Assessment of Management Strategies for Japanese knotweed (*Reynoutria japonica*) in Nova Scotia

by

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ABSTRACT

Japanese knotweed (*Reynoutria japonica*) is a problematic invasive species in Europe and North America that causes significant reductions in native plant diversity, increases soil erosion, leads to riverbank destruction, and can negatively affect property value and desirability. In Nova Scotia, knotweed has invaded numerous riparian zones, among other habitats, creating demand for effective and reliable management strategies. This project evaluated a variety of management strategies for their ability to reduce Japanese knotweed stem density, height, and diameter within the growing season and in the year after treatment. Chapter II evaluated the effect of cutting alone and integration of cutting and herbicide applications. Cutting significantly reduced knotweed stem height and diameter within the growing season, however stem density was not significantly reduced within the growing season or 1 YAIT. There was no significant interaction between cutting and herbicide 1 YAIT on knotweed stem density at either location. Applications of glyphosate to peak height growth and knotweed regrowth following cutting reduced knotweed stem density 1 YAIT. Applications of aminopyralid did not significantly reduce knotweed stem density 1 YAIT when applied at peak height or to regrowth. However, the re-application of treatments in the second growing season did lead to a significant reduction in knotweed stem density. Chapter III investigated if various herbicide application methods varied in their ability to control Japanese knotweed. Spot applications and stem injections of glyphosate or aminopyralid reduced knotweed stem height and diameter 1 YAIT, however knotweed stem density was only reduced by the end of the second growing season following a second application of treatments, indicating both require multiple applications to be successful strategies. A cut-stump application of glyphosate significantly reduced knotweed stem density by the end of the growing season, however knotweed completely recovered 1 YAIT. In contrast, a spot application of glyphosate resulted in stem density reductions 1 YAIT. Chapter IV evaluated if the seasonal timing of herbicide application impacted their ability to control knotweed. Peak height spot applications (mid-June) of imazapyr and glyphosate significantly reduced knotweed stem density 1 YAIT. Fall spot applications (end of September) of imazapyr, glyphosate and aminopyralid significantly reduced knotweed stem density by June of the following growing season, with aminocyclopyrachlor not providing significant stem density reductions in either timing. Japanese knotweed is a manageable species but requires considerable effort and commitment to control.

LIST OF ABBREVIATIONS USED

a.e	Acid equivalent
a.i	Active ingredient
AMPA	Aminomethylphosphonic acid
ANOVA	Analysis of variance
BASF	A German agrochemical company that produces herbicides
£	British pounds
cm	Centimeter
$	Dollars
g	Gram
ha	Hectare
kg	Kilogram
km	Kilometer
L	Liter
LAI	Leaf Area Index
LAR	Leaf Area Ratio
m	Meter
mg	Milligram
mL	Milliliter
n	Number (sample)
NSAGC	Nova Scotia Atlantic Gardening Club
NSTIR	Nova Scotia Transportation and Infrastructure Renewal
SD	Standard Deviation
UK	United Kingdom
US/USA	United States of America
USD	United States Dollar
YAT	Year After Treatment
YAIT	Year After Initial Treatment

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CHAPTER 1: INTRODUCTION

For as long as humans have practiced agriculture and horticulture, we have transported species vast distances to meet our demand for food, fuel, and fiber. This ability to domesticate plants and animals has made us a largely successful species, with a population in excess of seven billion across the earth's continents. As a result, the various species we brought with us have established in places they may never have reached without our intervention. While many species that we have introduced to new areas have been benign, there are several species introductions that have resulted in considerable economic, environmental and human consequences, including giant hogweed (*Heracleum mantegazzianum*), purple loosestrife (*Lythrum salicaria*), and my old friend and species of concern, Japanese knotweed (*Reynoutria japonica*).

Japanese knotweed was first introduced from Japan to Europe via the Netherlands, circa 1830s by Phillip Franz Balthasar von Siebold (Townsend 1997). From there, it spread to the UK and North America for its use in the horticultural trade. Knotweed was praised for characteristics such as creating groves, sheltering young plantings, and protecting sandy hills and dunes from erosion (Townsend 1997). Knotweed rapidly spread through North America via horticultural magazines and community sales. However, as early as 1884, there were reports of aggressive behavior by knotweed. William Robinson warned in *The Garden* magazine that knotweed should not be planted in border settings. "Not plants for the border, being of such spreading growth and being gross feeders would soon overrun and harm plants of weaker character" (Townsend 1997). This rapid spread and overpowering growth is verified by modern accounts. Vanderklien et al. (2013) found that knotweed could intercept up to 90% of incoming light, leading to a heavily shaded understory where few other plants can survive. By the 1920s knotweed had fallen out of favor, with more articles being written about how to kill it than propagate it (Townsend 1997), with varying degrees of success.

The dominant canopy knotweed produces prevents native plants from growing in affected areas, leading to an absence of ground cover (Beerling et al. 1994). The lack of ground cover is followed by an acceleration of soil erosion, sediment loading in streams, and riverbank destruction (Dawson and Holland 1999; Child and Wade 2000). As such, knotweed has gained a reputation as a major invasive species in riparian zones and wetlands. Further, in the UK knotweed can pose an economic hazard as well. In some cases the value of property has been reduced by as much as 84% due to the presence of Japanese knotweed (Elliot 2011), out of fear the plant may damage compromised building foundations. In preparation for the 2012 London Olympics, the UK government spent £70 million to clean up Japanese knotweed prior to building construction. This was achieved using a combination of spot applications of fluoxopyr and glyphosate, mass excavation, tissue incineration and burying tissue in membrane bags (Elliot 2011). These efforts were, in the short term successful, allowing construction to proceed. However, reports as of 2016 indicate the return of knotweed to the area (London Legacy Development Corporation (LLDC) 2016), requiring additional management efforts. This indicates knotweed management is an on-going activity, requiring many years of treatment, in addition to follow-up care to prevent re-infection of the managed area.

Given the persistence and difficulty of managing knotweed, considerable research has been conducted on its management. Chemical control is broadly considered the most effective means of control, with glyphosate and imazapyr providing the most reliable control of knotweed (Child and Wade 2000). Differing application methods have also been evaluated for niche environments such as near bodies of water, where spot applications may not be appropriate. Delbart et al. (2012)

found spot applications and injections of glyphosate reduced knotweed stem density up to 1 year after treatment application. Other herbicides evaluated included 2,4-D, fluoxopyr, and triclopyr, however these showed limited efficacy (Delbart et al. 2012). Further to this, Larsen (2013) found spot applications of imazapyr reduced knotweed stem density 1 YAIT in Nova Scotia, while early emergence applications of aminopyralid only reduced knotweed stem density for two to eight weeks. Mechanical control strategies such as cutting knotweed to ground level have been widely evaluated and found to be ineffective at causing long-term density reductions (De Waal 1995; Child and Wade 2000; Delbart et al. 2012), but it has been found to be effective for short term outcomes such as improving site lines and removing obstructions.

Despite existing research, Japanese knotweed is still a difficult species to manage, owing to its high stem density, widespread populations, and remaining uncertainty about what management strategies are most effective for long term control efforts. This project set out with three objectives, to fill the gaps in existing literature: (i) determine if integration of mechanical and chemical control methods improves stem density reductions compared to either in isolation, (ii) ascertain if any particular herbicide application method is more effective at reducing knotweed stem density and, (iii) determine if herbicide applications at either peak height (mid-June) or in the fall (September/ October) produce any differences in stem density reductions or herbicide efficacy.

1.1 Citations

Beerling, D. J., Bailey, J. P., Conolly, A. P. (1994). Biological flora of the British Isles, Fallopia japonica. *Journal of Ecology, 82: 959-979.*

Child, L., & Wade, P. M. (2000). *The Japanese knotweed manual: The management and control of an invasive alien weed*. Packard Publishing.

Dawson, F. H., & Holland, D. (1999). The distribution in bankside habitats of three alien invasive plants in the U.K. in relation to the development of Control Strategies. *Biology, Ecology and Management of Aquatic Plants*, 193–201. https://doi.org/10.1007/978-94-017-0922-4_27

de Waal, L. C. (1993, September). Treatment of Fallopia japonica near water a case study. Plant invasions: general aspects and special problems. In *Workshop held at Kostelec nad Cernymi lesy, Czech Republic* (pp. 16-19).

Delbart, E., Mahy, G., Weickmans, B., Henriet, F., Crémer, S., Pieret, N., Vanderhoeven, S., & Monty, A. (2012). Can land managers control Japanese knotweed? lessons from control tests in Belgium. *Environmental Management*, *50*(6), 1089–1097. https://doi.org/10.1007/s00267-012-9945-z

Elliot, V. (2018). *Couple are forced to demolish their £300k four-bed home after it was invaded by Japanese knotweed*. Couple are forced to demolish their £300k four-bed home after it was invaded by Japanese knotweed - Property Article : Property Services Directory - AllAboutProperty.com. Retrieved May 5, 2019, from http://www.surveyorsandvaluers.com/articles/item.php?id=3140

Larsen, T. (2013). *Biology, ecological impacts, and management of Japanese knotweed (Polygonum cuspidatum syn. fallopia japonica) in Nova Scotia*. Dalhousie University, Halifax, N.S., Canada, M.Sc thesis.

London Legacy Development Corporation. (2016). *Information Request Reference 17-046.* Information Request. Retrieved May 19, 2019, from https://www.queenelizabetholympicpark.co.uk/~/media/lldc/publication%20scheme/foi%20responses/201601/15078%20response.pdf

Townsend, A. (1997). Japanese knotweed: a reputation lost. *Arnoldia*, *57*(3), 13-19.

Vanderklein, D. W., Galster, J., & Scherr, R. (2013). The impact of Japanese knotweed on stream baseflow. *Ecohydrology*, *7*(2), 881–886. https://doi.org/10.1002/eco.1430

CHAPTER 2: INTEGRATING MECHANICAL AND CHEMICAL MANAGEMENT STRATEGIES FOR JAPANESE KNOTWEED CONTROL

2.1 Abstract

Japanese knotweed (*Reynoutria japonica*) is a problematic invasive species in Europe and North America that causes significant reductions in native plant diversity, increases soil erosion, leads to riverbank destruction, and negatively affects property value. Cost-effective management strategies for knotweed are therefore highly sought after. While chemical control methods have proven effective against knotweed, limited research has been done evaluating the efficacy of integrated management that combines mechanical and chemical control. Experiments were conducted to determine i) the effect of repeated cutting on Japanese knotweed shoot regeneration and ii) the main and interactive effects of mechanical and chemical control on Japanese knotweed. Repeated cutting reduced Japanese knotweed stem density, diameter, and height in the year of application ($P < 0.05$), but these responses were not consistently reduced at one year after cutting, or at the end of the second growing season when treatments were reapplied. Aminopyralid did not consistently reduce stem density in the year following initial treatment, however at Bible Hill, stem density was reduced 1 YAIT and again reduced following a second treatment application. Glyphosate was consistently effective at reducing knotweed stem density in the year following initial treatment, and caused further reductions when treatments were reapplied in the growing season following initial treatment. Cutting stems at peak height followed by herbicide application to stem regrowth did not significantly improve stem density reductions achieved in the year following treatment. We conclude that integrated management does not improve the degree of knotweed control achieved but does make the application of herbicides easier via height reduction of knotweed stems following cutting.

2.2 Introduction

Japanese knotweed is an invasive plant species in North America and Europe. The plant is native to Japan and other East Asian countries, where it behaves as a primary colonizer in many ecosystems (Child and Wade 2000). In this setting, it stabilizes loose soil aggregates, helps attract other plant species to areas, and allows ecological succession to proceed. It was introduced to North America in the 1800s as a horticultural species (Townsend 1997), which likely helped facilitate spread of this species. Issues associated with knotweed became apparent in the 1920s when the plant fell out of favor with American horticultural societies (Townsend 1997) due to aggressive growth and difficulty removing established plants.

Japanese knotweed develops dense, monotypic stands of stems that can exceed 300cm in height (Siemens and Blossey 2007; Larsen 2013). These stems prevent light from reaching understory areas, preventing native plant species from growing (Beerling et al. 1994). For example, Larsen (2013) measured leaf area index (LAI) of knotweed, shrub, and grass habitats and found that knotweed had a 65% greater LAI than these other habitats. Reductions in native plant diversity typically lead to a lack of ground cover, resulting in significant increases in soil erosion that can lead to increased sediment loading in water courses (Dawson and Holland 1999; Child and Wade 2000). Further, this loss of native species has been reported to negatively impact foraging success of animal species such as Green frogs (*Lithobates calmintans*) (Maerz et al. 2005) and leaf shredding insects (Cleason et al. 2013). Effects on animals are not always consistent though, as Larsen (2013) found no significant effect of knotweed on arthropod abundance or diversity in Nova Scotia.

In addition to environmental effects, knotweed can have significant economic impacts on individuals and land value. Knotweed can easily spread across property boundaries due to its rhizomatous nature (Bailey et al. 2009), and rhizomes are easily introduced to properties via contaminated soil or gravel. Soil and gravel contaminated with knotweed fragments are considered a controlled waste in the UK (Mclean 2010). This can delay construction projects and require legal action if planted knotweed results in significant damage (McLean 2012). Reduction of home values of up to 80% have been reported (Elliot 2011), and it cost the United Kingdom government £70 million to remediate knotweed-infested sites prior to the onset of the 2012 London Olympics (Oliver 2011; Middleton 2014). It is clear from these examples that cost of knotweed management is extremely high and often out of reach for many individuals.

The ecological, environmental, and economic impacts on areas invaded by Japanese knotweed necessitate effective management strategies for this species. Chemical control is generally most effective, with the active ingredients glyphosate and imazapyr proving most effective in research to date. Spot applications and stem injections of glyphosate reduce knotweed stem density up to 1 year after application (Delbart et al. 2012). Spot applications of imazapyr provide similar levels of control (Larsen 2013), and this herbicide is generally considered to be the most effective treatment for Japanese knotweed (De Waal 1995; Child and Wade 2000; Delbart et al. 2012). Other herbicides evaluated include phenoxy herbicides such as 2,4-D, fluoxopyr and triclopyr, though efficacy is generally lower than that achieved with glyphosate or imazapyr (Delbart et al. 2012). Mechanical control methods such as cutting appear to be less effective than chemical methods, but do contribute to management of this species. A single cutting applied to knotweed stems in July and August in the UK reduced shoot regrowth, with the additional benefit of reduced shoot height in the following growing season (De Waal 1995). Delbart et al. (2012) also found that a single cutting knotweed stems at peak height reduced stem density in the following year. However, when monthly cuttings were applied in conjunction with willow tree planting (and without), stem density was increased 1 YAT. While evidence is conflicting, it is possible combining mechanical methods with other methods can lead to improved knotweed control.

A review of the literature by Miller (2016) found that using mechanical control in combination with herbicide application was more effective than doing either alone for many perennial weed species. For example, cutting and glyphosate application to regrowth improved control of perennial pepperweed (*Lepidium latifolium*) relative to cutting or herbicide applications alone (Renz and DiTamaso 1998). Additionally, cutting or mowing followed by glyphosate application to common reed (*Phragmites australis*) resulted in improved control compared to any of those treatments alone (Monterio et al. 1999). Control of Japanese barberry was also improved by combining a cutting or burning treatment with a summer glyphosate application (Brown et al. 1999), further indicating that integrated control may be helpful against perennial species. Results, however, are not always consistent. Mowing of Bohemian knotweed (*Polygonum* X *bohemicum*) and herbicide application to regrowth did not improve control of this species (Davenport 2006), though mowing did reduce Bohemian knotweed canopy height, facilitating easier herbicide applications. Effects of similar combinations of control strategies on Japanese knotweed are not well documented.

The objectives of this research were to determine i) the effect of repeated cutting on knotweed stem regeneration and ii) the main and interactive effects of cutting and herbicide application on knotweed shoot regeneration. It was hypothesized that i) repeated cutting would reduce knotweed stem density, stem diameter, and height within the year of cutting, but that these responses would not be affected by cutting 1 year after treatment, and ii) cutting knotweed stems at peak height and applying herbicides to regrowth would improve control relative to cutting and herbicide applications alone.

2.3 Materials and Methods

2.3.1 Effect of repeated cutting on Japanese Knotweed Shoot Regeneration

The objective of this experiment was to determine the effect of repeated cutting on Japanese knotweed shoot regeneration. The experiment was arranged in a completely randomized design with three treatments and 6 replications and 1m X 1m plot size. Plots were placed such that obvious knotweed stem clumps, as defined by Adachi et al. (1996), were in the center of each plot. Treatments were 1) control (no cutting), 2) cut once, and 3) cut twice. The experiment was conducted at the Bible Hill, Avon Street site (Figure 1B) and the Antigonish Rights River site (Figure 1C) and plots were established in spring 2016 at each site. Initial cutting was conducted when plants reached peak height (~300 cm) and the subsequent cutting was conducted approximately 1 month after the initial cutting when stem regrowth appeared to reach maximum height. Cutting treatments were conducted in both the 2016 and 2017 growing seasons at each site. Initial cuttings were conducted on June 16 2016 and June 27 2017 in Bible Hill and June 22 2016 and June 29 2017 in Antigonish. Subsequent cuttings were conducted on July 27 2016 and August 29 2017 at Bible Hill and July 27 2016 and August 22 2017 at Antigonish. Cutting was conducted using machetes and knotweed stems were cut to ground level at each cutting.

Figure 1. A) Bible Hill Farm site with a total area of 515 m^{-2} located at 45°22'8.17"N 63°15'26.74"W, B) Bible Hill Avon Street site with a total area of 320 m^{-2} and located at 45°22'17.14"N 63°16'39.70"W, C) Antigonish, Rights River with a total area if 425 m^{-2} located at 45°37'21.91"N 61°58'32.17"W, D) South Maitland, Site 3 with a total area of 151 m^{-2} located at 45°15'15.34"N 63°28'23.18"W.

2.3.2 Main and Interactive Effects of Cutting and Herbicides on Japanese Knotweed Shoot Regeneration

The objective of this experiment was to determine the main and interactive effects of cutting and herbicides on Japanese knotweed shoot regeneration. The experiment was a 2 x 3 factorial arrangement of cutting at peak height (no, yes), and herbicide (none, glyphosate, aminopyralid) arranged in a completely randomized design with 6 replications and 1 m x 1 m plot size. Plots were placed such that obvious knotweed stem clumps, as defined by Adachi et al. (1996) were in the center of each plot. The experiment was conducted at the Bible Hill farm site (Figure 1A) and South Maitland (Figure 1D). Cutting was conducted using a machete and stems were cut to ground level. Glyphosate and aminopyralid were applied at a rate of 9.0 and 2.4 g a.e. L water^{-1}, respectively, using a CO_2 pressurized sprayer equipped with a single Teejet AI11002-VS air induction nozzle. Stems were sprayed until all leaf surfaces were covered and initial dripping of spray occurred. Treatments were conducted in both 2016 and 2017 at Bible Hill and in 2017 only at South Maitland. Initial cutting and herbicide applications were conducted at Bible Hill in 2016 on June 16 and herbicide applications to regrowth following cutting were applied on July 27. In 2017, initial cutting and herbicide applications at Bible Hill were conducted on June 16 with herbicide applications to regrowth following cutting occurring on July 27. In South Maitland in 2017, initial cutting and herbicide treatments were applied on July 4 with the second herbicide application to regrowth occurring on August 7.

2.3.4 Data Collection and Statistical Analysis

Data collection in each experiment included knotweed stem density, plant height, and stem diameter at the time of cutting and herbicide applications, the end of the season in which cutting and herbicide treatments were applied, as well as in the growing season after cutting and herbicide treatments were applied. Stem density was determined on a whole-plot basis. Stem height and stem diameter were determined on 5 randomly selected stems in each plot.

The effect of cutting and the main and interactive effects of cutting and herbicides on knotweed stem density, stem height, and stem diameter were determined using ANOVA in Minitab version 18 (Minitab LLC, State College, USA, 2019) at the 0.05 level of significance. Cutting and herbicide were modeled as fixed effects in the ANOVA. Means separation, where necessary, was conducted using Tukey's means separation test at the 0.05 level of significance. Minitab18 normality test was used to determine if data met required assumptions. If needed, data were transformed using square-root and log transformations where indicated to meet normality and constant variance assumptions. Back transformed means are presented in all tables.

