>th</sup> 2018 on all the treated areas.  
194 The assessments of the second run, treated in 2016, were carried out on October 12<sup>th</sup> 2016,  
195 July 7<sup>th</sup> 2017 and July 13<sup>th</sup> 2018.

196

197 *Basal bark application* Before the treatment, the circumference of the trunk of each plant was  
198 measured at a height of 50 cm from the ground. The measurement point was indicated with a  
199 label, stapled onto the bark, which contained an identification code in order to be able to  
200 determine the variations in circumference of each plant over time.

201 The assessments of the first run, treated in 2015, were conducted on May 26<sup>th</sup> 2016, August  
202 23<sup>rd</sup> 2016, July 7<sup>th</sup> 2017 and July 13<sup>th</sup> 2018. The assessments of the second run, treated in  
203 2016, were conducted on October 12<sup>th</sup> 2016, July 7<sup>th</sup> 2017 and July 13<sup>th</sup> 2018.

204

## 205 **2.4 Statistical analyses**

206 *Cut stump application* ANOVA and REGWF post-hoc tests ( $P \leq 0.05$ ) were conducted  
207 separately on the average number of resprouts per plant and on the plant height to find any  
208 differences between different treatments for each run and assessment date. Prior to the  
209 analysis, the data were square root transformed to satisfy the ANOVA assumptions. The zone  
210 in which each treatment was performed was considered as a block factor.

211 *Basal bark application* The trunk circumference of each treated plant measured on the  
212 different assessment dates was expressed as the variation in circumference (%) in comparison  
213 to the measurement taken immediately before the treatment; the circumference variations of  
214 all the plants pertaining to each treatment were averaged.

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



220 Geisser correction was used as Mauchly's test of sphericity was always significant ( $P \leq 0.05$ ),  
221 which means that the data did not satisfy the sphericity assumption. The interaction between  
222 treatment and assessment date was always significant ( $P \leq 0.05$ ) for both runs. The significant  
223 interactions that were found could have led to misinterpretation of the effects of the treatment  
224 on circumference variations, thus different ANOVA analyses were conducted for each  
225 sampling date to separate the effect of the treatments at each date (Constán-Nava *et al.*,  
226 2010). As even a small increase in circumference could have had a different impact, in terms  
227 of circumference variation, on small or large plants (with small or large circumferences  
228 before the treatment), the plants were grouped into three classes on the basis of their pre-  
229 treatment circumference: 5-10 cm class, 10-15 cm class, > 15 cm class. All the statistical  
230 analyses were conducted using IBM SPSS Statistics, version 25.

231

## 232 **3 RESULTS**

### 233 **3.1 Cut stump application**

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

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

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

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

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

258

259 *Table 1 near here*

260

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

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

280

281 *Table 2 near here*

282

283 The two runs of the cut stump treatments confirmed the higher efficacy of A+F,  
284 especially in the case of the summer application in the first run. In the second run, G showed  
285 a similar efficacy to that of A+F, while its efficacy was slightly lower in the first run. The  
286 technique of only cutting the *A. altissima* plants was not effective in reducing its ability to

287 resprout. Even though the number of resprouts in the control was similar to that of the other  
288 treatments, they were significantly taller than the treatment with A+F, with values reaching  
289 more than 490 cm in the last assessment.

290

### 291 **3.2 Basal bark application**

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

#### 297 **3.2.1 First run**

298 The repeated measure ANOVA showed an effect of the assessment (time) on the circumference  
299 variation (RM Anova: assessment,  $F_{2,3,556.0}=135.8$ ,  $P=0.00$ ). Interactions between the  
300 assessment and zone (RM Anova: assessment\*zone interaction,  $F_{2,3,556.0}=26.6$ ,  $P=0.00$ ) and  
301 between the assessment and treatment were found (RM Anova: assessment\*treatment  
302 interaction,  $F_{6,9,556.0}=7.2$ ,  $P=0.00$ ), thus showing that the circumference variation over time  
303 differed in relation to the treatment.

