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Can Land Managers Control Japanese Knotweed? Lessons from Control Tests in Belgium

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Abstract Japanese knotweed *Fallopia japonica* is an extremely abundant invasive plant in Belgium and surrounding countries. To date, no eradication method is available for land managers facing the invasion of this rhizomatous plant. We tested different chemical herbicides with two application methods (spraying and stem injection), as well as mechanical treatments, on knotweed clones throughout southern Belgium. The tested control methods were selected to be potentially usable by managers, e.g., using legally accepted rates for herbicides. Stem volume, height and density reduction were assessed after one or two years, depending on the control method. Labor estimations were made for each control method. No tested control method completely eradicated the clones. Stem injection with glyphosate-based herbicide (3.6 kg ha^{-1} of acid equivalent glyphosate) caused the most damage, i.e., no sprouting shoots were observed the year following the injection. The following year, though, stunted shoots appeared. Among the mechanical control methods, repeated cuts combined with native tree transplanting most appreciably reduced knotweed development. The most efficient methods we tested could curb knotweed invasion, but are not likely to be effective in eradicating the species. As such, they should be included in a more integrated restoration strategy, together with prevention and public awareness campaigns.

Keywords *Fallopia japonica* · *Polygonum cuspidatum* · *Reynoutria japonica* · Invasive plant management · Stem-injection · Herbicide · Control

Introduction

Invasive plant control is an increasingly important challenge for natural resource management. Several international policies, guidelines, agreements and conventions addressing the control of invasive alien species have been ratified and are being implemented (Shine and others 2000; Genovesi and Shine 2004; Heywood and Brunel 2008). Among others, states have expressed their concerns about the problem of invasions through the convention on biological diversity (CBD), which calls on the parties to "prevent the introduction of, control or eradicate those alien species which threaten ecosystems, habitats, or species" (Article 8h). More recent policy engagements, like the target 5 of the EU biodiversity strategy to 2020, even go further: "by 2020, invasive alien species and their pathways are identified and prioritized, priority species are controlled or eradicated, and pathways are managed to prevent the introduction and establishment of new invasive alien species". To achieve this, land managers need efficient and feasible control methods to set up management strategies.

Originating from Asia (China, Japan, parts of Korea and Taiwan) and introduced in Europe in the mid-1800s as

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ornamental plants and fodder (Bailey and Conolly 2000), Japanese knotweed [*Fallopia japonica* Houtt. (Ronse Decr.)] is a particularly troublesome invader (Weber 2003; Barney 2006). It thrives in various habitats, including riparian ecosystems and disturbed areas (Weber 2003). It enhances nutrient cycling rates and topsoil fertility, and has been shown to have detrimental effects on native plant and animal communities (Dassonville and others 2007; Gerber and others 2008; Vanderhoeven and others 2005). Despite the potential for sexual reproduction between related congeners (Tiébré and others 2007b), Japanese knotweed is known for its massive and particularly efficient vegetative reproduction (Hollingsworth and Bailey 2000). Rhizome fragments of as little as 0.7 g of fresh weight can give rise to new shoots (Brock and Wade 1992), and stem or leaf tissues have also been observed to regenerate new plants (Brabec 1997; Brock and others 1995; Child and others 1992). Natural dispersal involves transportation of rhizome and stem fragments along watercourses. However, human activities, such as the conveyance of contaminated soil for construction and transportation of stems and rhizomes as garden waste, play an important role in the long-distance dispersal of the species (Beerling and others 1994). When plants are cut as part of a control program, propagules can also be dispersed throughout the landscape (Beerling and others 1994).