2.4 Results

2.4.1 Effect of repeated cutting on Japanese Knotweed Shoot Regeneration

There was a significant effect of cutting on knotweed stem density (P = 0.002), height (P = 0.001), and diameter (P = 0.001) at the end of the first season in which treatments were applied at Bible Hill, but only a significant effect on stem height (P = 0.001) and diameter (P = 0.001) at the end of the first growing season at Antigonish. There was a significant effect of cutting on stem height (P = 0.002) and diameter (P = 0.009), but not density (P = 0.299) at Bible Hill at 1 year after initial cutting treatments (YAIT), but cutting had no effect on stem density (P = 0.058), height (P = 0.135), or diameter (P = 0.181) 1 YAIT at Antigonish. Cutting had a significant effect on knotweed stem density (P = 0.001), height (P = 0.001), and diameter (P = 0.001) at the end of the second season in which treatments were applied at Bible Hill, but only on stem height (P = 0.001) and diameter (P = 0.001) at Antigonish.

One and two cuttings significantly reduced stem density at the end of the first growing season at Bible Hill (Table 1), though there was no additional density reduction associated with two cuttings. In contrast, one and two cuttings did not cause a significant reduction in knotweed stem density at the end of first growing season at Antigonish (Table 2). Stem height and diameter, however, were consistently reduced by cutting at each site. Stem height was reduced by each cutting treatment, though two cuttings caused greater reductions in stem height than one cutting (Tables 1 and 2). In contrast, reductions in stem diameter were similar in each cutting treatment (Tables 1 and 2).

Cutting treatments conducted in 2016 did not significantly reduce stem density 1 YAIT at Bible Hill or Antigonish (Tables 1 and 2). Similarly, cutting treatments conducted in 2016 did not reduce stem height and diameter at Antigonish at 1 YAIT (Table 2). In contrast, two cuttings in

2016 reduced Japanese knotweed stem height and diameter at 1 YAIT at Bible Hill (Table 1). Stem height and diameter at Antigonish, however, were not reduced 1 YAIT (Table 2).

Re-application of cutting treatments in 2017 reduced stem density by the end of the season at Bible Hill (Table 1), though reductions in density were once again similar between cutting treatments. Re-application of cutting treatments was once again ineffective at reducing stem density at Antigonish (Table 2). One cutting significantly reduced stem height at both sites, though height reductions were improved by applying two cuttings at each site (Tables 1 and 2). One and two cuttings also reduced the diameter of regenerating stems at each site (Tables 2 and 3), though reductions in stem diameter were again similar between cutting treatments.

Table 1. Effect of cutting treatments on Japanese knotweed stem density, height, and diameter at Bible Hill, Nova Scotia, Canada in 2016 and 2017.

Treatment	Prior to onset of cutting treatments in 2016	End of 2016 growing season following first application of cutting treatments[a]	1 YAIT[b]	End of 2017 growing season following re-application of cutting treatments[c]
		Density (stems m^{-2})		
No cutting	18.7 ± 2.4 a[d]	19 ± 1.6 a	18.2 ± 2.9 a	16.7 ± 2.0 a
Cut once	20 ± 2.1 a	10.5 ± 1.8 b	10.7 ± 1.3 a	8.5 ± 1.3 b
Cut twice	20 ± 3.6 a	6 ± 0.8 b	17 ± 4.2 a	2.5 ± 1.0 b
		Stem height (cm)		
No cutting	199.4 ± 12.7 a	220.4 ± 15.8 a	232.8 ± 4.6 a	229 ± 9.6 a
Cut once	202 ± 9.4 a	124.1 ± 2.8 b	186.2 ± 13.6 ab	79.3 ± 6.4 b
Cut twice	191.9 ± 8.9 a	53.6 ± 7.3 c	151.8 ± 35.9 b	24.8 ± 7.4 c
		Stem diameter (cm)		
No cutting	1.9 ± 0.1 a	1.9 ± 0.2 a	1.7 ± 0.1 a	2.0 ± 0.1 a
Cut once	2.0 ± 0.1 a	0.64 ± 0.1 b	1.1 ± 0.1 b	0.3 ± 0.1 b
Cut twice	1.9 ± 0.1 a	0.2 ± 0.1 b	0.9 ± 0.1 b	0.1 ± 0.1 b

[a] Initial and second cutting treatments in 2016 were conducted on June 16 2016 and July 27 2016, respectively.

[b] YAIT, year after initial cutting treatments.

[c] Initial and second cutting treatments in 2017 were conducted on June 27 2017 and August 29 2017, respectively.

[d] Means within columns followed by the same letter do not differ significantly according to Tukey's means separation test at the 0.05 level of significance. Values represent the mean ± 1 SD ($n = 6$).

Table 2. Effect of cutting treatments on Japanese knotweed stem density, height, and diameter at Antigonish, Nova Scotia, Canada in 2016 and 2017.

Treatment	Prior to onset of cutting treatments in 2016	End of 2016 growing season following first application of treatments[a]	1 YAIT[b]	End of 2017 growing season following re-application of treatments[c]
Density (stems m^{-2})[d]				
No cutting	24 ± 2.6 a[e]	4.6 ± 0.1 a (21)	19 ± 2 a	19 ± 1.6 a
Cut once	26 ± 3.4 a	4.2 ± 0.3 a (18)	14 ± 1 a	16 ± 1.6 a
Cut twice	15 ± 3.3 a	4.5 ± 0.7 a (26)	11 ± 0.8 a	11 ± 4.0 a
Stem height (cm)				
No cutting	199 ± 9.5 a	199 ± 12.2 a	204 ± 9.6 a	201 ± 6.9 a
Cut once	180 ± 7.4 a	127 ± 7.3 b	178 ± 16 a	100 ± 6.2 b
Cut twice	196 ± 9.6 a	63 ± 5.2 c	156 ± 13.6 a	34 ± 2.8 c
Stem diameter (cm)[f]				
No cutting	1.9 ± 0.1 a	0.3 ± 0.02 a (2.1)	0.2 ± 0.04 a (1.9)	2 ± 1.1 a
Cut once	2.1 ± 0.2 a	-0.2 ± 0.04 b (0.5)	-0.3 ± 0.04 a (1.6)	0.5 ± 0.4 b
Cut twice	1.9 ± 0.1 a	-0.4 ± 0.02 b (0.3)	-0.5 ± 0.06 a (1.0)	0.3 ± 0.3 b

[a] Initial and second cutting treatments in 2016 were conducted on June 22 2016 and July 27 2016, respectively.

[b] YAIT, year after initial cutting treatments.

[c] Initial and second cutting treatments in 2017 were conducted on June 29 2017 and August 27 2017, respectively.

[d] Density data for end of 2016 growing season was log(*x*) transformed prior to analysis to satisfy the assumptions of the ANOVA analysis. Transformed means are presented for means comparisons, and back-transformed means are given in parentheses.

[e] Means followed by the same letter do not differ significantly according to Tukey's test at the 0.05 level of significance. Values represent the mean ±1 SD ($n = 6$).

[f] Diameter data for end of 2016 growing season and 1 YAIT were log(*x*) transformed prior to analysis to satisfy the assumptions of the ANOVA analysis. Transformed means are presented for means comparisons, and back-transformed means are given in parentheses.

2.4.2 Main and Interactive Effects of Cutting and Herbicides on Japanese Knotweed Shoot Regeneration

There was a significant effect of cutting, herbicide, and the cutting by herbicide interaction on knotweed stem density at the end of the first season in which treatments were applied at Bible Hill (Table 3), but only a significant effect of cutting on density by the end of the first season at South Maitland (Table 4). Similarly, only cutting had a significant effect on knotweed stem height and diameter at each site at the end of the first growing season (Tables 3 and 4).

There was a significant effect of herbicide, but not cutting or the herbicide by cutting interaction on Japanese knotweed stem density at 1 YAIT at each site (Tables 3 and 4). Both cutting and herbicide had a significant effect on Japanese knotweed stem height at 1 YAIT at Bible Hill (Table 3), but only cutting significantly affected height at South Maitland at 1 YAIT. There was also a significant effect of cutting and herbicide on stem diameter at 1 YAIT at each site (Tables 3 and 4).

Glyphosate application at peak height did not reduce knotweed stem density, height, or diameter by the end of the first growing season at either site (Tables 5 and 6). By 1 YAIT, however, all of these responses were reduced by the glyphosate treatment at Bible Hill (Table 5). Stem density and diameter were also reduced at 1 YAIT by glyphosate at South Maitland, though regenerating stems in this treatment were similar in height to the nontreated control (Table 6). Re-application of glyphosate at peak height in 2017 at Bible Hill further reduced stem density, height, and diameter (Table 5). Aminopyralid application at peak height significantly reduced stem density by the end of the first growing season and at 1 YAIT at Bible Hill (Table 5) but not at South Maitland (Table 6). Aminopyralid did not reduce stem height or diameter by the end of the first growing season at either site (Tables 5 and 6), though height and diameter of regenerating stems were reduced at 1 YAIT at Bible Hill (Table 5). Re-application of aminopyralid at peak height in 2017 at Bible Hill further reduced stem density, height, and diameter (Table 5).

Cutting at peak height significantly reduced stem density, height, and diameter in the year of cutting at Bible Hill (Table 5), but only reduced stem height and diameter at South Maitland (Table 6). Stem density was not reduced by cutting at 1 YAIT at either site (Tables 5 and 6), though height of regenerating knotweed stems was reduced by cutting at 1 YAIT at South Maitland (Table 6). Re-application of cutting at peak height in 2017 at Bible Hill further reduced stem density, height, and diameter (Table 5).

Cutting at peak height and subsequent treatment of knotweed regrowth with glyphosate significantly reduced knotweed stem density by the end of the first growing season at South Maitland (Table 6) but not at Bible Hill (Table 5). Stem height and diameter, however, were reduced by this treatment at the end of the first growing season at each site (Tables 5 and 6). Furthermore, cutting at peak height followed by glyphosate application to stem regrowth reduced stem density, height, and diameter at 1 YAIT at each site (Tables 5 and 6). Re-application of cutting at peak height followed by glyphosate application to stem regrowth in 2017 further reduced stem density, height, and diameter at Bible Hill (Table 5).

Cutting and subsequent treatment of knotweed regrowth with aminopyralid significantly reduced stem density, height, and diameter at the end of the first growing season and at 1 YAIT at each site (Tables 5 and 6). Re-application of cutting at peak height followed by aminopyralid application to stem regrowth in 2017 further reduced stem density, height, and diameter at Bible Hill (Table 5).

Table 3. Significance of main and interactive effects of cutting and herbicide applications on Japanese knotweed stem density, height, and diameter at Bible Hill, Nova Scotia, Canada.

Effect	Prior to onset of treatments in 2016	End of 2016 growing season following first application of cutting and herbicide treatments[a]	1 YAIT[b]	End of 2017 growing season following re-application of cutting and herbicide treatments[c]
		Density		
Cutting	0.141[d]	0.008	0.076	0.029
Herbicide	0.310	0.001	0.001	0.001
Cutting*Herbicide	0.314	0.002	0.067	0.385
		Stem height		
Cutting	0.058	0.001	0.023	0.029
Herbicide	0.050	0.731	0.001	0.001
Cutting*Herbicide	0.371	0.105	0.328	0.385
		Stem diameter		
Cutting	0.523	0.001	0.015	0.001
Herbicide	0.417	0.731	0.001	0.001
Cutting*Herbicide	0.295	0.218	0.777	0.001

[a] Initial treatments in 2016 were conducted on June 16 2016 with herbicide applications to knotweed regrowth on July 27 2016 in applicable treatments.

[b] YAIT, year after initial treatments.

[c] Initial and second treatments in 2017 were conducted on June 16 2017 and July 27 2017, respectively.

[d] Significance based on $P \leq 0.05$.

Table 4. Significance of main and interactive effects of cutting and herbicide applications on Japanese knotweed stem density, height, and diameter at South Maitland, Nova Scotia, Canada.

Effect	Prior to onset of cutting treatments in 2017	End of 2017 growing season following first application of treatments[a]	1 YAIT[b]	End of 2018 growing season following re-application of treatments[c]
		Density		
Cutting	0.340[d]	0.001	0.099	*
Herbicide	0.666	0.188	0.003	*
Cutting*Herbicide	0.466	0.893	0.184	*
		Stem height		
Cutting	0.176	0.001	0.001	*
Herbicide	0.078	0.737	0.765	*
Cutting*Herbicide	0.025	0.799	0.813	*
		Stem diameter		
Cutting	0.391	0.001	0.003	*
Herbicide	0.284	0.937	0.008	*
Cutting*Herbicide	0.210	0.551	0.351	*

[a] Initial treatments in 2017 were conducted on July 4 2017 with herbicide applications to regrowth occurring August 4 2017 in applicable treatments.

[b] YAIT, year after initial treatments.

[c] Second treatments were not conducted at this site.

[d] Significance based on P ≤0.05. *No data collected.

Table 5. Main and interactive effects of cutting and herbicides on Japanese knotweed stem density, height, and diameter at Bible Hill, Nova Scotia, Canada in 2016 and 2017.

Cutting	Herbicide	Prior to onset of treatments in 2016	End of 2016 growing season following first application of treatments[a]	1 YAIT[b]	End of 2017 growing season following re-application of treatments[c]
Density (stems m^{-2})[d]					
No	No	5.1 ± 0.1 a[e] (26)	4.9 ± 0.4 a (25)	5.1 ± 0.5 a (26)	1.3 ± 0.02 a (19.9)
No	Glyphosate	5 ± 0.2 a (26)	5 ± 0.2 a (25)	2.9 ± 0.3 b (8.4)	0.4 ± 0.1 bc (2.5)
No	Aminopyralid	5 ± 0.2 a (25)	2 ± 0.3 c (4)	3 ± 0.2 b (9)	0.3 ± 0.1 bc (1.9)
Yes	No	4.5 ± 0.2 a (21)	3 ± 0.1 bc (10)	3.9 ± 0.1 ab (15)	0.9 ± 0.05 b (7.9)
Yes	Glyphosate	5.1 ± 0.2 a (27)	4 ± 0.3 ab (16)	3.1 ± 0.4 b (9.6)	0.1 ± 0.1 c (1.2)
Yes	Aminopyralid	4.6 ± 0.1 a (21)	2.7 ± 0.1 bc (7)	2.7 ± 0.2 b (7.7)	0.2 ± 0.1 c (1.5)
Height (cm)[f]					
No	No	199.7 ± 4.8 a	14 ± 0.4 a (228)	14 ± 0.09 a (219)	2.3 ± 0.01 a (240)
No	Glyphosate	216.2 ± 5.3 a	15 ± 0.2 a (186)	7.5 ± 0.5 cd (73)	1.3 ± 0.1 b (16)
No	Aminopyralid	202.5 ± 9.8 a	14 0.3 a (202)	9.9 ± 0.6 b (100)	2.1 ± 0.04 bc (90)
Yes	No	197.4 ± 5.5 a	9 ± 0.6 b (91)	12.9 ± 0.2 a (168)	1.8 ± 0.02 bc (74)
Yes	Glyphosate	198.9 ± 14 a	10 ± 0.4 b (101)	6.3 ± 0.8 d (42)	1.6[g] bc (10)
Yes	Aminopyralid	191 ± 7.7 a	9 ± 0.1 b (88)	9.6 ± 0.5 bc (95)	1.7 ± 0.03 c (74)
Diameter (cm)[h]					
No	No	2.1 ± 0.1 a	0.3 ± 0.03 a (2)	0.3 ± 0.02 a (2.1)	2.2 ± 0.1 a
No	Glyphosate	2.2 ± 0.1 a	0.1 ± 0.03 a (1.6)	-0.2 ± 0.08 cd (0.7)	0.1 ± 0.1 b
No	Aminopyralid	2.2 ± 0.1 a	0.2 ± 0.06 a (1.6)	-0.09 ± 0.1 bc (0.8)	0.7 ± 0.3 b
Yes	No	2.3 ± 0.1 a	-0.3 ± 0.05 b (0.4)	0.1 ± 0.02 ab (1.4)	0.3 ± 0.1 b
Yes	Glyphosate	2.3 ± 0.1 a	-0.2 ± 0.05 b (0.5)	-0.3 ± 0.03 d (0.4)	0.1 b
Yes	Aminopyralid	2.1 ± 0.1 a	-0.3 ± 0.05 b (0.4)	-0.2 ± 0.03 cd (0.6)	0.1 ± 0.1 b

[a] Initial treatments in 2016 were conducted on June 16 2016 with herbicide applications to knotweed regrowth on July 27 2016 in applicable treatments.

[b] YAIT, year after initial cutting treatments.

[c] Initial and second cutting treatments in 2017 were conducted on June 16 2017 and July 27 2017, respectively.

[d] Density data from onset of treatments in 2016 thru 1 YAIT was squareroot(x), density data for end of 2017 growing season was log(x) transformed prior to analysis to satisfy the assumptions of the ANOVA analysis. Transformed means are presented for means comparisons, and back-transformed means are given in parentheses.

[e] Means within columns followed by the same letter do not differ significantly according to Tukey's means separation test at the 0.05 level of significance. Values represent the mean ± 1 SD ($n = 5$).

[f] Height data from the end of the 2016 growing season onward was log(x) transformed prior to analysis to satisfy the assumptions of the ANOVA analysis. Transformed means are presented for means comparisons, and back-transformed means are given in parentheses.

[g] SD could not be determined for height and diameter in this time period due to low stem density

[h] Diameter data for end of 2016 growing season and 1 YAIT were log(x) transformed prior to analysis to satisfy the assumptions of the ANOVA analysis. Transformed means are presented for means comparisons, and back-transformed means are given in parentheses.

Table 6. Main and interactive effects of cutting and herbicides on Japanese knotweed stem density, height, and diameter at South Maitland, Nova Scotia, Canada in 2017 and 2018.

Cutting	Herbicide	Prior to onset of treatments in 2017	End of 2017 growing season following first application of treatments[a]	1 YAIT	End of 2018 growing season following re-application of cutting treatments[b]
			Density (stems/ 1 m^{-1})[c]		
No	No	1 ± 0.04 a[d] (12)	3.7 ± 0.3 a (14)	15 ± 3.1 a	*
No	Glyphosate	1 ± 0.06 a (12)	2.5 ± 0.4 a (9)	4 ± 1.3 b	*
No	Aminopyralid	1 ± 0.09 a (15)	3 ± 0.5 a (6)	8.5 ± 2.1 ab	*
Yes	No	1 ± 0.05 a (13)	1.6 ± 0.6 b (3)	8.7 ± 1.3 ab	*
Yes	Glyphosate	1 ± 0.05 a (10)	0.9 ± 0.5 b (2)	5 ± 1.2 b	*
Yes	Aminopyralid	1 ± 0.07 a (11)	1.4 ± 0.5 b (0.8)	5.7 ± 1.38 b	*
			Height (cm)		
No	No	319.2 ± 4.0 ab	297.2 ± 7.5 a	205 ± 31.4 a	*
No	Glyphosate	277.2 ± 18.9 b	275.2 ± 25.6 a	94.7 ± 18.7 ab	*
No	Aminopyralid	306.7 ± 3.2 ab	298.8 ± 14.5 a	166.7 ± 13.3 ab	*
Yes	No	311 ± 4.7 a	100.1 ± 18.4 b	151.1 ± 21.4 b	*
Yes	Glyphosate	311 ± 4.7 ab	101.7 ± 14.2 b	78 ± 16.2 b	*
Yes	Aminopyralid	320 ± 5.2 a	106.3 ± 8.2 b	103.6 ± 9.3 ab	*
			Diameter (cm)		
No	No	2.48 ± 0.1 a	2.2 ± 0.1 a	2.0 ± 0.2 a	*
No	Glyphosate	2.1 ± 0.1 a	2.0 ± 0.2 a	1.04 ± 0.1 bc	*
No	Aminopyralid	2.0 ± 0.1 a	2.2 ± 0.1 a	1.8 ± 0.1 ab	*
Yes	No	2.2 ± 0.1 a	0.5 ± 0.1 b	1.2± 0.2 ab	*
Yes	Glyphosate	2.2 ± 0.1 a	0.6 ± 0.1 b	0.8 ± 0.2 c	*
Yes	Aminopyralid	2.2 ± 0.1 a	0.5 ± 0.5 b	1.0 ± 0.1 bc	*

[a] Initial treatments in 2017 were conducted on July 4 2018 with herbicide applications to regrowth occurring August 4 2017 in applicable treatments.