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

310 The lowest efficacy was generally observed for the G application, as the plants showed  
311 the greatest increase in the trunk circumference, which was even higher than the control plants,  
312 compared to the other treatments. Only in small plants (5-10 cm class) in the July 2017 and  
313 2018 assessments, did the application of T+F lead to the worst results and show an increase in  
314 the circumference of almost 25% and about 30%, respectively, compared to their  
315 circumference before the treatment. All the herbicides were generally able to limit plant growth  
316 in a similar way on small plants, except for T+F, which showed less efficacy.

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

320

321 *Figure 1 near here*

322

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

327

### 328 **3.2.2 Second run**

329 The repeated measure ANOVA analyses showed the absence of the effect of the assessment  
330 (time) on the circumference variation (RM Anova: assessment,  $F_{1,4,155.2}=0.8$ ,  $P=0.40$ ), as well  
331 as the absence of an interaction between the zone and assessment (RM Anova:  
332 assessment\*zone interaction,  $F_{1,4,155.2}=2.2$ ,  $P=0.13$ ). However, the interaction between the  
333 assessment and treatment was significant (RM Anova: assessment\*treatment interaction,  
334  $F_{4,1,556.0}=10.3$ ,  $P=0.00$ ) and the comparisons of the treatments were therefore again carried out  
335 separately for each assessment.

336 The assessment carried out a few months after the treatment (October 2016  
337 assessment) showed an absence of significance, in terms of circumference variation between  
338 the used herbicides and the control, as all the plants were able to grow similarly (Figure 2). In  
339 the following assessment, that is, in July 2017, a year after the treatment, A+F showed the  
340 lowest circumference growth, with maximum increase values of 4%. In the smallest plants  
341 (5-10 cm class), the control plants grew more than all the treated plants, while both the  
342 control plants and the plants treated with G showed a marked increase in circumference for  
343 the largest plants ( $> 15$  cm class). No difference in growth was observed for the intermediate  
344 class of plants (10-15 cm circumference) among all the compared treatments.

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

350 In general, the basal bark treatments carried out in 2016 were more effective than  
351 those performed in the first run (July 2015), as demonstrated by a generally more limited  
352 circumference growth. A slightly higher number of dead plants was observed in the last  
353 assessment of the second run than in the first one, with the highest mortality (about 50% of

354 the treated plants) and the presence of treatment symptoms (deformed buds and leaves, dead  
355 branches and bark detachments) on plants treated with A+F (data not shown; Figure S8).

356

357 *Figure 2 near here*

358

#### 359 **4 DISCUSSION**

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

365 In the present study, not only have different control methods been tested, but also  
366 different herbicides. Glyphosate was chosen as it is the most common means of managing  
367 weeds, including invasive species, in non-agricultural areas (Weidenhamer and Callaway,  
368 2010; Badalamenti *et al.*, 2015; Fogliatto *et al.*, 2020). However, being a non-selective  
369 herbicide, it can also damage other arboreous and herbaceous plants that are near the treated  
370 areas, and it can lead to a more difficult vegetation recovery after the treatment, which is not  
371 desirable in historical sites visited by people, as in the case of the Cittadella di Alessandria  
372 (Slopek and Lamb, 2017; Wagner *et al.*, 2017). For this reason, another two selective  
373 herbicides (T+F and A+F) were included in the study. The T+F mixture incorporated two  
374 herbicides that had previously been used to control exotic species, and their effects had been  
375 demonstrated to last for up to two seasons after the treatments; moreover, they degrade  
376 rapidly in the environment and are selective for grass species (Gibson *et al.*, 2019). The A+F  
377 mixture has similar characteristics, as these herbicides have successfully been used to  
378 eliminate several other difficult weeds, such as kudzu (*Pueraria montana*) (Weaver *et al.*,  
379 2016).