Managing such a widespread, detrimental and easily-dispersed invasive plant currently presents a considerable challenge for land managers. Attempts have been made to control Japanese knotweed using mechanical, chemical or biological techniques. Repeated mechanical cutting can reduce the resources of the rhizome if regularly performed over the growing season (Seiger and Merchant 1997). However, mechanical control is often insufficient and may even promote further invasion (Beerling and others 1994; McHugh 2006). Complete excavation can eliminate small stands, but the costs are significant and the efficiency questionable: the feasibility depends on soil type, digging the entire rhizome is generally difficult and the problem of plant material disposal still has to be solved (Soll 2004; Baker 1988). Chemical control by herbicides, notably glyphosate and imazapyr, has also been met with mixed success (reviewed in Bashtanova and others 2009). Child and Wade (2000) recommended triclopyr, imazapyr, glyphosate and picloram, and 2,4-D amine, but none of these herbicides can completely eradicate *F. japonica* stands. Overdosing, repeated application, as well as follow-up spot treatments of regrowth are often considered necessary. In addition, the herbicides tested in the literature cannot always be used by managers due to legal constraints and possible detrimental effects to the environment. For instance, Bashtanova and others (2009) suggested a good efficiency for imazapyr, but this active substance is excluded from Annex 1 of Directive EEC 91/414 and therefore, is no longer authorized for use in the European Union (EU). Herbicide application is generally performed by spraying the above-ground plant material. However, stem injection has been proposed as an encouraging alternative application method on *F.* x *bohemica* (Hagen and Dunwiddie 2008). *F. japonica* has been identified as a target for biological control in the UK (Shaw 2003; Shaw and others 2011), but large-scale application of biological control will not occur for years (Child and Wade 2000; de Waal 1995; Shaw and others 2011).

To date, land managers faced with knotweed invasion still lack information for decision making, and further research is needed on the types of control possible. Among other limitations, the following are the most problematic: (1) few studies have simultaneously compared chemical, mechanical and re-vegetation techniques, (2) the way efficiency is measured greatly varies among the studies, (3) several studies have only been performed over one growing season, so no information about the following years is given, (4) herbicide rates used in experimental studies are often higher than the authorized rates in a particular country, (5) not all experimental studies are validated in the field, and (6) most studies do not provide costs or labor estimates. In addition, constraints encountered by land managers are multiple and include site accessibility and/or herbicide prohibition in some areas (e.g., near rivers or catchments).

Thus, contrary to that observed with other problematic invasive plants in Belgium such as *Impatiens glandulifera* Royle or *Heracleum mantegazzianum* Sommier and Levier, there is still no consensus about how knotweed should be controlled (Delbart and others 2010), or if it should be controlled at all. Land managers cannot confidently predict the success and cost of a management campaign.

The present study fits within the practical conditions encountered by land managers. As recommended by Kabat and others (2006), we assessed the effectiveness of several control methods on a relatively long-term basis and evaluated post-treatment rhizome viability. We tested different chemical herbicides and two application methods, as well as mechanical treatments, on *F. japonica* clones throughout southern Belgium. The main working questions were: (i) among the chemical and mechanical control methods available to managers, which efficiently reduce stem height, stem volume and stem density of the controlled clones? (ii) For chemical treatments, is there a difference between spraying and stem injection? (iii) Do treatments leading to a 100 % volume reduction after 1 year really kill *F. japonica* clones? (iv) What do chemical and mechanical control methods represent in terms of labor? The assumption was made that managers would choose a single technique and apply it only once on a stand.

Material and Methods

Herbicide Control Experiment

Field Experiment

Forty clones were selected in Southern Belgium. They were selected based on the absence of previous management trials and the absence of waterways or buildings in the near vicinity. The distance between clones ranged from 5 to 106 km and the clone area ranged from 5.7 to 85.1 m^2 (mean: 31.2 m^2). A 1 × 1 m plot was installed in the central part of each clone, for measurements.

Eight commercial herbicides[1], or combinations thereof (Table 1), were used. All of them already contained at least one adjuvant/surfactant and no additional surfactants were used. Each was applied at the maximal authorized rate in Belgium (based on www.fytoweb.fgov.be). In addition, to assess whether the authorized rate was a limiting factor, two were applied at twice the authorized rate. This resulted in 10 different *herbicide treatments*. From mid-August to mid-September 2007, each herbicide treatment was applied to four clones, two by injection and two by spraying. As it was impossible to distinguish different individuals (i.e., independent clones, not connected underground) in a stand, a single treatment was applied to each stand to avoid interferences between treatments. The application period was chosen to maximize herbicide translocation to rhizomes (Beerling and others 1994; Bashtanova and others 2009; Price and others 2001). At this period of the year, stem height is more than 2 m and most knotweed plants are flowering. For both *application methods*, the same rate of active substances per hectare was used. To achieve this, the volume of water used was different between injection and spraying. In all case, the entire clone was treated. Herbicide application was made on intact stems (no cut before application).