[b] Follow-up treatments were not conducted at this location

[c] Density data for prior to treatment application in 2017 and end of 2017 growing season were squareroot(*x*) and log(*x*) transformed respectively prior to analysis to satisfy the assumptions of the ANOVA analysis. Transformed means are presented for means comparisons, and back-transformed means are given in parentheses.

[d] Means within columns followed by the same letter do not differ significantly according to Tukey's means separation test at the 0.05 level of significance. Values represent the mean ± 1 SD (*n* = 4).

[*] No data collected.

2.5 Discussion

The objectives of this research were to determine i) the effect of repeated cutting on knotweed stem regeneration, and ii) the main and interactive effects of cutting and herbicide application on knotweed shoot regeneration. Application of one or two cuttings reduced knotweed stem density in the year of cutting at Bible Hill but not Antigonish, though plant height and stem diameter of regenerating stems was consistently reduced in the year of cutting across sites (Tables 1 and 2). Density was not reduced at 1 YAIT by cutting at either site, though height and diameter of new stems was reduced at 1 YAIT at Bible Hill (Tables 1 and 2). Re-application of cutting treatments in 2017 at Bible Hill did not completely inhibit regeneration of new stems, however stem density, height and diameter were significantly reduced by the end of the second growing season (Table 1). Only stem height and diameter were significantly reduced compared to the control by the end of the second growing season at Antigonish (Table 2).

In both experiments, cutting alone only reliably caused reductions in Japanese knotweed stem height and diameter within the growing season in which cutting was conducted, and occasionally reduced stem height and diameter 1 YAIT (Tables 1, 2, 5 and 6). De Waal (1995) also reported limited regeneration of knotweed stems within the year of cutting in the UK, however the effects were not lethal. Delbart et al. (2012) found that a single cutting applied to Japanese knotweed at peak height was able to reduce stem density by 51% and stem height by 6.3% in the year following cutting, whereas multiple cuts caused an increase in stem density of about 45%. We did not find a significant increase or decrease in Japanese knotweed stem density in our experiments 1 YAIT. We did find a significant reduction in stem height and diameter 1 YAIT at Bible Hill when two cuttings were applied (Table 1). However, at Antigonish (table 2) density remained similar at all measured points. It is possible that underground biomass may have confounded the results in Antigonish (as there could have been more present there), leading to density remaining at levels similar to the control in treatment plots. Bible Hill and Antigonish are about 120 km apart and both sites were in a flood plain that received full-sun all day (Figure 1). At Bible Hill, significant reductions in Japanese knotweed stem density were achieved by the end of growing seasons in which treatments were applied, however at no time did this happen at Antigonish (Tables 1 and 2). I can only offer speculation as to why this is. It is possible that the Japanese knotweed in Antigonish is older and therefore has a greater amount of underground energy reserves to draw on when it comes under stress. However, the age of these stands is not known and no data is available on the relationship between age of a Japanese knotweed stand and relative stem density.

Further trials on the efficacy of mechanical control do not support it as an effective management strategy. Seiger and Merchant (1997) collected 1 cm rhizome fragments from Japanese knotweed in Washington D.C. They allowed these fragments to grow for 60 days under greenhouse conditions. Then a single or multiple cuttings were applied at 28-day intervals depending upon treatment. They determined that at least four cuttings would be required to cause net-depletion of Japanese knotweed below ground biomass, based on regression analysis (Seiger and Merchant 1997). However, they concluded that this would not be lethal to the knotweed, and suggested cutting be used in conjunction with herbicides (Seiger and Merchant 1997). Additionally, a review of the literature estimated that at least 17 cuttings per growing season would be required to achieve control of Japanese knotweed (McHugh 2006). Therefore, it is reasonable to conclude that mechanical cutting alone is not an effective means to achieve eradication of Japanese knotweed, however, it can be a useful suppression strategy.

Cutting of Japanese knotweed at peak plant height and application of herbicide to stem regrowth at four weeks after cutting did not significantly improve reductions in Japanese knotweed stem density, height, or diameter 1 YAIT at Bible Hill or South Maitland (Tables 5 and 6). At Bible Hill, glyphosate and aminopyralid both caused significant reductions in Japanese knotweed stem density 1 YAIT (Table 5). However, only glyphosate alone, cutting + glyphosate, and cutting + aminopyralid were able to cause significant density reductions at South Maitland (Tab le 6). Re-application of treatments at Bible Hill in 2017 did not result in stem density differences between cutting alone or cutting + herbicide treatments (Table 5), indicating there is no benefit, in terms of management outcome, to cutting Japanese knotweed prior to treatment with herbicides.

The efficacy of glyphosate in this experiment and across the literature is notable. Delbart et al. (2012) found glyphosate injected or spot applied at 7.2 kg a.i. ha $^{-1}$ or 3.6 kg a.i ha $^{-1}$ caused significant reductions in knotweed stem density 1 and 2 years after treatment (YAT). This is consistent with findings in this paper, where glyphosate consistently reduced stem density 1 YAIT (Tables 5 and 6). De Waal (1995) also found that glyphosate applied to Japanese knotweed in July and August in the UK significantly reduced stem regeneration in the following growing season. Our results are also consistent with Davenport (2006), who found no significant difference between density reductions when applying glyphosate to peak height growth or regrowth following cutting.

Aminopyralid, although promising, provided inconsistent control across sites. Aminopyralid reduced stem density, height, and diameter at Bible Hill within the season of initial application, as well as at 1 YAIT (Table 5). In South Maitland, density was only significantly reduced compared to the control when aminopyralid was utilized in conjunction with cutting (Table 6). However, height and diameter were significantly reduced 1 YAIT without the addition of cutting (Table 6). Given there was no significant interaction between cutting and herbicide for stem density, height, or diameter at South Maitland 1 YAIT (Table 4), there could be unaccounted factors contributing to lack of density reduction. One of note is the difference between herbicide application timing at South Maitland compared to Bible Hill. Initial herbicide applications were conducted at Bible Hill on June 16 2016, whereas initial treatments were conducted at South Maitland on July 4 2017. Ignoring the year, these dates are 18 days apart. Knotweed at Bible Hill was also considerably shorter at peak height compared to South Maitland (200 cm vs 307 cm). It is possible that the varied heights of the knotweed between sites altered their susceptibility to aminopyralid. I can only offer speculation on why heights are different, but South Maitland is not a flood prone area, whereas Bible Hill is subject to frequent flooding and these frequent disturbances may prevent knotweed there from attaining a greater height. It is also possible that knotweed has a time sensitive response to aminopyralid that may have affected our results. Larsen (2013) found that aminopyralid applied to knotweed stems during emergence caused young shoots to die, causing density reductions prior to knotweed reaching peak height. However, these results were not long lasting as knotweed plots treated with aminopyralid did not have significantly different density from control plots 1 YAT (Larsen 2013). Further, there is some evidence to suggest that synthetic auxins when used at certain times of year can promote the creation of storage organs, but not destroy them. This was found to be the case in potatoes, during the early stages of tuber formation (Borzenkova et al. 1998), when exogenous auxins were applied. Bashtanova et al. (2009) indicated synthetic auxins may increase rhizome development in Japanese knotweed when applied in the spring and summer, concluding that applications of synthetic auxins may be more effective when applied in the autumn (fall) when intensive basipetal movement of photo-assimilate is occurring (Price et al. 2002), as at this time, disruption of the phloem transport caused by synthetic auxins may significantly reduce assimilate transported to the rhizome.

The lack of a significant improvement from integrated management strategies is surprising, as previous literature indicated that efficacy may have been improved. Control of Canada thistle (*Cirsium arvense*) was improved by conducting tillage in late July followed by glyphosate application to regenerated rosettes when compared to applying glyphosate at bud stage alone (Hunter 1996). The author attributed this to a four-fold increase in glyphosate translocation while the Canada thistle was in a rosette form (Hunter 1995). This is substantially different from simply cutting the plants down as was done to knotweed in these experiments, as tillage causes substantial below ground biomass disturbance. Japanese knotweed in its native range of Japan and in its invasive ranges, does not form a rosette. Therefore, tillage of knotweed infested areas would not be advisable, due to its lack of efficacy, as well the increased risk of contaminating equipment and the environment with knotweed debris.

Renz and DiTomaso (1998) found that mowing followed by glyphosate application improved control of perennial pepperweed (*Lepidium latifolium*), whereas doing either alone was ineffective. This was attributed to an increase in the proportion of belowground meristematic sinks, relative to aboveground sinks. This would increase the quantity of glyphosate that is translocated to the roots and thus improve control of the pepperweed (Renz and DiTamaso 2006). In our experiments we found no significant differences in density reductions when applying herbicide to peak height growth or regrowth following cutting. Therefore, reducing the number of aboveground sinks in knotweed does not appear to be a factor of importance for management of this species. This lack of improved efficacy from integrated management strategies has also been observed by Farooq (2018), where cutting followed by glyphosate application to narrowleaf goldenrod

(*Euthamia graminifolia*) did not provide additional density reductions relative to applying glyphosate alone in wild blueberry fields. He concluded that integrated management would therefore not be cost-effective, due to the additional costs of performing cuttings and aforementioned lack of management benefit. However, it is important to consider the context of these infestations and growth habits of the species. Goldenrod typically reaches a peak height of 100 – 130 cm whereas knotweed stems can exceed 300 cm in height. Applying herbicides one month after initial cutting treatments in Bible Hill and South Maitland was considerably easier, as average stem height in treatments was reduced to 101 cm and 107 cm, respectively. Therefore, while cutting may not directly improve control achieved with herbicides, the reduction of knotweed height by cutting can facilitate easier application of herbicides to large knotweed patches.

2.6 Conclusions

Cutting alone did not reduce Japanese knotweed stem density in the year following treatment. While stem height and diameter were significantly reduced, our results indicate that cutting would be required each year to maintain suppression of knotweed. Two cuttings did not improve the level of control achieved, so use of cutting to reduce problems caused by knotweed should focus on one cutting conducted at peak height to reduce the labor required.

Integration of cutting and herbicide applications did not give greater reductions in knotweed stem density, height, or diameter relative to applying herbicides at peak height alone. From a purely results point of view, there is no benefit to conducting a cutting prior to herbicide application. However, the application of cutting reduces stem height, which makes applying herbicides easier. Therefore, while integration of mechanical and chemical control methods is not required for effective management of Japanese knotweed with herbicides, it may be necessary if the intention is to treat a large knotweed stand. Cutting would allow the area to become navigable and therefore treatable, as shorter stems could be walked over while spraying the canopy.

Glyphosate caused significant reductions in knotweed stem density, height, and diameter in the year following initial treatment and therefore continues to be an important herbicide for Japanese knotweed management. Aminopyralid was not consistently effective and may be better utilized as a suppression tool in early-post emergence herbicide applications as indicated by Boyd et al. (2017) or applied in the fall when translocation of assimilate is greater (Bashtanova et al. 2009).

2.7 Citations

Adachi, N., & Masayuki, T. (1996). Central die-back of monoclonal stands of Reynoutria japonicain an early stage of primary succession on Mount Fuji. *Annals of Botany*, *77*(5), 477–486. https://doi.org/10.1006/anbo.1996.0058

Bailey, J. P., Bímová, K., & Mandák, B. (2008). Asexual spread versus sexual reproduction and evolution in Japanese knotweed S.L. sets the stage for the "Battle of the Clones." *Biological Invasions*, *11*(5), 1189–1203. https://doi.org/10.1007/s10530-008-9381-4

Bashtanova, U. B., Beckett, K. P., & Flowers, T. J. (2009). Review:physiological approaches to the improvement of chemical control of Japanese knotweed (fallopia japonica). *Weed Science*, *57*(6), 584–592. https://doi.org/10.1614/ws-09-069.1

Beerling, D. J., Bailey, J. P., Conolly, A. P. (1994). Biological flora of the British Isles, Fallopia japonica. *Journal of Ecology, 82: 959-979.*

Borzenkova, R. A., & Borovkova, M. P. (2003). Developmental patterns of phytohormone content in the cortex and pith of potato tubers as related to their growth and starch content. *Russian journal of plant physiology*, *50*(1), 119-124.

Boyd, N. S., White, S. N., & Larsen, T. (2017). Sequential Aminopyralid and Imazapyr applications for Japanese knotweed (fallopia japonica) management. *Invasive Plant Science and Management*, *10*(3), 277–283. https://doi.org/10.1017/inp.2017.31

Brown, M. L., Duncan, C. A., & Halstvedt, M. B. (1999). Spotted knapweed management with integrated methods. In *Proceedings of the Western Society of Weed Science*.

Child, L., & Wade, P. M. (2000). *The Japanese knotweed manual: The management and control of an invasive alien weed*. Packard Publishing.

Claeson, S. M., LeRoy, C. J., Barry, J. R., & Kuehn, K. A. (2013). Impacts of invasive riparian knotweed on litter decomposition, aquatic fungi, and macroinvertebrates. *Biological Invasions*, *16*(7), 1531–1544. https://doi.org/10.1007/s10530-013-0589-6

Colleran, B. P., & Goodall, K. E. (2014). In situ growth and rapid response management of flood-dispersed Japanese knotweed (fallopia japonica). *Invasive Plant Science and Management*, *7*(1), 84–92. https://doi.org/10.1614/ipsm-d-13-00027.1

Davenport, R. (2006). Control of knotweed and other invasive species and experiences restoring native species in the Pacific Northwest us. *Native Plants Journal*, *7*(1), 20–26. https://doi.org/10.2979/npj.2006.7.1.20

Dawson, F. H., & Holland, D. (1999). The distribution in bankside habitats of three alien invasive plants in the U.K. in relation to the development of Control Strategies. *Biology, Ecology and Management of Aquatic Plants*, 193–201. https://doi.org/10.1007/978-94-017-0922-4_27 de Waal, L. C. (1993, September). Treatment of Fallopia japonica near water a case study. Plant invasions: general aspects and special problems. In *Workshop held at Kostelec nad Cernymi lesy, Czech Republic* (pp. 16-19).

Delbart, E., Mahy, G., Weickmans, B., Henriet, F., Crémer, S., Pieret, N., Vanderhoeven, S., & Monty, A. (2012). Can land managers control Japanese knotweed? lessons from control tests in Belgium. *Environmental Management*, *50*(6), 1089–1097. https://doi.org/10.1007/s00267-012-9945-z

Dommanget, F., Spiegelberger, T., Cavaillé, P., & Evette, A. (2013). Light availability prevails over soil fertility and structure in the performance of Asian knotweeds on riverbanks: New management perspectives. *Environmental Management*, *52*(6), 1453–1462. https://doi.org/10.1007/s00267-013-0160-3

Elliot, V. (2018). *Couple are forced to demolish their £300k four-bed home after it was invaded by Japanese knotweed*. Couple are forced to demolish their £300k four-bed home after it was invaded by Japanese knotweed - Property Article : Property Services Directory - AllAboutProperty.com. Retrieved May 5, 2019, from http://www.surveyorsandvaluers.com/articles/item.php?id=3140

Farooq, M.H. 2018. Management of goldenrods (Solidago spp.) in wild blueberry (Vaccinium angustifolium Ait.) fields. Dalhousie University., Halifax, N.S., Canada. M.Sc. thesis.

Larsen, T. (2013). *Biology, ecological impacts, and management of Japanese knotweed (Polygonum cuspidatum syn. fallopia japonica) in Nova Scotia*. Dalhousie University, Halifax, N.S., Canada, M.Sc thesis.

Lau, J. A., Puliafico, K. P., Kopshever, J. A., Steltzer, H., Jarvis, E. P., Schwarzländer, M., Strauss, S. Y., & Hufbauer, R. A. (2008). Inference of allelopathy is complicated by effects of activated carbon on plant growth. *New Phytologist*, *178*(2), 412–423. https://doi.org/10.1111/j.1469-8137.2007.02360.x

Maerz, J. C., Blossey, B., & Nuzzo, V. (2005). Green frogs show reduced foraging success in habitats invaded by Japanese knotweed. *Biodiversity and Conservation*, *14*(12), 2901–2911. https://doi.org/10.1007/s10531-004-0223-0

Manchester, S. J., & Bullock, J. M. (2000). The impacts of non-native species on UK biodiversity and the effectiveness of control. *Journal of Applied Ecology*, *37*(5), 845–864. https://doi.org/10.1046/j.1365-2664.2000.00538.x

Mclean, S. (2010). Identification of the presence and impact of Japanese knotweed on development sites. *Journal of Building Appraisal*, *5*(4), 289–292. https://doi.org/10.1057/jba.2010.2

Middleton, C. (2016, February 21). *Japanese knotweed: The invasive plant that eats the value of your home*. Newsweek. Retrieved May 5, 2019, from https://www.newsweek.com/japanese-knotweed-driving-men-murder-257257

Miller TW (2016) Integrated strategies for management of perennial weeds. Invasive Plant Science and Management. 9(2): 148-158

Monteiro, A., Moreira, I., & Sousa, E. (1999). Effect of prior common reed (phragmites australis) cutting on herbicide efficacy. *Biology, Ecology and Management of Aquatic Plants*, 305–308. https://doi.org/10.1007/978-94-017-0922-4_44

Oliver, K. (2011, October). Learning legacy: Lessons learned from the London 2012 Games construction project. London; London Legacy Development Agency.

Parepa, M., Schaffner, U., & Bossdorf, O. (2012). Sources and modes of action of invasive knotweed allelopathy: The effects of leaf litter and trained soil on the germination and growth of native plants. *NeoBiota*, *13*, 15–30. https://doi.org/10.3897/neobiota.13.3039

Renz, M. J., & DiTomaso, J. M. (1998). The Effectiveness of Mowing and Herbicides to Control Perennial Pepperweed in Rangeland & Roadside Habitats. In *Proceedings-Western Society Of Weed Science* (Vol. 51, pp. 129-129). *Western Society of Weed Science.*

Renz, M. J., & Ditomaso, J. M. (2006). Early season mowing improves the effectiveness of chlorsulfuron and glyphosate for control of perennial pepperweed (lepidium latifolium). *Weed Technology*, *20*(1), 32–36. https://doi.org/10.1614/wt-05-007r1.1

Siemens TJ, Blossey B (2007) An evaluation of mechanisms preventing growth and survival of two native species in invasive Bohemian knotweed (*Fallopia* × *bohemica, Polygonaceae*). American Journal of Botany 94(5): 776-783

Townsend A (1997) Japanese knotweed: A reputation lost. Arnoldia 57(3): 13-19

Ward, J. S., Worthley, T. E., & Williams, S. C. (2009). Controlling Japanese barberry (Berberis thunbergii DC) in Southern New England, USA. *Forest Ecology and Management*, *257*(2), 561–566. https://doi.org/10.1016/j.foreco.2008.09.032

CHAPTER 3: COMPARISON OF HERBICIDE APPLICATION METHODS FOR MANAGEMENT OF JAPANESE KNOTWEED

3.1 Abstract

Japanese knotweed (*Reynoutria japonica*) is a problematic invasive species in Europe and North America that causes significant reductions in native plant diversity and increases soil erosion, among other negative impacts. Chemical control has proven to be the most effective means to control Japanese knotweed within a reasonable amount of time. However, there is discord as well as limited literature available on the most effective application method for controlling Japanese knotweed. Experiments were conducted to determine i) the effect of herbicide spot applications and stem injections on Japanese knotweed shoot regeneration, and ii) the effect of cut-stump glyphosate applications on Japanese knotweed shoot regeneration. Spot applications of glyphosate and aminopyralid significantly reduced Japanese knotweed stem density 1 YAIT (year after initial treatment) at Bible Hill, but not at Antigonish ($P < 0.05$). Height and diameter of regenerating stems, however, were not consistently reduced in any treatment 1 YAIT. Injections of herbicides reduced Japanese knotweed stem height and diameter 1 YAIT, but only reduced stem density by the end of the second growing season in which treatments were applied at Bible Hill and Antigonish. Cut-stump glyphosate applications did not reduce Japanese knotweed stem density at 1 YAIT and were not as effective as spot applications of glyphosate. We concluded that spot applications and injections of herbicides can be effective for Japanese knotweed management if multiple applications are done over at least two growing seasons. Spot application should be the default application method due to the difficulty of performing injections. Cut-stump glyphosate applications cannot be reliably recommended for Japanese knotweed at this time due to inconsistent results, but additional research could be conducted to refine this potentially useful herbicide application method.