380 The study, which has confirmed our initial hypotheses, has demonstrated that the cut-  
381 stump treatment was effective in controlling *A. altissima*; a higher efficacy was in fact  
382 obtained when the A+F mixture was applied. In the treatment carried out in 2015 (run 1) and  
383 monitored for 3 years, the A+F treatment was able to limit the number of resprouts to fewer  
384 than one per plant, and its effect lasted for two years after the treatment. In 2016 (run 2), this  
385 treatment was again the most effective, even though non-significant differences with the  
386 other herbicides, in terms of number of resprouts, were detected after the first assessment.  
387 The higher efficacy observed in the first run than in the second one, for the A+F treatment,

388 can be attributed to the different periods in which the herbicide treatments were carried out:  
389 the first run in summer and the second run in spring. This behaviour is confirmed by the fact  
390 that woody species are generally better controlled when plants translocate the reserves to the  
391 roots, for winter storage, and not to the shoots as occurs in spring (DiTomaso and Kyser,  
392 2007; Badalamenti *et al.*, 2015; Enloe *et al.*, 2018). Moreover, the carbohydrates in the roots  
393 are at their lowest level mid-summer and the plants, once cut, cannot rely on these reserves to  
394 produce new shoot as they generally do, thus their regenerative capacity is at its lowest in this  
395 period (Kays and Canham, 1991).

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

404 The use of T+F and G after cutting showed an intermediate level of efficacy, in terms  
405 of number of resprouts and height. Contrasting results are reported in the literature about the  
406 effectiveness of G when used in the cut stump technique, as some studies found a high  
407 efficacy on small plants while the application of G to stumps was ineffective in others;  
408 however, in general, in agreement with the present study, G is able to partially limit *A.*  
409 *altissima* growth, especially on small plants, and if used at a high concentration, even though  
410 other herbicides can give better results (Meloche and Murphy, 2006; Constán-Nava *et al.*,  
411 2010; Badalamenti *et al.*, 2015).

412 Cutting the *A. altissima* plants without treating the stump with herbicides, a technique  
413 used as the control in this experiment, resulted in numerous and tall resprouts in both runs.  
414 Several previous studies found similar results, and not even annual cutting repeated for 5  
415 years permitted the density and height of *A. altissima* to be limited (Meloche and Murphy,  
416 2006; Constán-Nava *et al.*, 2010). In this study, the basal bark treatments showed a lower  
417 efficacy than cutting followed by a herbicide application to the stump. Previous studies  
418 generally found a quite high effectiveness in controlling many tree species, but only if the  
419 herbicides were applied together with an oil carrier that helps the herbicide to penetrate the  
420 bark (Burch and Zedaker, 2003; Bowker and Stringer, 2011). In the present study, the applied  
421 herbicides were diluted in water, as was also done for the cut stump, to obtain a better

422 comparison of the techniques. The absence of an oil carrier and the use of highly diluted  
423 products (formulated product at 10%) could be two of the reasons for the low efficacy found  
424 in our study.

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

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

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

456 be effective, such as those tested in the study, are particularly suitable for areas frequented by  
457 the public. Both the basal bark and cut stump techniques have low risks of off-site movement,  
458 as these products are applied to specific parts of the plants (Oneto *et al.*, 2010).

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

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

475

## 476 **ACKNOWLEDGEMENTS**

477 No conflict of interest has been declared.

478

## 479 **REFERENCES**

- 480 Almeida, M.T., Mouga, T. and Barracosa, P. (1994) The weathering ability of higher plants.  
481 The case of *Ailanthus altissima* (Miller) Swingle. *International Biodeterioration &*  
482 *Biodegradation*, 33, 333–343.
- 483 Badalamenti, E., Barone, E. and La Mantia, T. (2015) Seasonal effects on mortality rates and  
484 resprouting of stems treated with glyphosate in the invasive tree of heaven (*Ailanthus*  
485 *altissima* (Mill.) Swingle). *Arboricultural Journal*, 37, 180–195.
- 486 Bowker, D. and Stringer, J. (2011) Efficacy of herbicide treatments for controlling residual  
487 sprouting of tree-of-heaven. In: *Proceedings of the 17th Central Hardwood Forest*  
488 *Conference* (Ed F. Songlin, J.M. Lhotka, J.W. Stringer, K.W. Gottschalk, G.W. Miller) (April  
489 5-7 2010, Lexington, KY, USA). 128–133. Lexington, KY, USA: USDA Forest Service,  
490 Northern Research Station.