In the injection method, each stem exceeding 1.5 cm in diameter was injected with 5 ml of herbicide solution using a JK Injection Tool (Battle Ground, Washington, USA). As stems <1.5 cm in diameter could not be injected, the closest injectable stem was injected twice, so that the total number of injections was equal to the number of stems in the clone. Injection was performed between the first and second visible node. When a stem was injected twice, the internode higher up the stem was injected. Based on stem density and clone area measurements, the quantity of water in the injection mixture was regulated to give a constant herbicide rate per hectare. For instance, to inject 3.6 kg ha^{-1} of acid equivalent (AE) of herbicide when the stem density was 20 stem m^{-2} and clone area 10 m^2, the mixture had to be composed of 0.036 kg AE (based on the desired rate per ha and clone area) and 1,000 ml of water (based on the number of stems to inject).

Foliar application was performed with a backpack sprayer (Velmorel 2000 Pro®, Berthoud, Belleville, France) coupled to a red fan jet nozzle (Albuz®), using 1300 l ha^{-1} of water. The quantity of herbicide was adapted to reach the desired rate per ha. As for injection, the rate of foliar application stayed the same on a per area basis.

Plots were evaluated in August 2008. Height (h, cm) and diameter (d, cm, measured at the basis of the stem) of all stems in the plot were measured. Stem volume was calculated as $v = \pi/12 \times h \times d^2$ (volume of a cone shape). The number of stems was counted for each plot. Volume reduction (VR, cm^3 m^{-2}) was calculated for each plot as $(V_0 - V_1)/V_0$ where V_0 is the cumulated stem volume in the plot before herbicide application, and V_1 the cumulated stem volume the following year. Mean height reduction per plot (HR, cm) and density reduction (DR, stem number per m^2) were calculated the same way. VR, HR and DR were analyzed using a two-way ANOVA after rank transformation. Rank transformation was used because the data did not meet the assumption for parametric tests (Conover and Iman 1981). *Herbicide treatment* (fixed) and *application method* (fixed) were crossed. As a strong herbicide treatment effect was found, we performed a Dunnett simultaneous comparison of the 10 herbicide treatments with the most efficient one as control.

In September 2009, plots in the treatments that showed efficiencies comparable to the most efficient treatment were re-evaluated. Stem volume was calculated as before and the stem density was estimated. The two-year volume reduction (VR2, %) was calculated as $(V_0 - V_2)/V_0$ where V_2 is the cumulated stem volume in 2009. The mean height reduction per plot (HR2, %) and density reduction (DR2, %) were calculated the same way. VR2, HR2 and DR2 were analyzed using a two-way ANOVA on the data from the 20 re-measured clones, after rank transformation. *Herbicide treatment* (fixed) and *application method* (fixed) were crossed. In the case of a significant effect, Dunnett simultaneous comparisons were made with the most efficient herbicide treatment or application method.

When plots showed 100 % VR in August 2008, the clones where monitored in March 2009 to assess the presence of sprouting shoots outside of the plot. The presence of sprouting shoots would indicate that the clone was still alive. If no sprouting shoots were detected in March 2009, we collected pieces of rhizomes at three different locations from the periphery of the plot, at each of the following depths: 10, 20, 40 and 60 cm (resulting in 12 pieces of rhizome per clone).