3.2 Introduction

Japanese knotweed is a problematic invasive plant in Europe and North America. It was introduced to North America in the 1800s as a horticultural species (Townsend 1997), which likely facilitated its proliferation. Japanese knotweed develops dense, monotypic stands with stems that can exceed 300 cm in height (Siemens and Blossey 2007; Larsen 2013). These dense stands prevent up to 90% of incoming light from reaching the understory (Vanderklien et al. 2013), leading to a lack of ground cover plants in affected areas, increased soil erosion, sediment loading in streams, and riverbank destruction (Dawson and Holland 1999; Child and Wade 2000). Previous literature on Japanese knotweed management indicates that herbicides are the most effective means for controlling the species (De Waal 1995; Child and Wade 2000; Delbart et al. 2012;

Larsen 2013). Herbicide application method, however, may affect herbicide efficacy on Japanese knotweed. This plant can also occur in sensitive environments such as riparian zones where herbicide application methods that limit damage to non-target species or reduce the amount of herbicide required are important in facilitating the management of Japanese knotweed in these areas.

A range of herbicides and application methods have been evaluated for management of Japanese knotweed, though results to date have been inconsistent. Research has primarily focused on spot applications, with some exceptions. Delbart et al. (2012) assessed the efficacy of stem injections and spot applications of glyphosate, among other herbicides. Both injections and spot applications of glyphosate at 3.6 or 7.2 kg a.e. ha^{-1}, respectively, reduced stem density 1 and 2 years after treatment. However, Delbart et al. (2012) only had one 1m X 1m control plot at each site and did not test treatments multiple times at the same location, making some of their results difficult to compare to other studies. Hagen and Dunwiddie (2008) evaluated injections of both glyphosate concentrate (480 g a.e. L^{-1}) and 1:1 glyphosate: water solutions using injection volumes of 1, 3, or 5 mL of each treatment per stem. Hagen and Dunwiddie (2008) found that all injection treatments resulted in injury to knotweed four weeks post-treatment. Treated knotweed also had reduced stem density, height, and diameter in the following growing season. However, the authors indicated that follow up treatments would be required as knotweed shoot regeneration was not completely inhibited. Additionally, the authors indicated that needle breakage and stem destruction was common, reducing the efficacy and efficiency of treatments. Ford (2004) achieved similar reductions in knotweed stem density using stem injections of glyphosate, while also cutting a portion of the knotweed down (leaving some stem behind to inject) to improve access to the area.

Ford (2004) concluded that injections may not be practical for large scale usage, but useful in sensitive terrestrial or aquatic environments where prevention of off-target impact is desirable.

Spot applications of herbicides generally provide more consistent results than stem injections. De Waal (1995) found that a spot application of glyphosate at 5 L ha^{-1} with 2% adjuvant applied in July resulted in stem density reductions for two years following treatment. Early post spot applications of aminopyralid at 120 g a.e. ha^{-1} to knotweed in Nova Scotia resulted in stem density reductions within the growing season. However, knotweed completely recovered in the year following treatment (Larsen 2013). Larsen (2013) also found that spot applications of imazapyr at 720 g a.e. ha^{-1} reduced knotweed stem density when applied at peak height, flowering and prior to senescence.

The use of cut-stump herbicide applications may have utility for management of Japanese knotweed in addition to stem injections. A cut-stump treatment is where the plant stem is cut to a specific height and then immediately treated with a herbicide. While cut-stump has not been evaluated for use against knotweed, it has been found to be effective against similar species such as purple loosestrife (*Lythrum salicaria*) (Wahlers et al. 1997). (Wahlers et al. 1997) found that cut-stump herbicide applications were effective in greenhouse trials, but were not compared to other application methods, such as spot applications. Use of a similar application method on Japanese knotweed could improve ease of herbicide application, and may be more effective due to the hollow nature of the knotweed stem, allowing herbicide to pool in the hollow internode cavity. Further, as Ford (2004) indicated, cutting some knotweed tissue makes accessing all of a knotweed stand easier, allowing for a more uniform herbicide application.

The objectives of this research were to: (1) determine if spot applications of herbicides were more effective than injections of herbicides, and (2) determine if a cut-stump glyphosate application was more effective than a spot application of glyphosate in causing reductions in Japanese knotweed stem density, height and diameter.

3.3 Materials and Methods

3.3.1 Effect of herbicide spot applications and stem injections on Japanese knotweed shoot regeneration

The objective of this experiment was to compare efficacy of herbicide spot applications and stem injections on Japanese knotweed shoot regeneration. Glyphosate and aminopyralid were used as test herbicides in this experiment based on previous reports of efficacy and availability for use in Atlantic Canada (Larsen 2013). The experiment was arranged in a completely randomized design with five treatments: 1) control (do nothing), 2) spot apply glyphosate, 3) spot apply aminopyralid, 4) inject glyphosate, and 5) inject aminopyralid. Six and seven replications were used at Bible Hill and Antigonish, respectively. Plots were placed such that obvious Japanese knotweed stem clumps, as defined by Adachi et al. (1996), were in the center of each plot, with a plot size of 1m X 1m. Plots were spaced at least one meter apart. The experiment was conducted at Bible Hill, Nova Scotia, Canada (Figure 1A), and at Antigonish, Nova Scotia, Canada (Figure 1B).. Glyphosate and aminopyralid were applied at a rate of 9.0 and 2.4 g a.e. L water^{-1}, respectively. Spot applications were conducted using a CO_2 pressurized sprayer equipped with a single teejet AI11002-VS air induction nozzle. Stems were sprayed until all leaf surfaces were covered and initial run-off of spray solution occurred. Injections were conducted using a JK1000 injection gun (Japanese Knotweed Injection Systems^{TM}, Battle Ground, WA, USA) with a standard needle. All stems in the plot were injected between the first and second node above the soil surface with 5 mL of herbicide solution, as per the manufacturer instructions. Separate guns were used for glyphosate and aminopyralid to avoid cross contamination. Treatments were conducted in both 2016 and 2017 at Bible Hill and Antigonish. Initial herbicide applications were conducted on June 16, 2016 at Bible Hill and June 22, 2016 at Antigonish. Re-application of treatments in 2017 was conducted on June 16, 2017 at Bible Hill and June 20, 2017 at Antigonish.

Figure 1. A) Bible Hill Farmsite with a total area of ~ 313 m^{-2} located at 45°22'8.59"N 63°15'25.84"W, B) Antigonish Rights River location with a total area of ~ 907 m^{-2} located at 45°37'21.91"N 61°58'32.17"W, C) Truro Railsite with a total area of ~ 367 m^{-2} located at 45°21'59.95"N 63°16'10.90"W and CN Rail HQ with a total area of ~ 486 m^{-2} located at 45°21'56.88"N 63°16'8.45"W.

3.3.2 Effect of a Single Cut-Stump Glyphosate Application on Japanese Knotweed Shoot Regeneration

The objective of this experiment was to determine the effect of a cut-stump glyphosate application on Japanese knotweed shoot regeneration. The experiment was a 2 x 2 factorial arrangement of cut-stump (no, yes) and glyphosate application (no, yes), arranged in a completely randomized design with four replications. Plots were placed such that obvious knotweed stem clumps as defined by Adachi et al. (1996) were in the center of each plot, with a 1 m X 1 m plot size. Plots were spaced at least one meter apart. The experiment was conducted at the Truro Railsite and CN Rail HQ (Figure 1C). The Japanese knotweed stands used for this experiment were understory to a tree canopy and therefore had considerably lower stem density at the beginning of the experiment when compared to other locations used in this thesis. Cutting was conducted using machetes, and stems were cut between the third and fourth node above the soil surface when emerged stems were at approximately peak height (300 cm), prior to the development of flowers (mid-August). Glyphosate was applied immediately after cutting at a rate of 9.0 g a.e. L water^{-1} using a CO_2 pressurized sprayer equipped with a single teejet AI11002-VS air induction nozzle (Sprayer Supplies, Herndon, KY, USA). Stems in uncut treatments were treated on the same day as cutting treatments and were sprayed until all surfaces were covered using the same rate and application equipment as used for the cut-stump treatment. Treatments were applied on August 22, 2017 at both locations.

3.3.3 Data Collection and Statistical Analysis

Data collection in each experiment included Japanese knotweed stem density, stem height, and stem diameter. Stem density was determined by counting all stems in the plot. Height and diameter were determined by randomly measuring five stems per plot using a meter stick or a caliper, respectively. Average height and diameter was calculated on a per plot basis for analysis.

Data were collected at the time of treatment applications, end of the growing season in which treatments were applied, and the year following treatment.

The effect of spot applications and injections of glyphosate or aminopyralid on knotweed stem density, height, and diameter were determined using ANOVA (Analysis of Variance) in Minitab18 (Minitab LLC, State College, USA, 2019) at the 0.05 level of significance. The main and interactive effects of cut-stump and glyphosate application were determined using ANOVA in Minitab18 (Minitab LLC, State College, USA, 2019) at the 0.05 level of significance. Cutting and glyphosate application were modeled as fixed effects. Means separation for all experiments, where necessary, was conducted using Tukey's means separation test at the 0.05 level of significance. Minitab18 normality test was used to determine if data met required assumptions for the ANOVA analysis. If needed, data were transformed using squareroot, log, exponential or cube-root transformations where indicated to meet normality and constant variance assumptions. Back transformed means are presented in all tables where data transformations were used.

3.4 Results

3.4.1 Effect of Herbicide Spot Applications and Stem Injections on Japanese Knotweed Shoot Regeneration

There was a significant effect of herbicide treatment on stem height and diameter at Bible Hill at the end of the first growing season in which treatments were at applied ($P \leq 0.001$) but not at Antigonish ($P \geq 0.344$), and there was no effect of herbicide treatment on stem density in the year of treatment applications at either site ($P \geq 0.067$). There was a significant effect of herbicide treatment on knotweed stem density, height, and diameter at Bible Hill ($P \leq 0.001$) in the year following initial treatment. In Antigonish there was a significant effect of treatment on knotweed stem height ($P \leq 0.008$) in the year following initial treatments, however not on stem density or diameter ($P \geq 0.115$). Following the reapplication of treatments in 2017, there was a significant effect of herbicide treatment on knotweed stem density, height, and diameter by the end of the second growing season at both locations ($P \leq 0.008$)

Spot applied or stem injected glyphosate did not reduce Japanese knotweed stem density, height, or diameter at the end of the first growing season in which treatments were applied at Bible Hill or Antigonish (Tables 1 and 2). Spot applications and stem injections of aminopyralid did not reduce Japanese knotweed stem density by the end of the first growing season at Bible Hill or Antigonish (Tables 1 and 2). Stem injections of aminopyralid did, however, reduce Japanese knotweed stem diameter and height by the end of the first growing season at Bible Hill (Table 1).

Table 1. The effect of herbicide treatment on Japanese knotweed stem density, height, and diameter at Bible Hill, Nova Scotia, Canada in 2016 and 2017.

Application Method	Herbicide	Prior to treatment applications in 2016[a]	End of 2016 growing season following first application of treatments	1 YAIT[b]	End of 2017 growing season following re-application of treatments[c]
Density (stems m^{-2})[d]					
None	None	1.2 ± 0.03 a[e] (19)	1.1± 0.06 a (14)	1.3 ± 0.02 ab (24)	1.2 ± 0.06 a (18)
Spot application	Glyphosate	1.2 ± 0.05 a (19)	1.2 ± 0.06 a (17)	0.6 ± 0.16 c (4)	0.07 ± 0.07 b (0.3)
Spot application	Aminopyralid	1.3 ± 0.06 a (25)	0.92 ± 0.09 a (10)	0.85 ± 0.05 c (6)	0.345 ± 0.13 b (1.5)
Stem injection	Glyphosate	1.3 ± 0.05 a (23)	1.2 ± 0.14 a (20)	1.34 ± 0.09 a (23)	0.62 ± 0.07 b (3.4)
Stem injection	Aminopyralid	1.2 ± 0.06 a (19)	0.84 ± 0.13 a (9)	1.08 ± 0.12 bc (13)	0.76 ± 0.14 b (6)
Height (cm)[f]					
None	None	190 ± 6.6 a	225.6 ± 15.6 a	197.5 ± 20.7 a	2.42 ± 0.01 a (264)
Spot application	Glyphosate	186.2 ± 8.6 a	210.8 ± 7.4 a	37.8 ± 11.9 c	1.82[g] bc (65)
Spot application	Aminopyralid	178.1 ± 4 a	199.7 ± 10.3 a	88.6 ± 12.8 bc	2.13 ± 0.04 ab (137)
Stem injection	Glyphosate	190.2 5.9 a	222.8 ± 12.9 a	107.2 ± 14.7 b	1.82 ± 0.07 c (70)
Stem injection	Aminopyralid	180.9 ± 4.4 a	126.4 ± 6.5 b	178.1 ± 8.2 a	1.99 ± 0.06 bc (102)
Diameter (cm)[h]					
None	None	2.2 ± 0.2 a	4.60 ± 0.5 a (2.1)	2.2 ± 0.1 a	1.30 ± 0.02 a (2.2)
Spot application	Glyphosate	2.0 ± 0.1 a	3.9 ± 0.48 a (1.9)	0.7 ± 0.1 c	0.79 bc (0.5)
Spot application	Aminopyralid	1.9 ± 0.1 a	3.41 ± 0.41 a (1.8)	0.7 ± 0.1 c	1.03± 0.05 b (1.1)
Stem injection	Glyphosate	2.1 ± 0.1 a	4.39± 0.61 a (2.0)	1.3 ± 0.1 b	0.75 ± 0.05 c (0.4)
Stem injection	Aminopyralid	1.9 ± 0.1 a	0.57 ± 0.19 b (0.7)	1.5 ± 0.1 b	0.84 ± 0.06 bc (0.6)

[a] Initial treatments in 2016 were conducted on June 16 2016.

[b] YAIT, year after initial treatments.

[c] Treatments in 2017 were conducted on June 16, 2017.

[d] Density data at each timing were squareroot(*x*) transformed prior to analysis to satisfy the assumptions of the ANOVA analysis. Transformed means are presented for means comparisons, and back-transformed means are given in parentheses.

[e] Means within columns followed by the same letter do not differ significantly according to Tukey's means separation test at the 0.05 level of significance. Values represent the mean ± 1 SD (*n* = 6).

[f] Height data collected at the end of the 2017 growing season were log(x) transformed prior to analysis to satisfy the assumptions of the ANOVA analysis. Transformed means are presented for means comparisons, and back-transformed means are given in parentheses.

[g] SD could not be determined for height and diameter in this time period due to low stem density.

[h] Diameter data collected at the end of the 2016 growing season and end of the 2017 growing season were $(x)^2$ and cube-root transformed, respectively, prior to analysis to satisfy the assumptions of the ANOVA analysis. Transformed means are presented for means comparisons, and back-transformed means are given in parentheses.

Table 2. The effect of herbicide treatment on Japanese knotweed stem density, height, and diameter at Antigonish, Nova Scotia, Canada in 2016 and 2017.

Method	Herbicide	Prior to treatment applications in 2016[a]	End of 2016 growing season following first application of treatments	1 YAIT[b]	End of 2017 growing season following re-application of treatments[c]
			Density (stems m^{-2})[d]		
None	None	4.57 ± 0.25 a[e] (20)	18.9 ± 2.6 a	21.5 ± 3.7 a	4.54 ± 0.34 a (21)
Spot application	Glyphosate	4.63 ± 0.31 a (21)	22 ± 3.5 a	22.3 ± 1.9 a	2.20 ± 0.17 b (5)
Spot application	Aminopyralid	4.16 ± 0.43 a (17.4)	19 ± 4.2 a	11.83 ± 3.2 a	2.77 ± 0.31 ab (8.1)
Stem injection	Glyphosate	4.77 ± 0.37 a (22.6)	16.9 ± 2.3 a	17.6 ± 3.6 a	1.75 ± 0.53 b (4.2)
Stem injection	Aminopyralid	5.38 ± 0.56 a (32.2)	20.6 ± 5.3 a	15 ± 3.6 a	2.12 ± 0.77 b (6.3)
			Height (cm)[f]		
None	None	206 ± 13.6 a	2.27 ± 0.03 a (190)	198 ± 12.5 a	258.7 ± 11.2 a
Spot application	Glyphosate	226 ± 14.5 a	2.32 ± 0.03 a (215)	83.5 ± 13.1 b	97.4 ± 14.4 b
Spot application	Aminopyralid	203 ± 13.9 a	2.24 ± 0.03 a (177)	163.1 ± 29.3 a	148 ± 23.7 b
Stem injection	Glyphosate	209 ± 17.7 a	2.27 ± 0.04 a (190)	132.3 ± 17.2 ab	123.7 ± 30.6 b
Stem injection	Aminopyralid	206 ± 28.3 a	2.24 ± 0.03 a (174)	166.2 ± 12.6 ab	184.6 ± 40.6 ab
			Diameter (cm)		
None	None	1.9 ± 0.1 a	1.8 ± 0.1 a	1.92 ± 0.1 a	2.24 ± 0.1 a
Spot application	Glyphosate	2.1 ± 0.1 a	1.9 ± 0.2 a	1.38 ± 0.2 b	0.95 ± 0.2 b
Spot application	Aminopyralid	2.0 ± 0.1 a	2.1 ± 0.2 a	1.38 ± 0.2 ab	1.7 ± 0.3 ab
Stem injection	Glyphosate	1.9 ± 0.2 a	1.9 ± 0.1 a	1.23 ± 0.4 b	0.7 ± 0.2 b
Stem injection	Aminopyralid	1.8± 0.2 a	2.1 ± 0.3 a	1.45 ± 0.1 ab	1.3 ± 0.4 ab

[a] Initial treatments in 2016 were conducted on June 22 2016.

[b] YAIT, year after initial treatments.

[c] Treatments in 2017 were conducted on June 20 2017.

[d] Density data prior to treatment application in 2016 and at the end of the 2017 growing season were squareroot(x) transformed prior to analysis to satisfy the assumptions of the ANOVA analysis. Transformed means are presented for means comparisons, and back-transformed means are given in parentheses.

[e] Means within columns followed by the same letter do not differ significantly according to Tukey's means separation test at the 0.05

[f] Height data collected at the end of the 2016 growing season were log(*x*) transformed prior to analysis to satisfy the assumptions of the ANOVA analysis. Transformed means are presented for means comparisons, and back-transformed means are given in parentheses.

There was a significant effect of herbicide treatment on Japanese knotweed stem density, height, and diameter at 1 YAIT at Bible Hill ($P \leq 0.001$), though only a significant effect of herbicide treatment on stem height at Antigonish 1 YAIT ($P \leq 0.008$). Spot applications of glyphosate and aminopyralid reduced Japanese knotweed stem density 1 YAIT at Bible Hill (Table 1), but not at Antigonish (Table 2). Stem injections of aminopyralid reduced stem density at Bible Hill but not at Antigonish (Tables 1 and 2).

Japanese knotweed stem height was reduced at 1 YAIT by spot applications of glyphosate and aminopyralid and stem injections of glyphosate at Bible Hill, and all herbicide treatments at this site reduced diameter of regenerating stems at 1 YAIT (Table 1). Spot applications of glyphosate reduced stem height and diameter at Antigonish, with glyphosate stem injections also reducing stem diameter at 1 YAIT at this site (Table 2). Aminopyralid applications, regardless of application method, did not reduce Japanese knotweed stem height or diameter at 1 YAIT at Antigonish (Table 2).