- 491 Brundu, G. (2017) *Information on measures and related costs in relation to species*  
 492 *considered for inclusion on the Union list: Ailanthus altissima*. Technical note prepared by  
 493 IUCN for the European Commission. Available at: [https://circabc.europa.eu/sd/a/c5681406-](https://circabc.europa.eu/sd/a/c5681406-f3d4-4288-bfb3-40322df5c013/TSSR-2016-003%20Measures%20and%20Costs%20-%20Ailanthus%20altissima.pdf)  
 494 [f3d4-4288-bfb3-40322df5c013/TSSR-2016-003%20Measures%20and%20Costs%20-](https://circabc.europa.eu/sd/a/c5681406-f3d4-4288-bfb3-40322df5c013/TSSR-2016-003%20Measures%20and%20Costs%20-%20Ailanthus%20altissima.pdf)  
 495 [%20Ailanthus%20altissima.pdf](https://circabc.europa.eu/sd/a/c5681406-f3d4-4288-bfb3-40322df5c013/TSSR-2016-003%20Measures%20and%20Costs%20-%20Ailanthus%20altissima.pdf) [Accessed 2nd January 2020].
- 496 Burch, P.L. and Zedaker, S.M. (2003) Removing the invasive tree *Ailanthus altissima* and  
 497 restoring natural cover. *Journal of Arboriculture*, 29, 18–24.
- 498 Campagnaro, T., Brundu, G. and Sitzia, T. (2018) Five major invasive alien tree species in  
 499 European Union forest habitat types of the Alpine and Continental biogeographical regions.  
 500 *Journal for Nature Conservation*, 43, 227–238.
- 501 Caneva, G., Ceschin, S. and De Marco, G. (2006) Mapping the risk of damage from tree roots  
 502 for the conservation of archaeological sites: the case of the Domus Aurea, Rome.  
 503 *Conservation and Management of Archaeological Sites*, 7, 163–170.
- 504 Celesti-Grapow, L. and Blasi, C. (2004) The role of alien and native weeds in the  
 505 deterioration of archaeological remains in Italy. *Weed Technology*, 18, 1508–1513.
- 506 Constán-Nava, S., Bonet, A., Pastor, E. and Lledó, M.J. (2010) Long-term control of the  
 507 invasive tree *Ailanthus altissima*: Insights from Mediterranean protected forests. *Forest*  
 508 *Ecology and Management*, 260, 1058–1064.
- 509 Demasi, S., Caser, M., Vanara, F., Fogliatto, S., Vidotto, F., Negre, M., Trotta, F. and Scariot,  
 510 V. (2019) Ailanthone from *Ailanthus altissima* (Mill.) Swingle as potential natural herbicide.  
 511 *Scientia Horticulturae*, 257, 108702.
- 512 Ding, J., Wu, Y., Zheng, H., Fu, W., Reardon, R. and Liu, M. (2006) Assessing potential  
 513 biological control of the invasive plant, tree-of-heaven, *Ailanthus altissima*. *Biocontrol*  
 514 *Science and Technology*, 16, 547–566.
- 515 DiTomaso, J.M. and Kyser, G.B. (2007) Control of *Ailanthus altissima* using stem herbicide  
 516 application techniques. *Arboriculture & Urban Forestry*, 33, 55–63.
- 517 Enloe, S.F., O’Sullivan, S.E., Loewenstein, N.J., Brantley, E. and Lauer, D.K. (2018) The  
 518 Influence of treatment timing and shrub size on Chinese Privet (*Ligustrum sinense*) control  
 519 with cut stump herbicide treatments in the South eastern United States. *Invasive Plant*  
 520 *Science and Management*, 11, 49–55.
- 521 EPPO (2019) PM 9/29 (1) *Ailanthus altissima*. *EPPO Bulletin*, 1–8.
- 522 Fogliatto, S., Ferrero, A. and Vidotto, F. (2020) Current and future scenarios of glyphosate  
 523 use in Europe: Are there alternatives? In: Sparks, D.L. (Ed). *Advances in Agronomy*.  
 524 Cambridge, USA: Academic Press. pp. 219–278.
- 525 Gibson, D.J., Shupert, L.A. and Liu, X. (2019) Do no harm: efficacy of a single herbicide  
 526 application to control an invasive shrub while minimizing collateral damage to native species.  
 527 *Plants*, 8, 426.
- 528 Harmony, K.R. (2016) Controlling Honey Locust (*Gleditsia triacanthos*) with cut stump-  
 529 and basal bark-applied herbicides for grazed pasture. *Weed Technology*, 30, 801–806.