[1] 2,4-D (Aminex®, Agriphar S.A.), Fluroxypyr (Starane®, Dow AgroSciences), Fluroxypyr + aminopyralid (Bofort®, Dow AgroSciences), Glyphosate (Roundup® Max, Monsanto), Triclopyr (Garlon®, Dow AgroSciences), Triclopyr + aminopyralid (Garlon® Super, Dow AgroSciences)

Table 1 Details of the 10 herbicide treatments applied in the herbicide control experiment, each on two clones

Herbicide treatment	Active substances and concentrations (g l^{-1})		Authorized rate (kg AE ha^{-1})		Applied rate (kg AE ha^{-1})	
	Formulated herbicide 1	Formulated herbicide 2	Formulated herbicide 1	Formulated herbicide 2	Formulated herbicide 1	Formulated herbicide 2
1	Fluroxypyr (180)[a]	–	0.36	–	0.36	–
2	Fluroxypyr (100) + aminopyralid (30)[b]	–	0.2 + 0.06	–	0.2 + 0.06	–
3	Fluroxypyr (100) + aminopyralid (30)[b]	–	0.2 + 0.06	–	0.4 + 0.12	–
4	Triclopyr (480)[c]	–	7.2	–	7.2	–
5	Triclopyr (240) + aminopyralid (30)[d]	–	0.48 + 0.06	–	0.48 + 0.06	–
6	Glyphosate (450)[e]	–	3.6	–	3.6	–
7	Glyphosate (450)[e]	–	3.6	–	7.2	–
8	Glyphosate (450)[e]	2,4-D Amine (500)	3.6	1.2	3.6	1.2
9	Glyphosate (450)[e]	Triclopyr (480)[c]	3.6	0.72	3.6	0.72
10	Glyphosate (450)[e]	Triclopyr (240) + aminopyralid (30)[d]	3.6	0.48 + 0.06	3.6	0.48 + 0.06

Authorized rate refer to rates legally accepted in Belgium (www.fytoweb.be)

AE acid equivalent of active substance, *CAS* database code of adjuvants/surfactants

[a] CAS 26264-06-2: 1–5 % (w/w), CAS 000872-50-4: 5–10 % (w/w) and CAS 064742-94-5: 50–60 % (w/w)

[b] CAS 64742-94-5: 30–40 % (w/w), CAS 34590-94-8: 20–30 % (w/w), CAS 68585-34-2: <10 % (w/w) and CAS 00095-63-6: <5 % (w/w)

[c] CAS 008008-20-6 and confidential: 38.4 % (w/w)

[d] CAS 00057-55-6: <5 % (w/w)

[e] Confidential

Post-treatment Rhizome Viability Test

Rhizome pieces collected in the field were weighed to the nearest 0.1 g and planted in pots in a greenhouse in March 2009. Similar rhizome samplings were made on one non-treated clone, as a control. The mean mass (±standard error) of the collected pieces of rhizome was 40.2 ± 5.34 g. After five months, the cumulated stem volume produced by each rhizome piece was estimated. Rhizome pieces that did not produce stems were considered dead. Stem volume was analyzed using a two-way ANCOVA after rank transformation (covariate: rhizome piece mass, g). *Treatment* (the combination of herbicide treatment and application method) was fixed and crossed to *depth* (fixed). In the case of a significant effect, Dunnett simultaneous comparisons were made with the most efficient treatment.

Mechanical Control Experiment

Six additional clones were selected in southern Belgium, 5–87 km apart. The clone area ranged from 14.8 to 358 m^2 (mean: 128.6 m^2). A 1 × 1 m plot was installed in the central part of each clone for measurements.

Three mechanical control methods were tested, each on two clones: (1) single cut, performed at the biomass peak in August 2007, (2) monthly cuts from July to October in 2006, 2007 and 2008 and (3) monthly cuts from July to October 2006, followed by the plantation of native willow cuttings in spring 2007 (*Salix* sp., 50–80 cm long, collected in the vicinity of the clones and transplanted at a 30 cm depth) with a density of 5 cuttings m^{-2}, then by monthly cuts from May to October in 2007 and 2008. In the latter mechanical treatment, monthly cuts during the two last years started earlier to facilitate the growth of willows over *F. japonica*. The cuts were performed with a portable hand-held brush cutter the first year and then with an Italian sickle. The latter enabled the mechanical control of knotweed and did not cut the willows. *F. japonica* stems were amassed on the site. The resulting piles were surveyed for potential development of new roots, but no further development was observed.