All herbicide treatments reduced Japanese knotweed stem density by the end of the second year in which treatments were applied at Bible Hill, though spot applications of glyphosate were generally most effective at this site (Table 1). Reapplication of spot and stem injection applications of glyphosate, as well as stem injections of aminopyralid, in the second year also reduced Japanese knotweed stem density by the end of the second growing season at Antigonish (Table 2). Spot applications of aminopyralid, however, were not effective at Antigonish (Table 2). Spot applications of glyphosate and stem injections of both glyphosate and aminopyralid reduced Japanese knotweed stem height at Bible Hill by the end of the second growing season (Table 1). Both spot applications and injections of glyphosate also reduced stem height at Antigonish (Table 2), and spot applications of aminopyralid also reduced stem height at this site.

All herbicide treatments reduced knotweed stem diameter by the end of the second growing season at Bible Hill (Table 1). Both glyphosate treatments also reduced stem diameter at Antigonish (Table 2), but aminopyralid did not reduce stem diameter by the end of the second growing season at Antigonish (Table 2).

3.4.2 Effect of cut-stump glyphosate application on Japanese knotweed shoot regeneration

There was a significant effect of cut-stump and glyphosate on Japanese knotweed stem density at the end of the year in which treatments were applied at each site (Tables 3 and 4). There was a significant effect of cut stump, but not glyphosate, on Japanese knotweed stem height and diameter at the end of the year in which treatments were applied at the rail site (Table 3), but only an effect of cut stump on stem height at rail HQ (Table 4).

Table 3. Significance of main and interactive effects of cut-stump and glyphosate application on Japanese knotweed stem density, height, and diameter at the rail site in 2017 and 2018 at Bible Hill, Nova Scotia.

Effect	Prior to treatment applications in 2017[a]	End of 2017 growing season following application of treatments	1 YAIT[b]
		Density	
Cut-stump	0.620[c]	0.011	0.426
Glyphosate	0.275	0.026	0.045
Cut-stump*glyphosate	0.329	0.948	0.004
		Stem height	
Cut-stump	0.173	0.001	0.655
Glyphosate	0.176	0.133	0.168
Cut-stump*glyphosate	0.196	0.082	0.157
		Stem diameter	
Cut-stump	0.268	0.012	0.002
Glyphosate	0.754	0.053	0.158
Cut-stump*glyphosate	0.268	0.032	0.629

[a] Initial treatments were conducted on August 22, 2017.
[b] YAIT, year after initial treatments.
[c] P-values obtained from ANOVA in Minitab18™. Values less than 0.05 are significant.

Table 4. Significance of main and interactive effects of cut-stump and glyphosate application on Japanese knotweed stem density, height, and diameter at the rail HQ site in 2017 and 2018 at Bible Hill, Nova Scotia.

Effect	Prior to treatment applications in 2017[a]	End of 2017 growing season following application of treatments	1 YAIT[b]
		Density	
Cut-stump	0.879[c]	0.001	0.686
Glyphosate	0.078	0.008	0.001
Cut-stump*glyphosate	0.039	0.839	0.012
		Stem height	
Cut-stump	0.810	0.001	0.203
Glyphosate	0.977	0.289	0.001
Cut-stump*glyphosate	0.647	-[d]	0.032
		Stem diameter	
Cut-stump	0.159	0.157	0.387
Glyphosate	0.790	0.758	0.001
Cut-stump*glyphosate	0.808	-	0.022

[a] Initial treatments in 2017 were conducted on August 22 2017.

[b] YAIT is year after initial treatments.

[c] P-values obtained from ANOVA in Minitab18™. Values less than 0.05 are significant.

[d] Could not determine effect due to insufficient stem density.

Cut-stump or glyphosate spot applications alone did not reduce Japanese knotweed stem density in the year of treatment applications at the Rail site (Table 5), though the cut-stump treatment did reduce density at the Rail HQ site (Table 6). In contrast, cut-stump plus glyphosate applications reduced density in the year of treatment applications at each site (Tables 5 and 6). The cut-stump treatment reduced height of regenerating stems at each site (Tables 5 and 6), though diameter of these stems was not consistently reduced across sites. Cut-stump plus glyphosate applications also reduced height and diameter of regenerating stems at the Rail site (Table 5), though density was too low for these measurements at the Rail HQ site (Table 6).

There was a significant effect of glyphosate and the cut-stump by glyphosate interaction on Japanese knotweed stem density 1 YAIT at each site (Tables 3 and 4). Glyphosate applications alone consistently reduced density at 1 YAIT at each site (Tables 5 and 6), with height and diameter of stems regenerating at 1 YAIT also reduced at the Rail HQ site (Table 6). Insufficient stem density in the glyphosate treatment at 1 YAIT at the Rail Site precluded inclusion of this treatment in the stem height and diameter analysis. Cut-stump alone or in combination with glyphosate applications did not reduce stem density, height, or diameter at 1 YAIT at Rail site (Table 5). However, Cut-stump with glyphosate application reduced stem density and stem diameter 1 YAIT at Rail HQ site (Table 6).

Table 5. The main and interactive effects of cut-stump and glyphosate application on Japanese knotweed stem density, height, and diameter in 2017 and 2018 at the Rail site, Bible Hill, Nova Scotia.

Cut-Stump	Glyphosate	Prior to treatment applications in 2017[a]	End of 2017 growing season following application of treatments	1 YAIT[b]
			Density (stems m^{-2})[c]	
No	No	9 ± 1.8 a[d]	3.4 ± 0.3 a (11)	13 ± 2 a
No	Yes	9 ± 2.1 a	2.0 ± 0.6 ab (5)	1 ± 1.7 b
Yes	No	8 ± 2 a	2.2 ± 0.4 ab (5)	4.5 ± 1.2 b
Yes	Yes	12 ± 1.9 a	0.9 ± 0.3 b (1)	7.5 ± 2.5 ab
			Height (cm)	
No	No	305 ± 16.4 a	292 ± 8.9 a	192 ± 20.1 a
No	Yes	306 ± 6.2 a	303 ± 4.1 a	-[e]
Yes	No	253 ± 28.6 a	87 ± 16.4 b	126 ± 18.5 a
Yes	Yes	304 ± 14.5 a	41 ± 15.7 b	128 ± 53 a
			Diameter (cm)[f]	
No	No	2.3 ± 0.1 a	4.8 ± 0.3 a (2.2)	2.0 ± 0.1 a
No	Yes	2.1 ± 0.1 a	5.4 ± 0.2 a (2.3)	-
Yes	No	1.9 ± 0.2 a	4.1 ± 0.8 a (2.0)	1.1 ± 0.2 b
Yes	Yes	2.1 ± 0.2 a	1.5 1.46 b (0.7)	1.3 ± 0.3 a

[a] Initial treatments in 2017 were conducted on August 22 2017.

[b] YAIT, year after initial treatments.

[c] Density data at the end of the 2017 growing season were squareroot(*x*) transformed prior to analysis to satisfy the assumptions of the ANOVA analysis. Transformed means are presented for means comparisons, and back-transformed means are given in parentheses.

[d] Means within columns followed by the same letter do not differ significantly according to Tukey's means separation test at the 0.05 level of significance. Values represent the mean ± 1 SD ($n = 4$).

[e] Height and diameter data could not be determined 1 YAIT due to low stem density.

[f] Diameter data collected at the end of the 2017 growing season were $(x)^2$ transformed prior to analysis to satisfy the assumptions of the ANOVA. Transformed means are presented for means comparisons, and back-transformed means are given in parentheses.

Table 6. The main and interactive effects of cut-stump and glyphosate application on Japanese knotweed stem density, height, and diameter in 2017 and 2018 at CN Rail HQ, Bible Hill, Nova Scotia.

Cut-Stump	Glyphosate	Prior to treatment applications in 2017[a]	End of 2017 growing season following application of treatments	1 YAIT[b]
			Density (stems m^{-2})[c]	
No	No	163.8 ± 15.6 a[d] (12)	3.6 ± 0.3 a (13)	12 ± 1.3 a
No	Yes	105.2 ± 5.2 b (9)	1.1 ± 0.4 a (7)	1 ± 0.9 c
Yes	No	85 ± 20.8 b (10)	2.6 ± 0.4 b (1.7)	9 ± 0.9 ab
Yes	Yes	118.8 ± 21.5 ab (10)	0 ± 0 b (0)	5 ± 1.1 bc
			Height (cm)[e]	
No	No	6.5 ± 0.2 a (287)	17.2 ± 0.1 a (295)	225 ± 25.3 a
No	Yes	6.4 ± 0.3 a (275)	16.5 ± 0.6 a (275)	32 ± 3.17 b
Yes	No	6.4 ± 0.2 a (267)	6.9 ± 0.4 b (48)	194 ± 16.1 a
Yes	Yes	6.5 ± 0.1 a (281)	-[f]	134 ± 28.0 ab
			Diameter (cm)[g]	
No	No	2.3 ± 0.3 a	5 ± 1.2 a (2.2)	1.9 ± 0.2 a
No	Yes	2.3 ± 0.3 a	5.7 ± 0.9 a (2.3)	0.1 ± 0.1 c
Yes	No	2.1 ± 0.1 a	2.6 ± 2.1 a (1.2)	1.5 ± 0.2 ab
Yes	Yes	2 ± 0.1 a	-	0.9 ± 0.2 bc

[a] Initial treatments in 2017 were conducted on August 22 2017.

[b] YAIT year after initial treatments.

[c] Density data prior to treatment applications in 2017 and at the end of the growing season in 2017 were $(x)^2$ and squareroot(x) transformed, respectively, prior to analysis to satisfy the assumptions of the ANOVA. Transformed means are presented for means comparisons, and back-transformed means are given in parentheses.

[d] Means within columns followed by the same letter do not differ significantly according to Tukey's means separation test at the 0.05 level of significance. Values represent the mean ± 1 SD ($n = 4$).

[e] Height data collected at the end of the 2017 growing season were log(x) transformed prior to analysis to satisfy the assumptions of the ANOVA analysis. Transformed means are presented for means comparisons, and back-transformed means are given in parentheses.

[f] Could not determine height or diameter at the end of the 2017 growing season due to low stem density.

[g] Diameter data collected at the end of the 2017 growing season were $(x)^2$ transformed prior to analysis to satisfy the assumptions of the ANOVA. Transformed means are presented for means comparisons, and back-transformed means are given in parentheses.

3.5 Discussion

Spot applications of glyphosate and aminopyralid reduced Japanese knotweed stem density, height, and diameter 1 YAIT at Bible Hill (Table 3). These treatments did not reduce these responses at 1 YAIT at Antigonish, though re-application of treatments in 2017 reduced these responses in 2018 (Table 4). Injections of glyphosate and aminopyralid did not result in significant reductions in Japanese knotweed stem density, height, or diameter 1 YAIT at Bible Hill or Antigonish (Tables 3 and 4). However, the re-application of treatments in the 2017 growing season reduced Japanese knotweed stem density at Bible Hill and Antigonish (Tables 3 and 4). These results highlight the importance of repeated treatment applications in Japanese knotweed management. With persistent treatment, density reductions acquired from spot applications or injections could be considered large enough to begin re-establishment of native species.

Our findings are similar to those in existing knotweed management literature. Delbart et al. (2012) found that both glyphosate injections and spot applications reduced knotweed stem density, and Ford (2004) found that cutting Japanese knotweed stems followed by injecting 10 mL of Roundup Pro-Biactive per stem at 96 g a.e. L water^{-1}, resulted in 60 – 95% kill of Japanese knotweed in treated areas. We did not see such large reductions until the fall of the second growing season in which treatments were applied (Tables 1 and 2). While these results are promising, Ford (2004) noted that this application method should be considered for use primarily in areas where conservation of co-existing plants is a priority to provide competition against knotweed and prevent invasion by other invasive species.

We found that stem injections were required over at least two consecutive years for consistent reductions in stem density (Tables 1 and 2) as new stems regenerated in the year after the first stem injection. Ford (2004) noted that some knotweed regeneration occurred following stem injections as well and was most effectively treated with spot applications of glyphosate as regenerating stems were too small for additional injections. Diameter of regenerating stems at 1 YAIT was also reduced in our study (Tables 1 and 2), though not to the extent that additional stem injections were prohibited. As such, the ability to conduct subsequent injections may vary across locations or potentially be affected by the severity of injury from the initial stem injection. Hagen and Dunwiddie (2008) also found that knotweed stem regrowth occurred following stem injections of glyphosate, further indicating the general need for subsequent treatments when using this application method. Our data suggest that spot applications are generally more effective than stem injections (Tables 1 and 2), and we would therefore recommend that use of stem injections as an herbicide application method be limited to environmentally sensitive areas where preservation of surrounding plants is a priority and use of spot applications may be prohibited.

Cut-stump glyphosate applications did not consistently reduce Japanese knotweed stem density at either site at 1 YAIT (Tables 5 and 6). In contrast, spot applications of glyphosate reduced knotweed stem density, height, and diameter at 1 YAIT at each site (Tables 5 and 6). These results are consistent with other experiments conducted with glyphosate for this thesis (Chapter 2, Tables 5 and 6)) and from other reports in the literature (De Waal 1995; Child and Wade 2000; Delbart et al. 2012). It is possible that the rapid lethal effects of the cut-stump glyphosate application damaged above ground tissues too quickly, preventing the herbicide from translocating to the rhizomes. Stems treated with cut-stump glyphosate turned completely black and died by the end of the growing season. This in-season lethality was not characteristic of other glyphosate applications conducted in this thesis or in pre-existing literature, wherein glyphosate injury took at least a year to cause stem density reductions (Chapter 2 - Tables 5 and 6; Child and Wade 2000; Delbart et al. 2012). Further to this, Kabat et al. (2006) found through a meta-analysis that 1.5 to 23.75 months was the average time for glyphosate to cause a significant reduction in knotweed stem density. Nice (2007) noted knotweed stem regrowth can occur 1 YAIT following a spot application of glyphosate at 840 g a.e. ha^{-1} to Japanese knotweed stems within 30 minutes of cutting. We also observed regrowth 1 YAIT in plots where cut-stump glyphosate treatments were applied (Tables 5 and 6). Nice (2007) suggests a follow up application of glyphosate to regenerating stems. Given this method did not have significantly different density from control plots 1 YAIT, it does not seem like an efficient way to manage knotweed given that a spot application of glyphosate provided greater reductions in stem density 1 YAIT (Tables 5 and 6). Results from the Rail HQ site, however, do suggest potential for use of cut-stump herbicide applications to control Japanese knotweed (Table 6), and perhaps additional work to examine alternative glyphosate concentrations or other herbicides, including those that are apoplastically translocated. Further research into this method could yield a useful means for control on roadsides or other areas where lines of sight must be maintained. For example, James et al. (2006) found in greenhouse trials that performing a cut-stump application of 0.15 mL picloram gel (Vigilant$^{\text{TM}}$) to knotweed cut at 50 or 200 mm above ground level resulted in 100% control of top growth 135 days after treatment.

One additional aspect of the cut-stump experiment that warrants discussion is the fact that the knotweed stands used in this experiment were in the understory of a tree canopy, which may have affected knotweed response to treatments. Dommanget et al. (2013) found that light availability was the most significant factor contributing to knotweed biomass generation. This effect may have occurred at the rail site as cut-stump alone significantly reduced stem density 1 YAIT (Table 5), which is not consistent with the general response of Japanese knotweed to cutting alone (Chapter 2, tables 1 thru 6; Child and Wade 2000; Delbart et al. 2012). Delbart et al. (2012)

also found competitive willow planting combined with cutting was also able to significantly reduce knotweed stem density better than cutting alone. Initial stem density was also considerably lower at the rail site and rail HQ site relative to other knotweed stands in this chapter (Tables 3 and 4), potentially further indicating the suppressive effects of the overstory canopy on knotweed growth at these sites.

3.6 Conclusions

Spot application of glyphosate and aminopyralid at peak height reduced knotweed stem density, height, and diameter at 1 YAIT and should remain the standard treatment for Japanese knotweed management in areas where these treatments are acceptable. Efficacy of herbicide stem injections and cut-stump herbicide applications was variable, and at least two consecutive years of injections were required to cause significant reductions in stem density. We also found injections to be slow and labor-intensive, limiting practicality of this herbicide application method to highly sensitive areas where damage to associated vegetation or environmental contamination must be avoided. Cut-stump applications of glyphosate caused rapid death of above ground knotweed tissues and the use of this treatment could be considered in situations where immediate restoration of sight lines (e.g., roadsides) is required.

3.7 Citations

Barney, J. N. (2006). North American history of two invasive plant species: Phytogeographic distribution, dispersal vectors, and multiple introductions. *Biological Invasions*, *8*(4), 703–717. https://doi.org/10.1007/s10530-005-3174-9

Child, L., & Wade, P. M. (2000). *The Japanese knotweed manual: The management and control of an invasive alien weed*. Packard Publishing.

Dawson, F. H., & Holland, D. (1999). The distribution in bankside habitats of three alien invasive plants in the U.K. in relation to the development of Control Strategies. *Biology, Ecology and Management of Aquatic Plants*, 193–201. https://doi.org/10.1007/978-94-017-0922-4_27

de Waal, L. C. (1993, September). Treatment of Fallopia japonica near water a case study. Plant invasions: general aspects and special problems. In *Workshop held at Kostelec nad Cernymi lesy, Czech Republic* (pp. 16-19).

Delbart, E., Mahy, G., Weickmans, B., Henriet, F., Crémer, S., Pieret, N., Vanderhoeven, S., & Monty, A. (2012). Can land managers control Japanese knotweed? lessons from control tests in Belgium. *Environmental Management*, *50*(6), 1089–1097. https://doi.org/10.1007/s00267-012-9945-z

Dommanget, F., Spiegelberger, T., Cavaillé, P., & Evette, A. (2013). Light availability prevails over soil fertility and structure in the performance of Asian knotweeds on riverbanks: New management perspectives. *Environmental Management*, *52*(6), 1453–1462. https://doi.org/10.1007/s00267-013-0160-3

Ford, S. (2004). Cut and inject herbicide control of Japanese knotweed Fallopia japonica at Rocky Valley, Cornwall, England. *Conservation Evidence*, *1*, 1-2.

Hagen, E. N., & Dunwiddie, P. W. (2008). Does stem injection of glyphosate control invasive knotweeds (Polygonum spp.)? A comparison of four methods. *Invasive Plant Science and Management*, *1*(1), 31–35. https://doi.org/10.1614/ipsm-07-013r.1

James, T. K., Rahman, A., & Popay, A. I. (2006). Control of the environmental weeds Reynoutria japonica and Bryonia cretica. In *Proceedings of the 15th Australian Weeds Conference, eds C. Preston, JH Watts and ND Crossman* (pp. 195-8).

Japanese Knotweed Injection Systems. (2017). *Articles and videos*. JK Injection Systems. Retrieved September 15, 2019, from https://www.jkinjectiontools.com/news/articles-and-videos/

Kabat, T. J., Stewart, G., & Pullin, A. (2006). Are Japanese Knotweed (Fallopia japonica) control and eradication interventions effective? Systematic Review n 21. *Center for Evidence-Based Conservation, Birmingham*.

Larsen, T. (2013). *Biology, ecological impacts, and management of Japanese knotweed (Polygonum cuspidatum syn. fallopia japonica) in Nova Scotia*. Dalhousie University, Halifax, N.S., Canada, M.Sc thesis.

Nice, G. (2007). (publication). *Japanese Knotweed (Polygonum cuspidatum)*. West Lafayette, IN: Purdue University Extension.

Siemens, T. J., & Blossey, B. (2007). An evaluation of mechanisms preventing growth and survival of two native species in invasive bohemian knotweed (Fallopia xbohemica, Polygonaceae). *American Journal of Botany*, *94*(5), 776–783. https://doi.org/10.3732/ajb.94.5.776

Townsend, A. (1997). Japanese knotweed: a reputation lost. *Arnoldia*, *57*(3), 13-19.