- 530 Harris, P.T., Cannon, G.H., Smith, N.E. and Muth, N.Z. (2013) Assessment of plant  
531 community restoration following Tree-of-Heaven (*Ailanthus altissima*) control by  
532 *Verticillium albo-atrum*. *Biological Invasions*, 15, 1887–1893.
- 533 Kasson, M.T., O’Neal, E.S. and Davis, D.D. (2014) Expanded host range testing for  
534 *Verticillium nonalfalfae*: potential biocontrol agent against the invasive *Ailanthus altissima*.  
535 *Plant Disease*, 99, 823–835.
- 536 Kays, J.S. and Canham, C.D. (1991) Effects of time and frequency of cutting of hardwood  
537 root reserves and sprout growth. *Forest Science*, 37, 524–539.
- 538 Kochenderfer, J.D., Kochenderfer, J.N. and Miller, G.W. (2012) *Manual herbicide*  
539 *application methods for managing vegetation in Appalachian hardwood forests*. General  
540 Technical Report NRS-96NRS-GTR-96. Available at:  
541 <https://www.fs.usda.gov/treearch/pubs/40792>. [Accessed 26<sup>th</sup> June 2020].
- 542 Kowarik, I. and Säumel, I. (2007) Biological flora of Central Europe: *Ailanthus altissima*  
543 (Mill.) Swingle. *Perspectives in Plant Ecology, Evolution and Systematics*, 8, 207–237.
- 544 Maschek, O. and Halmschlager, E. (2017) Natural distribution of *Verticillium* wilt on  
545 invasive *Ailanthus altissima* in eastern Austria and its potential for biocontrol. *Forest*  
546 *Pathology*, 47, e12356.
- 547 Meloche, C. and Murphy, S.D. (2006) Managing Tree-of-Heaven (*Ailanthus altissima*) in  
548 parks and protected areas: a case study of Rondeau provincial park (Ontario, Canada).  
549 *Environmental Management*, 37, 764–772.
- 550 Motard, E., Dusz, S., Geslin, B., Akpa-Vinceslas, M., Hignard, C., Babiar, O., Clair-  
551 Maczulajtys, D. and Michel-Salzat, A. (2015) How invasion by *Ailanthus altissima*  
552 transforms soil and litter communities in a temperate forest ecosystem. *Biological Invasions*,  
553 17, 1817–1832.
- 554 Nelson, L.R., Ezell, A.W. and Yeiser, J.L. (2006) Imazapyr and triclopyr tank mixtures for  
555 basal bark control of woody brush in the southeastern United States. *New Forests*, 3, 173–  
556 183.
- 557 Oneto, S.R., Kyser, G.B. and DiTomaso, J.M. (2010) Efficacy of mechanical and herbicide  
558 control methods for Scotch Broom (*Cytisus scoparius*) and cost analysis of chemical control  
559 options. *Invasive Plant Science and Management*, 3, 421–428.
- 560 Pisuttu, C., Marchica, A., Bernardi, R., Calzone, A., Cotrozzi, L., Nali, C., Pellegrini, E. and  
561 Lorenzini, G. (2020) *Verticillium* wilt of *Ailanthus altissima* in Italy caused by *V. dahliae*:  
562 new outbreaks from Tuscany. *iForest - Biogeosciences and Forestry*, 13, 238.
- 563 Sladonja, B., Sušek, M. and Guillermic, J. (2015) Review on invasive Tree of Heaven  
564 (*Ailanthus altissima* (Mill.) Swingle) conflicting values: assessment of its ecosystem services  
565 and potential biological threat. *Environmental Management*, 56, 1009–1034.
- 566 Slopek, J.I. and Lamb, E.G. (2017) Long-term efficacy of glyphosate for Smooth Brome  
567 control in native prairie. *Invasive Plant Science and Management*, 10, 350–355.