In September 2009, VR, DR and HR were calculated as above. A one-way ANOVA was used to compare mechanical control modalities, after rank transformation. In the case of a significant effect, Dunnett simultaneous comparisons were made with the most efficient method.

Labor Estimation

The area of all the 46 clones, as well as the time and the number of people needed to manage them, was recorded.

Only the effective treatment time, not the preparation, was taken into account. Labor was estimated as the mean area treated per person and per hour. Note that one person alone performed stem injection, whereas spraying was performed by two workers together. For repeated cuttings, 10–11 operations were made throughout the season that consisted of both cutting the stems and piling them up, whereas 12–15 operations were necessary for repeated cutting followed by native willow transplanting. The time required for each operation was recorded.

Results

Herbicide Control Experiment

Field Experiment

One year after treatment (YAT), knotweed VR, DR and HR were significantly different ($F_{9,20} = 10.05$; 5.16 and 11.96, respectively; all P values ≤ 0.001). In contrast, the application method and the interaction with herbicide treatment did not significantly affect the results. The herbicide treatment that led to the greatest volume reduction was the injection of 3.6 kg AE ha^{-1} of glyphosate (Table 2). Dunnett simultaneous comparisons showed that all glyphosate treatments and fluroxypyr + aminopyralid at twice the authorized rate were comparable to the best treatment control (Table 2). Similarly, height was reduced the most by glyphosate at 3.6 kg ha^{-1} and other glyphosate applications (Table 2). All treatments, except for fluroxypyr at 0.36 kg ha^{-1} and triclopyr at 7.2 kg ha^{-1}, reduced stem density one YAT, comparable to glyphosate at 3.6 kg ha^{-1} (Table 2).

Only the clones treated with glyphosate alone or in combination were re-evaluated 2 YAT. While neither herbicide treatment nor application technique affected VR2 or DR2 2 YAT, HR2 was affected by both. The interaction between herbicide treatment and application technique was not significant. Stem injection shortened knotweed stems by 9.2 cm compared to foliar application (data not shown). HR2 was similar among all glyphosate treatments (average height 2 YAT: 69.1 cm), except for glyphosate + 2,4-D (average height 2 YAT: 163.5 cm).

Post-treatment Rhizome Viability Test

VR 1 YAT was 100 % in eight out of the 40 field plots. Among the eight clones, five had sprouting shoots developing on the field the following year, whereas three showed no shoot development. Rhizomes were collected from the latter three clones that had been injected with glyphosate at 3.6 kg ha^{-1} (two clones) or foliarly-treated with glyphosate at 7.2 kg ha^{-1} (1 clone).

After 5 months in the glasshouse, at least one rhizome piece from all clones showed sprout development, indicating that no clone had been killed. However, Dunnett simultaneous comparisons revealed that injection of 3.6 kg ha^{-1} of glyphosate led to a highly significantly lower proportion of rhizome pieces producing shoots. With this treatment, 41.7 % of rhizome pieces still sprouted. From the no treatment control clone, 83.3 % of rhizomes sprouted. The cumulated stem volume produced from the 12 rhizome pieces per clone significantly differed according to the treatment ($F_{2,35} = 19.98$; p value <0.001) and the interaction between treatment and depth ($F_{6,35} = 2.62$; p value $= 0.034$). The lowest cumulated stem volume corresponded to the injection of 3.6 kg ha^{-1} of glyphosate. For glyphosate-treated clones, deeper rhizome pieces tended to produce lower stem volume, but this was not the case for the control clone. The mass of the rhizome pieces, used as a covariate, also significantly influenced stem volume ($F_{1,35} = 30.70$; p value <0.001). Heavier rhizome pieces tended to lead to higher cumulated stem volumes.

Mechanical Control Experiment

Mechanical treatments were generally less effective than the best chemical treatments (Table 2). VR differed according to the mechanical treatment ($F_{2,3} = 16$; p value $= 0.025$), but density and height reduction did not. Stem volume was reduced the most by repeated cutting followed by willow transplanting, although repeated cutting alone resulted in a similar VR (Dunnett simultaneous comparisons; p value $= 0.107$). After 1 year, the average height of transplanted willow cuttings was 1.9 m. After 2 years, the average height was 2.6 m. It has to be noted that a single cut performed at the biomass peak actually led to stem volume and stem density increase 1 YAT (Table 2).