Vanderklein, D. W., Galster, J., & Scherr, R. (2013). The impact of Japanese knotweed on stream baseflow. *Ecohydrology*, *7*(2), 881–886. https://doi.org/10.1002/eco.1430

Wahlers, R. L., Burton, J. D., Maness, E. P., & Skroch, W. A. (1997). A stem cut and blade delivery method of herbicide application for weed control. *Weed Science*, *45*(6), 829–832. https://doi.org/10.1017/s0043174500089037

CHAPTER 4: EFFECT OF SEASONAL HERBICIDE APPLICATION TIMING ON JAPANESE KNOTWEED SHOOT REGENERATION

4.1 Abstract

Japanese knotweed (*Reynoutria japonica*) is a problematic invasive species in Europe and North America that causes significant reductions in native plant diversity, increases soil erosion, among other negative impacts. Chemical control has proven to be the most effective means of control for Japanese knotweed. Evaluations of multiple herbicides at different application timings, however, are limited. Experiments were conducted to determine i) the effect of spot applications of herbicides when applied at peak height and ii) the effect of spot applications of herbicides when applied in the fall. In both (i) and (ii) imazapyr, aminopyralid, glyphosate and aminocyclopyrachlor were evaluated for their ability to reduce Japanese knotweed stem density, height, and diameter. Spot applications of glyphosate and imazapyr at peak height caused significant reductions in knotweed stem density, height, and diameter 1 year after initial treatment (YAIT) at South Maitland and Antigonish ($P < 0.05$). Aminopyralid and aminocyclopyrachlor did not reduce stem density, height, or diameter when applied at peak height ($P > 0.05$). Spot applications of imazapyr, aminopyralid and glyphosate in fall caused significant reductions in Japanese knotweed stem density, height, and diameter at South Maitland and Antigonish 1 YAIT ($P > 0.05$), though aminocyclopyrachlor was once again ineffective. Glyphosate and imazapyr should be the primary herbicides used for the management of Japanese knotweed, and our data support an imazapyr registration for use in Canada. Aminopyralid should be utilized for fall applications, when efficacy is improved, and to allow for selective control of Japanese knotweed when species such as grasses are present.

4.2 Introduction

Japanese knotweed (*Reynoutria japonica*) is a problematic invasive plant in Europe and North America. It was introduced to North America in the 1800s as a horticultural species (Townsend 1997), which likely facilitated its proliferation. Japanese knotweed develops dense, monotypic stands and produces stems that can exceed 300 cm in height (Siemens and Blossey 2007; Larsen 2013). These stems develop a dense canopy that can prevent up to 90% of light from reaching its understory (Vanderklien et al. 2013), thus preventing native plants from growing and leading to a lack of ground cover (Beerling et al. 1994). This lack of ground cover can lead to significant increases in soil erosion, sediment loading, and riverbank destruction (Dawson and Holland 1999; Child and Wade 2000). Management of this species is therefore a priority for a range of landowners. Homeowners in the UK, for example, have incentive to manage knotweed due to the negative effect on real estate value (Elliot 2011). Although it is now known that the frequency of foundation penetration by knotweed rhizomes is ~ 2 – 6% of cases, where shrinkable clay soil is also present (Bacon 2018), the potential cost of home repairs tends to encourage management. Prior to hosting the 2012 Olympics, the UK government spent ~ $123 million CAD, on the management of knotweed, as it was present in areas designated for building construction (Elliot 2011).

Literature on Japanese knotweed management indicates that herbicides are the most effective means for controlling the species (De Waal 1995; Child and Wade 2000; Delbart et al. 2012; Larsen 2013). De Waal (1995) and Child and Wade (2000) both recommend mid-summer glyphosate applications to reduce knotweed stem density. While effective, multiple glyphosate applications would be required, as reemergence of stems tends to occur, however their vigor is reduced (De Waal 1995; Child and Wade 2000; Delbart et al. 2012). Larsen (2013) found that spot applications of aminopyralid conducted prior to knotweed reaching peak height caused stem density reductions within the growing season, however failed to cause density reductions in the year following treatment. Knotweed stem density was reduced in the year following treatment, however, when spot applications of imazapyr were conducted at peak height and after flowering (Larsen 2013). A new group 4 herbicide aminocyclopyrachlor (Truvist$^{\text{TM}}$) was found to be effective in a greenhouse experiment for control of Japanese knotweed (Rudenko and Hulting 2010), but there is limited data to suggest that it is effective under field conditions. While it is clear that herbicides are an essential tool in the management of Japanese knotweed, efficacy of herbicides may be affected by application timing due to seasonally dependent changes in source-sink relationships in Japanese knotweed.

The primary target organ in the management of Japanese knotweed is rhizomes (Child and Wade 2000), which need to be destroyed to prevent re-establishment of Japanese knotweed following treatment, but also to prevent movement of Japanese knotweed out of infested areas (Colleran and Goodall 2014). Therefore, herbicides need to be applied to Japanese knotweed when rhizomes are a strong sink, which is not the case at all times during the growing season. Price et al. (2002) showed, via radiotracer carbon, that rhizomes are a strong sink near the end of the growing season (August and September). This is supported by Jones et al. (2018), who modeled seasonal source-sink relationships in Japanese knotweed and found that rhizomes become an increasingly stronger sink after above ground stems reach maximum growth in early summer. During the fall (August, September, October) rhizomes become a strong sink (Jones et al. 2018), indicating an herbicide application at this time could cause significant damage. Comparisons of herbicide treatments at application timings when rhizomes are a sink, however, are generally lacking in the literature. Information regarding differential efficacy of common herbicides at relevant application timings is also lacking and could be useful for guiding management efforts given the potential effects of different herbicides on non-target desirable plants.

The objective of this research was to determine the effect of peak height (summer) and fall applications of glyphosate, aminopyralid, aminocyclopyrachlor, and imazapyr on Japanese knotweed shoot regeneration.

4.3 Materials and Methods

4.3.1 Effect of Herbicide Applications at Peak Stem Height on Japanese Knotweed Shoot Regeneration

The objective of this experiment was to determine the effect of herbicide applications at peak stem height on Japanese knotweed shoot regeneration. The experiment was conducted at South Maitland site 1 (Figure 1A) and Antigonish site 1 (Figure 1 C). The experiment was arranged in a completely randomized design with five treatments, six and eight replications at Antigonish and South Maitland respectively, and 1 m X 2 m plot size. Plots were placed such that obvious Japanese knotweed stem clumps, as defined by Adachi et al. (1996), were in the center of each plot was at least one meter apart from each other. Treatments were 1) nontreated control, 2) imazapyr, 3) aminopyralid, 4) glyphosate, and 5) aminocyclopyrachlor. Herbicide application rates were 12, 4.8, and 9 g a.e. L water^{-1} for imazapyr, aminopyralid, and glyphosate, respectively and 1 g a.i. L water^{-1} for aminocyclopyrachlor. Aminocyclopyrachlor was applied in conjunction with a non-ionic surfactant at 0.2% v/v prior to application. Treatments were conducted once plants reached peak height (~ 300 cm). Treatments were applied using a CO_2 pressurized sprayer with a single teejet AI11002-VS air induction nozzle. Stems were sprayed until all leaf surfaces were covered and initial run-off of spray occurred. Treatments were conducted in 2017 and were applied at South Maitland on June 15, 2017 and at Antigonish on June 20, 2017.

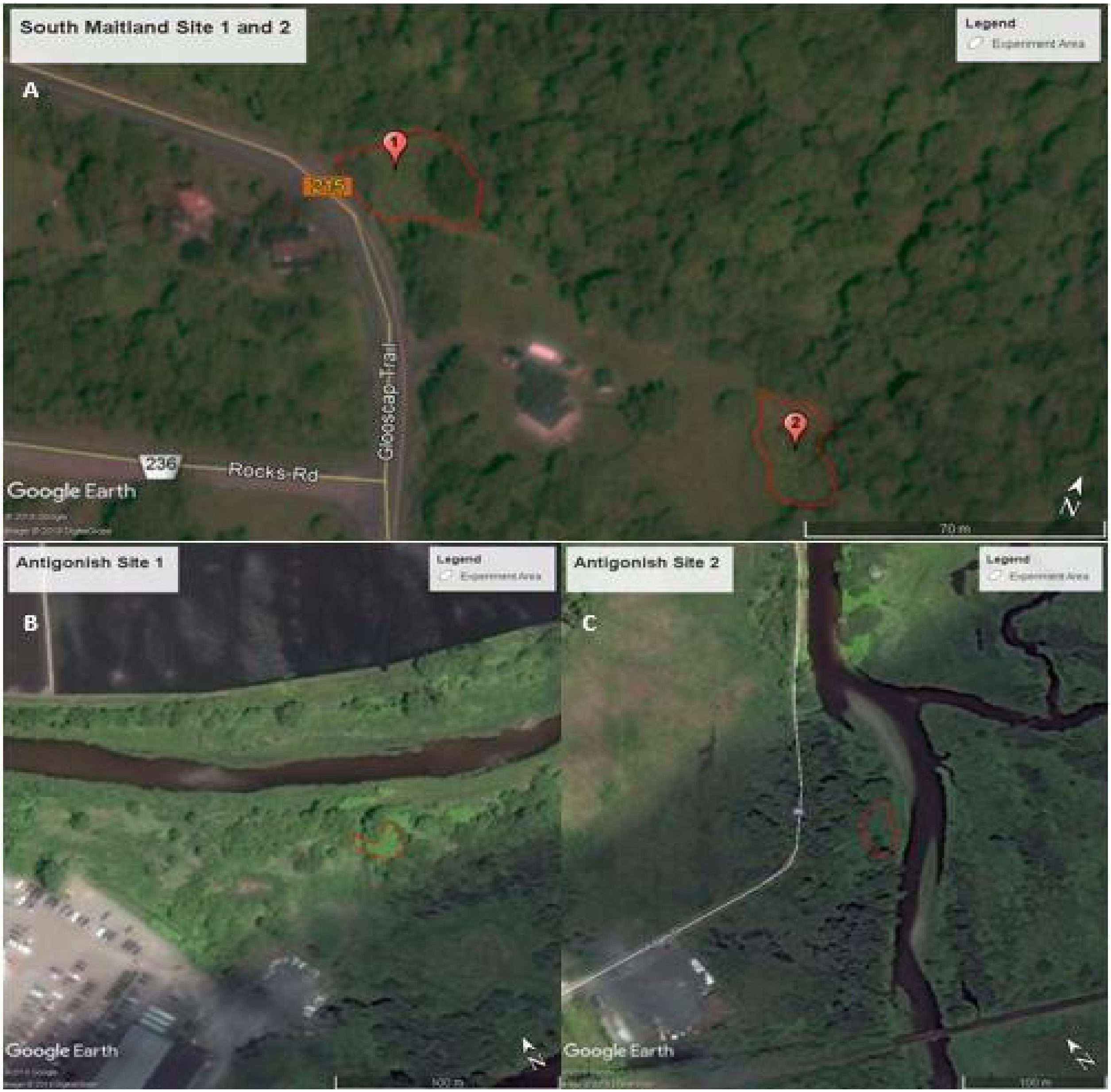

Figure 1. A) South Maitland site 1, total area of 604 m^{-2}, located at 45º15’15.89”N 63º28’23.48”W and South Maitland site 2, total area of 439 m^{-2} located at 45º15’14.66”N 63º28’18.07”W, B) Antigonish site 1, total area of 228 m^{-2}, located at 45º37’20.51”N 61º58’42.87”W, C) Antigonish site 2, total area of 780 m^{-2}, located at 45º37’30.50”N 61º58’26.59”W.

4.3.2 Effect of Fall Herbicide Application on Japanese Knotweed Shoot Regeneration

The objective of this experiment was to determine the effect of fall herbicide applications on Japanese knotweed shoot regeneration. The experiment was conducted at South Maitland site 2 (Figure 1A) and at Antigonish site 2 (Figure 1C). The experiment was arranged in a completely randomized design with five treatments, 4 and 6 replications at South Maitland and Antigonish, respectively, and 1m X 2m plot size. Plots were placed such that obvious Japanese knotweed stem clumps, as defined by Adachi et al. (1996), were in the center of each plot. Treatments and application methods were as indicated for the peak stem height experiment. Stems were sprayed until all leaf surfaces were covered and initial run-off of spray occurred. Treatments were conducted in 2017 and were applied at South Maitland on September 26 2017 and at Antigonish on October 2 2017.

4.3.3 Data Collection and Statistical Analysis

Data collection in each experiment included knotweed stem density, height, and diameter at the time of herbicide applications, at the end of the growing season in which treatments were applied, and in the season after herbicide treatments were applied. Stem density was determined on a whole-plot basis. Stem height and stem diameter were determined on 5 randomly selected stems in each plot.

The effect of herbicide treatment on Japanese knotweed stem density, stem height, and stem diameter in each experiment was determined using ANOVA in Minitab18 (Minitab LLC 2019) at the 0.05 level of significance. Herbicide treatment was modeled as a fixed effect in the ANOVA. Means separation, where necessary, was conducted using Tukey’s means separation test at the 0.05 level of significance. Minitab18 normality test was used to determine if data met required assumptions. If needed, data were transformed using squareroot and log transformations where indicated to meet normality and constant variance assumptions. Back transformed means are presented where transformations were used.

4.4 Results

4.4.1 Effect of Peak Height Herbicide Application on Japanese Knotweed Shoot Regeneration

There was a significant effect of herbicide treatment on knotweed stem density at South Maitland and Antigonish ($P \leq 0.001$) by the end of the first growing season in which treatments were applied. There was also a significant effect of herbicide treatment on stem height at South Maitland ($P = 0.004$) but not at Antigonish ($P = 0.094$) at the end of the first growing season. There was a significant effect of herbicide treatment on stem diameter at South Maitland ($P = 0.001$), but not Antigonish ($P = 0.084$) by the end of first growing season.

Imazapyr applications at peak height reduced Japanese knotweed stem density by the end of the season in which treatments were applied at each site (Tables 1 and 2). Aminopyralid, glyphosate, and aminocyclopyrachlor did not consistently reduce density in the year of treatment applications at either site (Tables 1 and 2), though aminopyralid reduced density at South Maitland (Table 1). Japanese knotweed stem height and diameter were not consistently reduced in the year of application (Tables 1 and 2), though once again aminopyralid caused reductions at South Maitland (Table 1) but not at Antigonish (Table 2).

There was a significant effect of herbicide treatment on Japanese knotweed stem density, height, and diameter 1 YAIT at each site ($P \leq 0.003$). Imazapyr and glyphosate applications at peak height reduced Japanese knotweed stem density at 1 YAIT at each site (Tables 1 and 2). Aminopyralid reduced density at 1 YAIT at South Maitland but not at Antigonish, and aminocyclopyrachlor did not reduce density at 1 YAIT at either site (Tables 1 and 2). Reductions in stem height and diameter at 1 YAIT followed similar trends as occurred with stem density. All herbicide treatments reduced height of regenerating stems at 1 YAIT at South Maitland, with diameter of these stems also reduced by imazapyr, aminopyralid, and glyphosate (Table 1). In contrast, only imazapyr and glyphosate applications reduced height and diameter of regenerating stems at 1 YAIT at Antigonish (Table 2).

Table 1. Effect of herbicide applications at peak Japanese knotweed stem height on Japanese knotweed stem density, height, and diameter at South Maitland, Nova Scotia, Canada in 2017 and 2018.

Herbicide	Prior to onset of treatment applications in 2017	End of 2017 growing season following application of treatments[a]	1 YAIT[b]
		Density (stems plot^{-1})[c]	
None	30 ± 3.9 a[d]	22 ± 2.7 a	4.8 ± 0.3 a (24)
Imazapyr	32 ± 5.1 a	0 ± 0.1 c	0.1± 0.1c (0)
Aminopyralid	26 ± 4.1 a	4 ± 3.1 bc	2.5 ± 0.2 b (6)
Glyphosate	25 ± 4.1 a	14 ± 3 ab	2.1 ± 0.5 b (7)
Aminocyclopyrachlor	28 ± 3.6 a	19 ± 3.6 a	4.1 ± 0.3 a (18)
		Height (cm)	
None	285 ± 5.1 a	297 ± 4.4 a	172 ± 13 a
Imazapyr	280 ± 7.5 a	*	*
Aminopyralid	339 ± 65 a	123 ± 49.5 b	102 ± 12.1 b
Glyphosate	264 ± 10.7 a	262 ± 11.9 ab	72 ± 14.7 b
Aminocyclopyrachlor	277 ± 6.8 a	349 ± 56 a	115 ± 27.1 b
		Diameter (cm)	
None	2.3 ± 0.1 a	2.5 ± 0.1 a	2.1 ± 0.1 a
Imazapyr	2.3 ± 0.1 a	*	*
Aminopyralid	2.1 ± 0.1 a	1.0 ± 0.4 b	1.2 ± 0.2 bc
Glyphosate	2.1 ± 0.1 a	2.0 ± 0.1 a	0.7 ± 0.4 c
Aminocyclopyrachlor	2.2 ± 0.1 a	2.2 ± 0.4 a	1.7 ± 0.2 ab

[a] Initial treatments were conducted on June 15 2017.

[b] YAIT, year after initial treatments.

[c] Density data for 1 YAIT was squareroot(*x*) transformed prior to analysis to satisfy the assumptions of the ANOVA analysis. Transformed means are presented for means comparisons, and back-transformed means are given in parentheses.

[d] Means within columns followed by the same letter do not differ significantly according to Tukey's means separation test at the 0.05 level of significance. Values represent the mean ± 1 SD (*n* = 8).

[*] Inadequate number of surviving stems were available to determine stem height or diameter.

Table 2. Effect of peak height herbicide application on Japanese knotweed stem density, height, and diameter at Antigonish, Nova Scotia, Canada in 2017 and 2018.

Herbicide	Prior to onset of treatment applications in 2017	End of 2017 growing season following application of treatments[a]	1 YAIT[b]
		Density (stems plot^{-1})[c]	
None	30[c] ± 5.4 a[d]	26 ± 3.5 a	4.5 ± 0.2 a (19)
Imazapyr	23 ± 0.9 a	6 ± 2.2 b	1 ± 0 b (0)
Aminopyralid	23 ± 2 a	20 ± 2.1 a	3.8 ± 0.4 a (15)
Glyphosate	25 ± 2.8 a	20 ± 3.2 a	1.8 ± 0.1 b (2)
Aminocyclopyrachlor	29 ± 3.7 a	26 ± 2.2 a	4.7 ± 0.2 a (22)
		Height (cm)	
None	271 ± 7.9 a	293 ± 7.1 a	251 ± 14.5 a
Imazapyr	272 ± 4.6 a	205 ± 54 a	*
Aminopyralid	272 ± 7.9 a	275 ± 6.6 a	168 ± 54 ab
Glyphosate	274 ± 7.7 a	289 ± 13 a	83 ± 6.6 b
Aminocyclopyrachlor	264 ± 11 a	297 ± 2.2 a	171 ± 2.2 ab
		Diameter (cm)	
None	2.5 ± 0.1 a	2.7 ± 0.1 a	2.3 ± 0.2 a
Imazapyr	2.5 ± 0.1 a	1.9 ± 0.5 a	*
Aminopyralid	2.5 ± 0.1 a	2.7 ± 0.1 a	1.6 ± 0.2 a
Glyphosate	2.6 ± 0.1 a	2.7 ± 0.1 a	0.4 ± 0.1 b
Aminocyclopyrachlor	2.5 ± 0.1 a	2.5 ± 0.1 a	1.9 ± 0.1 a

[a] Initial treatments were conducted on June 20 2017.

[b] YAIT year after initial treatments.

[c] Density data for 1 YAIT was squareroot(*x*) transformed prior to analysis to satisfy the assumptions of the ANOVA analysis. Transformed means are presented for means comparisons, and back-transformed means are given in parentheses.

[d] Means within columns followed by the same letter do not differ significantly according to Tukey's means separation test at the 0.05 level of significance. Values represent the mean ± 1 SD ($n = 8$).

[*] Inadequate number of surviving stems were available to determine stem height or diameter.