568 Wagner, V., Antunes, P.M., Irvine, M. and Nelson, C.R. (2017) Herbicide usage for invasive  
569 non-native plant management in wildland areas of North America. *Journal of Applied*  
570 *Ecology*, 54, 198–204.

571 Weaver, M.A., Boyette, C.D. and Hoagland, R.E. (2016) Rapid kudzu eradication and  
572 switchgrass establishment through herbicide, bioherbicide and integrated programmes.  
573 *Biocontrol Science and Technology*, 26, 640–650.

574 Weber, E. and Gut, D. (2004) Assessing the risk of potentially invasive plant species in  
575 central Europe. *Journal for Nature Conservation*, 12, 171–179.

576 Weidenhamer, J.D. and Callaway, R.M. (2010) Direct and indirect effects of invasive plants  
577 on soil chemistry and ecosystem function. *Journal of Chemical Ecology*, 36, 59–69.

578 Wickert, K.L., O’Neal, E.S., Davis, D.D. and Kasson, M.T. (2017) Seed Production,  
579 Viability, and reproductive limits of the invasive *Ailanthus altissima* (Tree-of-Heaven) within  
580 invaded environments. *Forests*, 8, 226.

581

## 582 **Supporting Information**

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

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

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

588 **FIGURE S4** Details of an area in which the cut stump technique was implemented in  
589 2015. A control area (only cut plants) with *A. altissima* resprouts about a month after the  
590 treatment is visible in the foreground.

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

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

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

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

599

600

601 Figure legends

602

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

613

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

623

624

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

627

Assessments	treatment	Assessment dates				
		September 2015	May 2016	July 2016	June 2017	July 2018
<i>average</i>	control (cut only)	4.7 $\pm$ 1.88 b	2.1 $\pm$ 1.21 b	3.9 $\pm$ 0.46 b	2.4 $\pm$ 0.52 b	1.8 $\pm$ 0.28 ns
<i>number of</i>	G	2.1 $\pm$ 0.15 ab	0.7 $\pm$ 0.34 b	2.4 $\pm$ 0.50 ab	2.6 $\pm$ 0.84 b	1.3 $\pm$ 0.57
<i>resprouts/plant</i>	T+F	1.4 $\pm$ 0.22 b	1.0 $\pm$ 0.36 b	1.6 $\pm$ 0.54 ab	1.2 $\pm$ 0.58 ab	0.9 $\pm$ 0.06
$\pm$ SE	A+F	0.1 $\pm$ 1.88 a	0.0 $\pm$ 0.00 a	0.7 $\pm$ 0.09 a	0.4 $\pm$ 0.19 a	0.7 $\pm$ 0.21
<i>Average height</i>	control (cut only)	58.5 $\pm$ 3.00 c	125.8 $\pm$ 7.92 c	180.4 $\pm$ 19.66 d	296.0 $\pm$ 31.71 c	431.0 $\pm$ 34.49 b
<i>(m) <math>\pm</math> SE</i>	G	45.0 $\pm$ 4.05 b	83.7 $\pm$ 8.09 b	59.7 $\pm$ 5.27 b	183.7 $\pm$ 19.87 b	352.0 $\pm$ 25.23 ab
	T+F	43.9 $\pm$ 2.85 b	106.2 $\pm$ 6.34 c	85.6 $\pm$ 4.81 c	157.5 $\pm$ 11.53 b	330.0 $\pm$ 42.52 ab
	A+F	13.8 $\pm$ 8.51 a	0.0 $\pm$ 0.00 a	29.8 $\pm$ 3.46 a	87.8 $\pm$ 12.73 a	283.3 $\pm$ 33.04 a

628 Analyses were conducted separately on a number of resprouts per plant and resprout height values between treatments for each assessment date.

629 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

630 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.

631

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

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

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

640  
 641  
 642  
 643  
 644