Labor Estimation

The least laborious treatment was stem injection (87.9 m^2 h^{-1} men^{-1}), followed by spraying (74.3 m^2 h^{-1} men^{-1}). Labor was 26.2 m^2 h^{-1} men^{-1} for single cuts, 9.1 m^2 h^{-1} men^{-1} for repeated cuttings (10–11 operations) and only 1.8 m^2 h^{-1} men^{-1} for repeated cuttings combined with willow transplanting (12–15 operations).

Discussion

Among the different control methods considered in the present study, none resulted in the complete elimination of the clones 1 or 2 YAT. These clones were relatively small in area, so the control methods tested here should not be

Table 2 Japanese knotweed (*F. japonica*) stem volume reduction (VR and VR2), height reduction (HR and HR2) and density reduction (DR and DR2), one (1 YAT) and two (2 YAT) years after the different control methods (means ± standard deviation)

Control methods	1Yat			2Yat		
	VR (%) ± SD	HR (%) ± SD	DR (%) ± SD	VR2 (%) ± SD	HR2 (%) ± SD	DR2 (%) ± SD
0.36 kg AE ha^{-1} fluroxypyr—injected	32.4 ± 9.8[b]	13.3 ± 7.7[b]	7.9 ± 19.1[b]			
0.36 kg AE ha^{-1} fluroxypyr—sprayed	64.1 ± 0.2	16.2 ± 17.4	23.5 ± 13.9			
0.2 kg AE ha^{-1} fluroxypyr + 0.06 kg AE ha^{-1} aminopyralid—injected	91.8 ± 8.5[b]	51.5 ± 4.0[b]	70.7 ± 29.3[a]			
0.2 kg AE ha^{-1} fluroxypyr + 0.06 kg AE ha^{-1} aminopyralid—sprayed	59.9 ± 54.7	45.5 ± 40.1	50.3 ± 19.7			
0.4 kg AE ha^{-1} fluroxypyr + 0.12 kg AE ha^{-1} aminopyralid—injected	94.9 ± 7.2[a]	68.6 ± 44.4[b]	86.4 ± 19.3[a]			
0.4 kg AE ha^{-1} fluroxypyr + 0.12 kg AE ha^{-1} aminopyralid—sprayed	71.9 ± 32.2	27.3 ± 14.5	67.9 ± 21.9			
7.2 kg AE ha^{-1} triclopyr—injected	−55.5 ± 193.3[b]	13.2 ± 22.1[b]	−27.2 ± 55.8[b]			
7.2 kg AE ha^{-1} triclopyr—sprayed	−88.9 ± 67.4^{-1}	−18.7 ± 12.2^{-1}	−18.6 ± 17.2			
0.48 kg AE ha^{-1} triclopyr + 0.06 kg AE ha^{-1} aminopyralid—injected	87.2 ± 12.0[b]	31.9 ± 0.3[b]	75.4 ± 20.6[a]			
0.48 kg AE ha^{-1} triclopyr + 0.06 kg AE ha^{-1} aminopyralid—sprayed	40.3 ± 11.6	9.3 ± 22.8	37.0 ± 24.1			
3.6 kg AE ha^{-1} glyphosate—injected	**100.0 ± 0.0[a]**	**100.0 ± 0.0[a]**	**100.0 ± 0.0[a]**	99.9 ± 0.0	**99.2 ± 1.2[a]***	37.5 ± 88.4
3.6 kg AE ha^{-1} glyphosate—sprayed	**99.9 ± 0.1**	**94.4 ± 3.0**	**67.7 ± 32.0**	97.7 ± 0.6	**75.0 ± 6.9**	74.6 ± 22.3
7.2 kg AE ha^{-1} glyphosate—injected	95.3 ± 5.4[a]	62.6 ± 42.1[a]	52.8 ± 41.4[a]	93.4 ± 8.6	55.1 ± 48.5[a]	77.0 ± 3.5
7.2 kg AE ha^{-1} glyphosate—sprayed	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	99.4 ± 0.0	74.9 ± 5.7	71.9 ± 22.0
3.6 kg AE ha^{-1} glyphosate + 1.2 kg AE ha^{-1} 2,4-D amine—injected	100.0 ± 0.0[a]	80.0 ± 1.8[a]	95.8 ± 0.5[a]	80.2 ± 26.2	13.4 ± 34.1[b]	84.4 ± 16.6
3.6 kg AE ha^{-1} glyphosate + 1.2 kg AE ha^{-1} 2,4-D amine—sprayed	98.7 ± 0.1	81.0 ± 9.1	73.3 ± 10.6	91.7 ± 0.3	56.1 ± 2.3	52.8 ± 1.5
3.6 kg AE ha^{-1} glyphosate + 0.72 kg AE ha^{-1} triclopyr—injected	99.7 ± 0.5[a]	96.8 ± 4.5[a]	72.4 ± 39.0[a]	99.6 ± 0.5	90.9 ± 3.8[a]	13.5 ± 56.3
3.6 kg AE ha^{-1} glyphosate + 0.72 kg AE ha^{-1} triclopyr—sprayed	99.8 ± 0.3	95.6 ± 6.2	72.7 ± 38.6	84.9 ± 20.7	56.9 ± 14.7	59.1 ± 32.1
3.6 kg AE ha^{-1} glyphosate + 0.48 kg AE ha^{-1} triclopyr + 0.06 kg AE ha^{-1} aminopyralid—injected	100.0 ± 0.0[a]	92.4 ± 10.7[a]	99.2 ± 1.2[a]	99.3 ± 0.0	80.4 ± 8.5[a]	16.4 ± 85.6
3.6 kg AE ha^{-1} glyphosate + 0.48 kg AE ha^{-1} triclopyr + 0.06 kg AE ha^{-1} aminopyralid—sprayed	98.8 ± 1.2	74.1 ± 3.6	79.5 ± 8.8	54.9 ± 39.9	30.1 ± 7.3	48.6 ± 12.1
Summer cut	−63.8 ± 139.5[β]	6.3 ± 15.1	−51.1 ± 37.0			
Repeated cuts	78.6 ± 12.9[α]	9.4 ± 3.2	45.8 ± 17.7			
Repeated cuts + native tree transplanting	**99.7 ± 0.0[α]**	76.0 ± 5.7	60.5 ± 5.7			