4.4.2 Effect of Fall Herbicide Application on Japanese Knotweed Shoot Regeneration

There was a significant effect of herbicide treatment on Japanese knotweed stem density, height, and diameter ($P \leq 0.006$) in summer 2018 following application of treatments at South Maitland and Antigonish in fall 2017. Imazapyr, glyphosate, and aminopyralid applications reduced stem density by summer 2018 at both South Maitland and Antigonish (Tables 3 and 4). Only glyphosate reduced stem height by summer 2018 at South Maitland (Table 3), though applications of imazapyr, glyphosate, and aminopyralid reduced stem height by summer 2018 at Antigonish (Table 4). Applications of imazapyr and glyphosate reduced stem diameter by summer 2018 at both South Maitland and Antigonish, with aminopyralid also reducing stem height at Antigonish but not South Maitland (Tables 3 and 4). Applications of aminocyclopyrachlor did not reduce any measured response by summer 2018 at either site (Tables 3 and 4).

Table 3. Effect of fall herbicide applications on Japanese knotweed stem density, height, and diameter at South Maitland, Nova Scotia, Canada in 2018.

Herbicide	Prior to onset of treatment applications in fall 2017[a]	Summer 2018[b]
	Density (stems plot^{-1})[c]	
None	16 ± 1.8 a[d]	5.1 ± 0.1 a (26)
Imazapyr	16 ± 1.8 a	0.6 ± 0.3 c (0.7)
Aminopyralid	20 ± 2.9 a	2.3 ± 0.4 b (6)
Glyphosate	20 ± 2.5 a	1.3 ± 0.4 bc (2)
Aminocyclopyrachlor	23 ± 2.0 a	4.8 ± 0.3 a (24)
	Height (cm)[e]	
None	2.4 ± 0.008 a (301)	171 ± 4.9 a
Imazapyr	2.4 ± 0.01 a (293)	83 ± 38.0 ab
Aminopyralid	2.4 ± 0.005 a (300)	82 ± 7.0 ab
Glyphosate	2.4 ± 0.003 a (302)	45 ± 15.7 b
Aminocyclopyrachlor	2.4 ± 0.002 a (305)	137 ± 20.4 ab
	Diameter (cm)[f]	
None	2.6 ± 0.1 a	5.4 ± 0.5 a (2.3)
Imazapyr	2.6 ± 0.1 a	0.1 ± 0.06 b (0.3)
Aminopyralid	2.6 ± 0.1 a	5.4 ± 1.22 a (2.3)
Glyphosate	2.6 ± 0.1 a	1 ± 1.22 b (0.8)
Aminocyclopyrachlor	2.6 ± 0.1 a	6.7 ± 0.9 a (2.6)

[a] Treatments were applied on September 26 2017.

[b] Plots were evaluated on July 4 2018.

[c] Density data for summer 2018 was squareroot(*x*) transformed prior to analysis to satisfy the assumptions of the ANOVA analysis. Transformed means are presented for means comparisons, and back-transformed means are given in parentheses.

[d] Means within columns followed by the same letter do not differ significantly according to Tukey's means separation test at the 0.05 level of significance. Values represent the mean ± 1 SD ($n = 4$).

[e] Height data for prior to onset of treatments and summer 2018 were log(x) and squareroot(x) transformed respectively, prior to analysis to satisfy the assumptions of the ANOVA analysis. Transformed means are presented for means comparisons, and back-transformed means are given in parentheses.

[f] Diameter data for summer 2018 was $(x)^2$ transformed prior to analysis to satisfy the assumptions of the ANOVA analysis. Transformed means are presented for means comparisons, and back-transformed means are given in parentheses.

Table 4. Effect of fall herbicide application on Japanese knotweed stem density, height, and diameter at Antigonish, Nova Scotia, Canada in 2018.

Herbicide	Prior to onset of treatments in fall 2017[a]	Summer 2018[b]
	Density (stems plot^{-1})[c]	
None	22 ± 2.8 a[d]	1.3 ± 0.09 a (23)
Imazapyr	20 ± 3.1 a	0.06 ± 0.06 b (0.2)
Aminopyralid	24 ± 2.8 a	0.1 ± 0.1 b (0.75)
Glyphosate	23 ± 2.9 a	0.1 ± 0.09 b (0.6)
Aminocyclopyrachlor	25 ± 2.1 a	1.3 ± 0.07 a (20)
	Height (cm)[e]	
None	2.4 ± 0.01 a (292)	14 ± 0.8 a (201)
Imazapyr	2.4 ± 0.01 a (293)	*
Aminopyralid	2.4 ± 0.005 a (293)	4.2 ± 2.8 bc (41)
Glyphosate	2.4 ± 0.007 a (289)	2.9 ± 1.9 bc (23)
Aminocyclopyrachlor	2.4 ± 0.004 a (297)	9.5 ± 0.6 ab (93)
	Diameter (cm)	
None	2.3 ± 0.1 a	2.4 ± 0.2 a
Imazapyr	2.4 ± 0.1 a	*
Aminopyralid	2.6 ± 0.1 a	1.7 ± 0.3 ab
Glyphosate	2.4 ± 0.1 a	0.4 ± 0.1 c
Aminocyclopyrachlor	2.5 ± 0.1 a	2.0 ± 0.2 a

[a] Initial treatments were conducted on October 2 2017

[b] Plots were evaluated on June 28 2018

[c] Density data for summer 2018 squareroot(*x*) transformed prior to analysis to satisfy the assumptions of the ANOVA analysis. Transformed means are presented for means comparisons, and back-transformed means are given in parentheses.

[d] Means within columns followed by the same letter do not differ significantly according to Tukey's means separation test at the 0.05 level of significance. Values represent the mean ± 1 SD (*n* = 6).

[e] Height data for prior to onset of treatments and summer 2018 were log(x) and squareroot(x) transformed respectively, prior to analysis to satisfy the assumptions of the ANOVA analysis. Transformed means are presented for means comparisons, and back-transformed means are given in parentheses.

[*] Inadequate number of surviving stems were available to determine stem height or diameter.

4.5 Discussion

The objective of this research was to determine the effect of peak height and fall applications of imazapyr, glyphosate, aminopyralid, and aminocyclopyrachlor on Japanese knotweed shoot regeneration. There was a significant effect of herbicide, regardless of application timing or location, by summer 2018 ($P < 0.05$). Imazapyr and glyphosate consistently reduced Japanese knotweed stem density across application timings and locations. Peak height applications of aminopyralid did not consistently reduce Japanese knotweed stem density, height, or diameter. In contrast, fall applications of aminopyralid caused consistent reductions in knotweed stem density across sites by summer 2018. Peak height and fall aminocyclopyrachlor applications did not reduce Japanese knotweed stem density, height, or diameter at either site.

The efficacy of glyphosate on Japanese knotweed is well documented. However, it should be noted that multiple applications are typically required for complete control (Roblin 1988; De Waal 1995; Child and Wade 2000; Barney et al. 2006). Our results support the requirement for multiple glyphosate applications as stem regrowth was reduced but not completely inhibited by a single glyphosate application, regardless of time of application (Tables 1, 2, 3, and 4). Given the efficacy and domestically acceptable use of glyphosate for invasive plant control in Nova Scotia, this herbicide should be considered for routine knotweed management. In addition to efficacy, it is a relatively affordable herbicide at \$8.70 - \$12.84 per L, compared to aminopyralid at \$79.25 per L or imazapyr at \$43.73 per L (Ferrell and Sellers 2018). From an environmental standpoint, glyphosate is low risk to non-target organisms such as mammals, as they lack the EPSP synthase pathway that is the target site of glyphosate (Carlise and Trevors 1988). Further, it is rapidly degraded by microorganisms such as *Pseudomonas spp.* as a phosphorus source, leading to its degradation (Carlisle and Trevors 1988). Further to this, glyphosate can also be degraded by abiotic processes. Blake and Pallet (2018) reviewed literature and found that if glyphosate reaches water, it is primarily deposited into sediments where it is mineralized into AMPA (aminomethylphosphonic acid), then to carbon dioxide and phosphate compounds. Given the accessibility, low price, and minimal environmental impact makes glyphosate a good herbicide for the management of knotweed.

Imazapyr efficacy on Japanese knotweed has also been documented (Figueroa 1989; Larsen 2013). Our results show very limited shoot regeneration occurred in treated plots by summer 2018 (Tables 1, 2, 3, and 4), indicating more rapid control of Japanese knotweed likely occurs with imazapyr relative to glyphosate. Miller (2006) found that over 90% control could be achieved using a single application of imazapyr in Washington State. Panke and Renz (2012) reported 70 – 90% control of knotweed when imazapyr was spot applied in the fall. This herbicide should therefore be considered for use in situations where rapid kill of knotweed is required, such as where the presence of knotweed is a threat to infrastructure, or areas where glyphosate would not be appropriate. Imazapyr is not recommended for use within twice the drip line (e.g. twice the diameter of the tree canopy), as it is possible for the herbicide to leach from the roots of treated plants and be absorbed by non-target plants via roots (Tu et al. 2001). This is problematic as imazapyr has a half-life in soil as long as 141 days, where it remains available for uptake by plants (Tu et al 2001). There is therefore greater risk to non-target plants when treating Japanese knotweed with imazapyr relative to glyphosate, which would need to be considered when using this herbicide. That said, imazapyr would be an excellent choice when knotweed is near or in aquatic environments with limited tree cover as it is highly efficacious and rapidly degraded via microbes and photodegradation in water (Tu et al. 2001). As such, the current United States imazapyr registration permits the use of imazapyr products, such as Timberland Imazapyr Pro 4

Forestry, in the aforementioned environments for control of knotweed (Durkin 2009). No imazapyr herbicide products, however, are currently registered for Japanese knotweed control in Canada. The registration of an aquatic formulation of imazapyr in Canada would be beneficial as it would provide managers with an additional herbicide to control knotweed in sensitive environments, and this study provides relevant efficacy data to support such a registration.

Aminopyralid efficacy was inconsistent when applied at peak height (Tables 1 and 2), but this herbicide consistently reduced stem density when applied in the fall (Tables 3 and 4). Jones et al. (2018) suggested that applications of group 4 herbicides (such as aminopyralid), should be conducted prior to knotweed reaching its peak height, for the purpose of disrupting growth and development. That said, previous literature indicates that group 4 herbicides such as aminopyralid have limited efficacy against knotweed (Child and Wade 2000; Larsen 2013). Beerlin (1990) evaluated glyphosate and 2,4-D amine applications on knotweed. 2,4-D amine applications caused reductions in LAR (leaf area ratio) two weeks following treatment. While knotweed biomass was reduced 10 weeks following treatment, shoots began to recover from herbicide injury (Beerlin 1990). When a second application of glyphosate and 2,4-D amine was conducted, stems died within four weeks of treatment (Beerlin 1990). Delbart et al. (2012) found that triclopyr did not cause significant density reductions at 1 year after treatment following spot applications or stem injections. However, it did cause density reductions when tank mixed with aminopyralid and injected into knotweed stems. Fluroxypyr similarly performed poorly on its own, but efficacy improved when tank mixed with aminopyralid. These applications were all conducted in or around knotweeds peak height (mid-July to August) in Belgium (Delbart et al. 2012). Our results indicate that fall applications of aminopyralid would be useful for management of knotweed. These results are significant as aminopyralid selectively injures broadleaf plants and does not damage grass species if they are also treated with the herbicide (Wagner 2017). This would be useful if trying to conserve existing grasses on infested sites, or if considering the re-introduction of native grasses to sites subject to management. While reintroducing native species to infested areas is a ubiquitous and somewhat obvious recommendation (Zanette 2018), the exact species that should be used are less clear. Claeson and Bisson (2013) found that residual tree cover was negatively correlated with knotweed stem density, therefore tree establishment should be a priority in the long term. However, during the short term/ periods of on-going management, Zanette (2018) indicates shade and moisture tolerant species should be utilized. Grass species native to Nova Scotia such as bottlebrush grass (*Elymus hystrixi*) switchgrass (*Panicum virgatum*), and Indian grass (*Sorghastrum nutans*), among other species, should be evaluated for this purpose in future research.

Aminocyclopyrachlor did not reduce Japanese knotweed stem density, stem height, or stem diameter, regardless of application timing (Tables 1, 2, 3, and 4). Aminocyclopyrachlor applications of 0.56 kg a.i. ha^{-1} reduced Japanese knotweed biomass under greenhouse conditions (Rudenko and Hulting 2010), indicating potential efficacy of this herbicide on Japanese knotweed. Aminocyclopyrachlor applications of 0.168 kg a.i. ha^{-1} used in our study, however, were not effective, indicating that currently labelled aminocyclopyrachlor application rates for Canada are not high enough to allow this herbicide to be used for Japanese knotweed management. This herbicide is commonly used in non-crop areas in Canada (Bell et al. 2011), including Nova Scotia for control of wild chervil on dyke lands (Beaton 2014). It would be beneficial to consider higher application rates of this herbicide for Japanese knotweed control in Canada given previous reports of efficacy at higher application rates (Rudenko and Hulting 2010).

4.6 Conclusion

Both peak height and fall applications of herbicides provided effective control of Japanese knotweed. Imazapyr and glyphosate consistently reduced stem density, height, and diameter at both timings and should be considered for Japanese knotweed management in Canada. Peak height aminopyralid applications were inconsistent, but fall aminopyralid applications reduced stem density, height, and diameter. These results suggest that fall aminopyralid applications can be used to control Japanese knotweed on sites with sensitive grasses species, provided the herbicide is applied in the fall. Aminocyclopyrachlor applications, regardless of application timing, did not reduce stem density, height, or diameter, and cannot be recommended for Japanese knotweed management at this time. Future research should consider evaluations of higher application rates if this herbicide is to be pursued further for Japanese knotweed management.

4.7 Citations

Adachi, N., & Masayuki, T. (1996). Central die-back of monoclonal stands of Reynoutria japonicain an early stage of primary succession on Mount Fuji. *Annals of Botany*, *77*(5), 477–486. https://doi.org/10.1006/anbo.1996.0058

Bacon, K. (2018, July 9). *Japanese knotweed is no more of a threat to buildings than other plants – new study*. The Conversation. Retrieved May 12, 2019, from https://theconversation.com/japanese-knotweed-is-no-more-of-a-threat-to-buildings-than-other-plants-new-study-99580

Barney, J. N., Tharayil, N., DiTommaso, A., & Bhowmik, P. C. (2006). The biology of invasive alien plants in Canada. 5. Polygonum cuspidatum sieb. & zucc. [= fallopia japonica (Houtt.) Ronse Decr.]. *Canadian Journal of Plant Science*, *86*(3), 887–906. https://doi.org/10.4141/p05-170

Bashtanova, U. B., Beckett, K. P., & Flowers, T. J. (2009). Review: physiological approaches to the improvement of chemical control of Japanese knotweed (fallopia japonica). *Weed Science*, *57*(6), 584–592. https://doi.org/10.1614/ws-09-069.1

Beaton, E. F. (2014). *Wild chervil (anthriscus sylvestris (L.) Hoffm.) management on nova scotia dykes*. M.Sc thesis. Dalhousie University, Halifax, NS.

Beerling, D. J. (1990). The use of non-persistent herbicides, glyphosate, and 2,4-D amine, to control riparian stands of Japanese knotweed (Reynoutria Japonica Houtt.). *Regulated Rivers: Research & Management*, *5*(5), 413–417. https://doi.org/10.1002/rrr.3450050506

Bell, J. L., Burke, I. C., & Prather, T. S. (2011). Uptake, translocation and metabolism of aminocyclopyrachlor in prickly lettuce, rush skeletonweed and Yellow Starthistle. *Pest Management Science*, *67*(10), 1338–1348. https://doi.org/10.1002/ps.2197

Boyd, N. S., White, S. N., & Larsen, T. (2017). Sequential Aminopyralid and Imazapyr applications for Japanese knotweed (fallopia japonica) management. *Invasive Plant Science and Management*, *10*(3), 277–283. https://doi.org/10.1017/inp.2017.31

Carlisle, S. M., & Trevors, J. T. (1988). Glyphosate in the environment. *Water, Air, and Soil Pollution*, *39*(3-4), 409–420. https://doi.org/10.1007/bf00279485

Child, L., & Wade, P. M. (2000). *The Japanese knotweed manual: The management and control of an invasive alien weed*. Packard Publishing.

Claeson, S. M., & Bisson, P. A. (2013). Passive reestablishment of riparian vegetation following removal of invasive knotweed (Polygonum). *Invasive Plant Science and Management*, *6*(2), 208–218. https://doi.org/10.1614/ipsm-d-12-00070.1

Delbart, E., Mahy, G., Weickmans, B., Henriet, F., Crémer, S., Pieret, N., Vanderhoeven, S., & Monty, A. (2012). Can land managers control Japanese knotweed? lessons from control tests in Belgium. *Environmental Management*, *50*(6), 1089–1097. https://doi.org/10.1007/s00267-012-9945-z

Duke, S. O. (2020). Glyphosate: Environmental fate and impact. *Weed Science*, *68*(3), 201–207. https://doi.org/10.1017/wsc.2019.28

Durkin, P. R. (2009). *Imazapyr - US Forest Service*. Human Health and Ecological Risk Assessment. Retrieved May 19, 201AD, from https://www.fs.fed.us/foresthealth/pesticide/pdfs/Imazapyr_TR-052-29-03a.pdf

Ferrell, J. A. (2018). *SS-AGR-16/WG056: Approximate herbicide pricing*. Approximate herbicide pricing. Retrieved May 19, 2019, from https://edis.ifas.ufl.edu/wg056

Figueroa, P. F. (1989). Japanese knotweed herbicide screening trial applied as a roadside spray. In *Proceedings of the Western Society of Weed Science.* (Vol. 42, pp. 288-298).

Jones, D., Bruce, G., Fowler, M. S., Law-Cooper, R., Graham, I., Abel, A., Street-Perrott, F. A., & Eastwood, D. (2018). Optimizing physiochemical control of invasive Japanese knotweed. *Biological Invasions*, *20*(8), 2091–2105. https://doi.org/10.1007/s10530-018-1684-5

London Legacy Development Corporation. (2016). *Information Request Reference 17-046*. Information Request. Retrieved May 19, 2019, from https://www.queenelizabetholympicpark.co.uk/~/media/lldc/publication%20scheme/foi%20responses/201601/15078%20response.pdf

Miller, T. W., Evaluation of knotweed control projects in southwestern Washington (2006). Olympia, Wash. Washington State Dept. of Agriculture.

Panke, B., & Renz, M. (2012). Japanese knotweed Information Sheet. Madison; University of Wisconsin Extension.

Price, E. A., Gamble, R., Williams, G. G., & Marshall, C. (2002). Seasonal patterns of partitioning and remobilization of 14c in the invasive rhizomatous perennial Japanese knotweed (Fallopia japonica (Houtt.) Ronse Decraene). *Ecology and Evolutionary Biology of Clonal Plants*, 125–140. https://doi.org/10.1007/978-94-017-1345-0_7

Roblin, E. (1988). Chemical control of Japanese knotweed (Reynoutria japonica) on river banks in South Wales. *Aspects Of Applied Biology*, *16*, 201 – 206.

Rudenko, M., & Hulting, A. (2010). Integration of chemical control with restoration techniques for management of Fallopia japonica populations. *Management of Biological Invasions*, *1*(1), 37–49. https://doi.org/10.3391/mbi.2010.1.1.06

Tu, M., Herd, C., & Randall, J. M. (2001, April). Weed Control Methods Handbook: Tools & Techniques for Use in Natural Areas . Logan; Utah State University.

Wagner, V., Antunes, P. M., Irvine, M., & Nelson, C. R. (2016). Herbicide usage for invasive non-native plant management in wildland areas of North America. *Journal of Applied Ecology*, *54*(1), 198–204. https://doi.org/10.1111/1365-2664.12711

White, S. N., Boyd, N. S., & Van Acker, R. C. (2016). Evaluation of aminocyclopyrachlor applied aloneand in combination with registered herbicides for crop tolerance and weed control in wild blueberry (Vaccinium angustifolium AIT.). *Canadian Journal of Plant Science*, *96*(1), 11–16. https://doi.org/10.1139/cjps-2015-0214

Zanette, M. (2018). Mapping Knotweed Species Along Rivers in the District of North Vancouver. *Ecorestoration: RNS Technical Series*, (1).