Control methods in the table correspond to a combination of the factors herbicide treatment and application method for the herbicide control experiment, and to the different control modalities in the mechanical control experiment. In the case of a significant effect of herbicide treatment: Latin letters correspond to Dunnett simultaneous comparisons with the best herbicide treatment (in bold) for the herbicide control experiment; Greek letters correspond to Dunnett simultaneous comparisons with the best mechanical treatment (in bold) for the mechanical treatment experiment

* Indicates that application methods were significantly different

expected to eliminate Japanese knotweed in southern Belgium, where much larger clones occur. Although the most effective treatment eliminated knotweed shoots 1 YAT, peripheral rhizomes were able to sprout, indicating the plant's ability to survive treatment and potentially establish new clones.

Some of the control methods tested reduced the harmfulness of the clones through important reductions in volume, height and density, sometimes coupled with a reduction of the vigor of sprouting shoots produced by peripheral rhizomes. Through the use of an efficient active substance, herbicide application can curb the development of Japanese knotweed. This can reduce the nuisance caused by the species, where it is acceptable from an environmental point of view. Where the hazard linked to herbicide use makes it unacceptable, mechanical solutions still exist to hamper the development of the clones.

Since (i) each isolated stand was considered a single clone and experienced a single control treatment to avoid interferences between treatments through rhizome

exchanges, and (ii) a large number of control methods were considered, only a limited number of replicates were available for each treatment. It has to be noted, however, that all *F. japonica* individuals in southern Belgium are known to be of the same genotype (Tiébré and others 2007a). Therefore, all treatments were applied to the same genotype and replicated in different environmental conditions. Based on the present results, future experiments should focus on a lower number of better replicated control treatments. As pointed out in the recent literature, combinations of treatments (e.g., herbicide that interrupts protein synthesis followed by herbicide that interrupts phloem transport) could also represent opportunities to better curb *F. japonica* clone development (Bashtanova and others 2009).

Glyphosate was the most effective herbicide tested. Efficacy was not affected by combination with 2,4-D, triclopyr, or triclopyr + aminopyralid. Fluroxypyr + aminopyralid and triclopyr alone did not adequately control Japanese knotweed. Our results are consistent with a recent study by Rudenko and Hulting (2010) that showed that triclopyr and 2,4-D hardly affected knotweed clones.

Stem injection and spraying did not yield different volume, height or density reductions the year following the treatment. However, several aspects made stem injection a more promising method for glyphosate application, as already demonstrated in the literature (Hagen and Dunwiddie 2008). First, even if volume, height and density reductions did not differ the year following application, peripheral rhizomes were less vigorous when the clone was stem-injected than sprayed, as indicated by the post-treatment rhizome viability test. This suggests that stem injection allowed a better translocation of the chemical through the rhizome. Second, two years after application, stem injection reduced stem height to a greater extent than spraying. Third, in our case, glyphosate treatment led to a 100 % volume reduction the following year for three clones: two stem-injected and one sprayed. The last had received twice the rate compared to the former two, indicating that stem injection requires less quantity of herbicide and that the authorized rate in Belgium is sufficient to efficiently curb clone development. Moreover, stem injection was a little less laborious than spraying. Operators in our case needed to work above the canopy for homogeneous and complete spraying, which induced the use of a ladder and slowed the operator's movement in the field. It has to be noted, however, that spraying may have been faster on a lower canopy, e.g., if preliminary cuts had been performed before herbicide application. Finally, as pesticide drifts can have dramatic effects on the native flora and fauna, spraying can hardly be performed on windy days, but applying injections remains possible.

Stem injection with 3.6 kg AE ha^{-1} of glyphosate was the most efficient treatment in this study. When controlled with this method, central parts of the clones did not produce any sprouting shoot the year following application. The second year, however, abundant sprouting shoots were present that were very short, stunted and non-injectable. Our results with this active ingredient are comparable to those found in the literature, even though the dose we injected per stem was much lower (approximately 0.15 g AE $stem^{-1}$ in our trials against 0.72 to 2.4 g AE $stem^{-1}$ in the literature (Hagen and Dunwiddie 2008)). Such clone development is likely not to have the same detrimental or undesirable effects than that of non-treated clones. Longer-term studies are needed to identify even more efficient techniques, notably by combining stem injection and mechanical or chemical treatments the following years.

If glyphosate-based herbicide injection is a promising method to limit knotweed development and potentially slow down the species' spread, it cannot be applied everywhere due to its potential detrimental effect on natural ecosystems. Most glyphosate-containing products are either made or used with a surfactant, which help glyphosate penetrate plant cells. Glyphosate and the surfactants it is formulated with have been shown to have detrimental effects on many non-target organisms such as bacteria, protozoa, microalgae, crustaceans (Tsui and Chu 2003), earthworms (Casabé and others 2007), and fish (Folmar and others 1979; Nešković and others 1996). As such, it is forbidden in Belgium along rivers. In that case, repeated cuttings can be an interesting alternative for managers, especially if they are followed by transplanting with native trees such as willow (*Salix* spp). Those techniques require much more time and labor than chemical control, but are not detrimental to natural habitats and allow a substantial decrease of clone development.

Even if not all possible control methods for Japanese knotweed were assessed in the present study, our results indicate that eradicating Japanese knotweed from Belgium is probably an unmanageable task. Land managers facing Japanese knotweed invasion should therefore see the mechanical and chemical control methods as tools to curb clone development and slow invasion, where objectively necessary. These techniques should however be integrated in a more global, integrated strategy at the landscape level. Managers should consider maintaining *F. japonica* stands at low densities and integrating them among the native flora as a reasonable goal. Management strategies should include the prevention of rhizome movement (through soil movement and river floods, etc.) and public awareness campaigns, in addition to control methods.

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