CHAPTER 5: CONCLUSION

5.1 Summary

This thesis set out with the objective to assess effective management strategies for Japanese knotweed. The effectiveness of these strategies was based on their ability to reduce knotweed stem density, height, and diameter. Mechanical control (cutting) occasionally provided in season density reductions, and reliably reduced stem height and diameter within the growing season when cutting was conducted (Chapter 2, tables 1 - 6). Knotweed recovered in the year following treatment applications, however, indicating limited long-term suppression of knotweed by cutting. While cutting can be useful for immediately reducing obstructions caused by knotweed stems, it is not an efficient means to achieve long term stem density reductions (Chapter 2, tables 1 and 2).

Spot applications of glyphosate and aminopyralid significantly reduced knotweed stem density 1 YAIT (Chapter 2, tables 5 and 6), regardless of whether they were applied to knotweed stems at peak height or to stem regrowth one month following cutting. While glyphosate consistently reduced stem density, height, and diameter at Bible Hill and South Maitland 1 YAIT, aminopyralid was more variable unless combined with cutting (Chapter 2, tables 5 and 6). While cutting prior to herbicide applications did not result in greater density reductions compared to using herbicides alone, it does make performing the treatment application easier by reducing knotweed stem height. This method would also be useful when the size of a knotweed stand prevents some leaf and stem surfaces from being treated with herbicide.

A comparison of spot applications and injections of glyphosate and aminopyralid showed that both could significantly reduce stem density in the year following initial treatment (Chapter 3, tables 3 and 4). However, regardless of initial treatment method, a second herbicide application would be required 1 YAIT as stem density was not reduced to zero by either treatment (Chapter 3, tables 3 and 4). Stem injections, while potentially less damaging to non-target vegetation, would not be practical in large knotweed infestations due to high stem density limiting mobility and access to affected areas. Literature to date has indicated that stem injections should be used for the treatment of knotweed on the periphery of sensitive areas such as bodies of water or desirable plant species, to protect them from off-target drift (Ford 2004). Ford (2004) also indicates that cutting stems back to the 3rd or 4th node prior to injections can improve access to the site while leaving enough knotweed tissue behind to conduct treatments. We did not investigate this approach to stem injections, but this approach would be logical to improve accessibility to knotweed stems based on difficulties we encountered injecting dense clumps of stems. From a purely practical stand point, spot applications should be the first choice of methods when dealing with Japanese knotweed infestations due to the low cost of spot application versus injection equipment, (injection guns can cost upwards of $250 USD – Japanese Knotweed Injection Systems, Battle Ground, WA, USA 2020), the general ready availability of spot application equipment, the speed at which treatments can be applied, and the low amount of preparation required to perform treatments compared to injections. Our results are in line with existing literature, and reaffirm this practice.

The efficacy of a cut-stump glyphosate application was also evaluated. At approximately peak height (August), knotweed was cut to between the 3rd and 4th node above ground level with a machete. Glyphosate at 9.0 g a.e. L $^{-}$1 was immediately spot applied to these stems. Cut-stump glyphosate treatments reduced knotweed stem density to zero by the end of the growing season in which treatments were applied (Tables 3.5 and 3.6). Knotweed stem density was not significantly different from the control 1 YAIT in cut-stump treatments at both locations (Tables 3.5 and 3.6). Spot applications of glyphosate to knotweed at peak height, at the same rate, reduced knotweed stem density 1 YAIT at both locations (Tables 3.5 and 3.6). The in-season lethality of glyphosate in the cut-stump treatment was not characteristic of other glyphosate treatments throughout this thesis (Tables Chapter 2 tables 5 and 6, Chapter 3, tables 1 and 2, Chapter 4, Tables 1 and 2), wherein at least one growing season was required to cause significant reductions in stem density. This makes cut-stump applications useful for areas where maintaining line of site without performing multiple treatments is a priority, such as rail-crossings or intersections. However, the lack of long term density reductions from this method warrants further investigation into alternative glyphosate application rates, or alternative herbicides that could improve long term results.

In evaluating the effect of seasonal application timing, we found peak height and fall spot applications of imazapyr and glyphosate effective in reducing knotweed stem density 1 YAIT (Chapter 4, Tables 1 thru 4). The efficacy of imazapyr to control knotweed should further the case for an expansion of the Canadian label as Arsenal Powerline is currently only labeled for use on Japanese knotweed in the USA (BASF 2011). However, of great interest was how application timing changed the efficacy of aminopyralid. Fall applications of aminopyralid consistently reduced knotweed stem density 1 YAIT at both locations (Chapter 4, Tables 3 and 4), whereas peak height applications of aminopyralid reduced stem density at South Maitland (Chapter 4, Table 1), but not at Antigonish (Table 4.2). The inconsistent effects of aminopyralid were evident in chapters 2 and 3 as well, where it would reduce stem density 1 YAIT at one location but not the other (Chapter 2, Tables 5 and 6, Chapter 3, Tables 1 and 2). However, in previous chapters this lack of efficacy was corrected for by spraying regrowth instead of peak height growth (Chapter 2), or by performing a second round of treatment applications on surviving knotweed stems (Chapter 3). Based on our results, a fall application of aminopyralid could be used to suppress knotweed while maintaining or establishing native grass species in knotweed stands. This would help restore some ecological value to an infested area and while potentially insulating it against invasion from another invasive species.

5.2 Practical Applications

With an improved understanding of effective management strategies for knotweed, we can expand upon them to see how they could be applied in a management scenario. In figure 1 there are multiple large knotweed stands on this property that require varying treatment strategies.

Figure 1. Area layout at 10377 HWY 215, South Maitland, Nova Scotia. There are three large knotweed populations: (A) front yard area (~ 600 m^2), (B) roadside area (~ 67 m^2), and (C) backyard area (~ 439 m^2). Three smaller populations also occur on the property (D) (~ 23 m^2). Average stem density in stands A & C is 30 stems m^2 and in B & D is 15 stems m^2.

5.2.1 Alternating Rows

To treat stands A and C, an applicator could utilize the findings of Chapter 2 by creating alternating treatment rows (in this example we will use A and B). In A-rows, cut knotweed down to ground level using a machete in as straight of a line as possible to create a one meter wide strip that runs the length of the stand. Alternate this with one meter width B-rows, where knotweed is left standing. In B-rows, herbicide treatment can be conducted at peak height or in the fall. One month following cutting in A-rows, spot apply glyphosate until all leaf surfaces of the regenerating knotweed are covered.

This method allows applicators to treat the entirety of a knotweed stand in one growing season by opening up the knotweed canopy to treatment throughout the infested site. By setting a row width of one meter, this allows the applicator to treat all leaf surfaces in uncut rows, by treating either side of the B-row from the A-rows. The other benefit is that treatments in rows can be staggered throughout the growing season, spacing out the workload. Further, herbicides do not need to be applied all at once, reducing the risk of off-target impacts and limiting the applicator exposure to pesticides.

5.2.2 Stem Injection

Stand B requires a different approach than the previous example as knotweed has grown into a culvert. This would make spot application of glyphosate inappropriate as sprays are likely to land in water. Spot applications of imazapyr would also be inappropriate due to the pine trees growing near the knotweed stand and being at risk of imazapyr exposure through contaminated soil. Protecting these trees is a priority to the landowner for aesthetic reasons and they are shading the knotweed, possibly reducing stem density. As previously indicated by Dommengant et al. (2013), reducing light availability is key to the suppression of knotweed. Utilizing the findings of chapter 3, stem injections of glyphosate could be conducted here as stem density is relatively low and most stems are accessible by walking down the middle of the culvert. Stem injections are less likely to cause off-target impacts as the herbicide is trapped inside the knotweed stem following injection. Stem injection would also be appropriate for stand D as it has other woody and herbaceous plants in competition with the knotweed due to overhead canopy cover. Stem injections would minimize the risk of off-target impacts and preserve the surrounding plants to succeed the area from knotweed.

5.2.3 Selective Herbicide Treatments to Aid Site Re-colonization by Native Plant Species

In the year following initial treatment using imazapyr or glyphosate, knotweed stem density should be reduced. This creates the need to re-establish native plant species in the area, however herbicide treatments still need to be conducted to suppress the surviving knotweed. An applicator could utilize the findings of chapter 4 by establishing a native grass species in the area and conducting a selective herbicide application using fall aminopyralid applications to suppress surviving knotweed plants. Group 4 herbicides such as aminopyralid generally do not damage grasses and a fall application would maximize the efficacy of aminopyralid. While this may not be appropriate for all areas, it would give managers a tool to start restoring the area.

5.3 The Endgame

Japanese knotweed, with time and effort, is a manageable species. However, when assessing the feasibility of management, what comes after needs to be a consideration. In many infested areas, native plants have been displaced for a significant period of time, meaning we may not know what an area ‘should’ look like. Therefore, setting a clear objective from the outset for what an area should become is crucial. Management for the sake of management isn’t good enough. There are plenty of ecological, economic, and human reasons to manage knotweed. If one hopes to make any progress in the management of invasive species; making sure managers and the general public see those reasons through the dense stands of knotweed crucial. With a well understood reason to conduct management, it will give us the motivation, buy-in, and social license to keep our machetes swinging and the treatments flowing. Hopefully with all of this, we will have the tools to make our little chunk of the world a slightly better place.

5.4 Citations

BASF. (2011). *Arsenal powerline herbicide*. BASF Professional Vegetation Management. `Retrieved May 5, 2019, from https://www.better-vegetation-management.basf.ca/en/products/arsenal-powerline-herbicide.html

Dommanget, F., Spiegelberger, T., Cavaillé, P., & Evette, A. (2013). Light availability prevails over soil fertility and structure in the performance of Asian knotweeds on riverbanks: New management perspectives. *Environmental Management*, *52*(6), 1453–1462. https://doi.org/10.1007/s00267-013-0160-3

Ford, S. (2004). Cut and inject herbicide control of Japanese knotweed Fallopia japonica at Rocky Valley, Cornwall, England. *Conservation Evidence*, *1*, 1-2.

Japanese Knotweed Injection Systems. (2017). *Articles and videos*. JK Injection Systems. Retrieved September 15, 2019, from https://www.jkinjectiontools.com/news/articles-and-videos/

References

Adachi, N., & Masayuki, T. (1996). Central die-back of monoclonal stands of Reynoutria japonicain an early stage of primary succession on Mount Fuji. *Annals of Botany*, *77*(5), 477–486. https://doi.org/10.1006/anbo.1996.0058

Bacon, K. (2018, July 9). *Japanese knotweed is no more of a threat to buildings than other plants – new study*. The Conversation. Retrieved May 12, 2019, from https://theconversation.com/japanese-knotweed-is-no-more-of-a-threat-to-buildings-than-other-plants-new-study-99580

Bailey, J. P., Bímová, K., & Mandák, B. (2008). Asexual spread versus sexual reproduction and evolution in Japanese knotweed S.L. sets the stage for the "Battle of the Clones." *Biological Invasions*, *11*(5), 1189–1203. https://doi.org/10.1007/s10530-008-9381-4

Barney, J. N. (2006). North American history of two invasive plant species: Phytogeographic distribution, dispersal vectors, and multiple introductions. *Biological Invasions*, *8*(4), 703–717. https://doi.org/10.1007/s10530-005-3174-9

Barney, J. N., Tharayil, N., DiTommaso, A., & Bhowmik, P. C. (2006). The biology of invasive alien plants in Canada. 5. Polygonum cuspidatum sieb. & zucc. [= fallopia japonica (Houtt.) Ronse Decr.]. *Canadian Journal of Plant Science*, *86*(3), 887–906. https://doi.org/10.4141/p05-170

BASF. (2011). *Arsenal powerline herbicide*. BASF Professional Vegetation Management. `Retrieved May 5, 2019, from https://www.better-vegetation-management.basf.ca/en/products/arsenal-powerline-herbicide.html

Bashtanova, U. B., Beckett, K. P., & Flowers, T. J. (2009). Review:physiological approaches to the improvement of chemical control of Japanese knotweed (fallopia japonica). *Weed Science*, *57*(6), 584–592. https://doi.org/10.1614/ws-09-069.1

Beaton, E. F. (2014). *Wild chervil (anthriscus sylvestris (L.) Hoffm.) management on nova scotia dykes* (dissertation). Dalhousie University, Halifax, NS.

Beerling, D. J. (1990). The use of non-persistent herbicides, glyphosate, and 2,4-D amine, to control riparian stands of Japanese knotweed (Reynoutria Japonica Houtt.). *Regulated Rivers: Research & Management*, *5*(5), 413–417. https://doi.org/10.1002/rrr.3450050506

Beerling, D. J., Bailey, J. P., Conolly, A. P. (1994). Biological flora of the British Isles, Fallopia japonica. *Journal of Ecology, 82: 959-979.*

Bell, J. L., Burke, I. C., & Prather, T. S. (2011). Uptake, translocation and metabolism of aminocyclopyrachlor in prickly lettuce, rush skeletonweed and Yellow Starthistle. *Pest Management Science*, *67*(10), 1338–1348. https://doi.org/10.1002/ps.2197

Borzenkova, R. A., & Borovkova, M. P. (2003). Developmental patterns of phytohormone content in the cortex and pith of potato tubers as related to their growth and starch content. *Russian journal of plant physiology*, *50*(1), 119-124.

Boyd, N. S., White, S. N., & Larsen, T. (2017). Sequential Aminopyralid and Imazapyr applications for Japanese knotweed (fallopia japonica) management. *Invasive Plant Science and Management*, *10*(3), 277–283. https://doi.org/10.1017/inp.2017.31

Brown, M. L., Duncan, C. A., & Halstvedt, M. B. (1999). Spotted knapweed management with integrated methods. In *Proceedings of the Western Society of Weed Science*.

Carlisle, S. M., & Trevors, J. T. (1988). Glyphosate in the environment. *Water, Air, and Soil Pollution*, *39*(3-4), 409–420. https://doi.org/10.1007/bf00279485

Child, L., & Wade, P. M. (2000). *The Japanese knotweed manual: The management and control of an invasive alien weed*. Packard Publishing.

Claeson, S. M., & Bisson, P. A. (2013). Passive reestablishment of riparian vegetation following removal of invasive knotweed (Polygonum). *Invasive Plant Science and Management*, *6*(2), 208–218. https://doi.org/10.1614/ipsm-d-12-00070.1

Claeson, S. M., LeRoy, C. J., Barry, J. R., & Kuehn, K. A. (2013). Impacts of invasive riparian knotweed on litter decomposition, aquatic fungi, and macroinvertebrates. *Biological Invasions*, *16*(7), 1531–1544. https://doi.org/10.1007/s10530-013-0589-6

Colleran, B. P., & Goodall, K. E. (2014). In situ growth and rapid response management of flood-dispersed Japanese knotweed (fallopia japonica). *Invasive Plant Science and Management*, *7*(1), 84–92. https://doi.org/10.1614/ipsm-d-13-00027.1

Davenport, R. (2006). Control of knotweed and other invasive species and experiences restoring native species in the Pacific Northwest us. *Native Plants Journal*, *7*(1), 20–26. https://doi.org/10.2979/npj.2006.7.1.20

Dawson, F. H., & Holland, D. (1999). The distribution in bankside habitats of three alien invasive plants in the U.K. in relation to the development of Control Strategies. *Biology, Ecology and Management of Aquatic Plants*, 193–201. https://doi.org/10.1007/978-94-017-0922-4_27

Delbart, E., Mahy, G., Weickmans, B., Henriet, F., Crémer, S., Pieret, N., Vanderhoeven, S., & Monty, A. (2012). Can land managers control Japanese knotweed? lessons from control tests in Belgium. *Environmental Management*, *50*(6), 1089–1097. https://doi.org/10.1007/s00267-012-9945-z

Dommanget, F., Spiegelberger, T., Cavaillé, P., & Evette, A. (2013). Light availability prevails over soil fertility and structure in the performance of Asian knotweeds on riverbanks: New management perspectives. *Environmental Management*, *52*(6), 1453–1462. https://doi.org/10.1007/s00267-013-0160-3

Durkin, P. R. (2009). *Imazapyr - US Forest Service*. Human Health and Ecological Risk Assessment. Retrieved May 19, 201AD, from https://www.fs.fed.us/foresthealth/pesticide/pdfs/Imazapyr_TR-052-29-03a.pdf

Elliot, V. (2018). *Couple are forced to demolish their £300k four-bed home after it was invaded by Japanese knotweed*. Couple are forced to demolish their £300k four-bed home after it was invaded by Japanese knotweed - Property Article : Property Services Directory - AllAboutProperty.com. Retrieved May 5, 2019, from http://www.surveyorsandvaluers.com/articles/item.php?id=3140

Farooq, M.H. 2018. Management of goldenrods (Solidago spp.) in wild blueberry (Vaccinium angustifolium Ait.) fields. Dalhousie University., Halifax, N.S., Canada. M.Sc. thesis.

Ferrell, J. A. (2018). *SS-AGR-16/WG056: Approximate herbicide pricing*. Approximate herbicide pricing. Retrieved May 19, 2019, from https://edis.ifas.ufl.edu/wg056

Figueroa, P. F. (1989). Japanese knotweed herbicide screening trial applied as a roadside spray. In *Proceedings of the Western Society of Weed Science.* (Vol. 42, pp. 288-298).

Ford, S. (2004). Cut and inject herbicide control of Japanese knotweed Fallopia japonica at Rocky Valley, Cornwall, England. *Conservation Evidence*, *1*, 1-2.

Hagen, E. N., & Dunwiddie, P. W. (2008). Does stem injection of glyphosate control invasive knotweeds (Polygonum spp.)? A comparison of four methods. *Invasive Plant Science and Management*, *1*(1), 31–35. https://doi.org/10.1614/ipsm-07-013r.1

James, T. K., Rahman, A., & Popay, A. I. (2006). Control of the environmental weeds Reynoutria japonica and Bryonia cretica. In *Proceedings of the 15th Australian Weeds Conference, eds C. Preston, JH Watts and ND Crossman* (pp. 195-8).

Japanese Knotweed Injection Systems. (2017). *Articles and videos*. JK Injection Systems. Retrieved September 15, 2019, from https://www.jkinjectiontools.com/news/articles-and-videos/

Jones, D., Bruce, G., Fowler, M. S., Law-Cooper, R., Graham, I., Abel, A., Street-Perrott, F. A., & Eastwood, D. (2018). Optimizing physiochemical control of invasive Japanese knotweed. *Biological Invasions*, *20*(8), 2091–2105. https://doi.org/10.1007/s10530-018-1684-5

Kabat, T. J., Stewart, G., & Pullin, A. (2006). Are Japanese Knotweed (Fallopia japonica) control and eradication interventions effective? Systematic Review n 21. *Center for Evidence-Based Conservation, Birmingham*.

Larsen, T. (2013). *Biology, ecological impacts, and management of Japanese knotweed (Polygonum cuspidatum syn. fallopia japonica) in Nova Scotia*. Dalhousie University, Halifax, N.S., Canada, M.Sc thesis.

Lau, J. A., Puliafico, K. P., Kopshever, J. A., Steltzer, H., Jarvis, E. P., Schwarzländer, M., Strauss, S. Y., & Hufbauer, R. A. (2008). Inference of allelopathy is complicated by effects of activated carbon on plant growth. *New Phytologist*, *178*(2), 412–423. https://doi.org/10.1111/j.1469-8137.2007.02360.x

London Legacy Development Corporation. (2016). *Information Request Reference 17-046*. Information Request. Retrieved May 19, 2019, from https://www.queenelizabetholympicpark.co.uk/~/media/lldc/publication%20scheme/foi%20responses/201601/15078%20response.pdf

Maerz, J. C., Blossey, B., & Nuzzo, V. (2005). Green frogs show reduced foraging success in habitats invaded by Japanese knotweed. *Biodiversity and Conservation*, *14*(12), 2901–2911. https://doi.org/10.1007/s10531-004-0223-0

Manchester, S. J., & Bullock, J. M. (2000). The impacts of non-native species on UK biodiversity and the effectiveness of control. *Journal of Applied Ecology*, *37*(5), 845–864. https://doi.org/10.1046/j.1365-2664.2000.00538.x

Mclean, S. (2010). Identification of the presence and impact of Japanese knotweed on development sites. *Journal of Building Appraisal*, *5*(4), 289–292. https://doi.org/10.1057/jba.2010.2

Middleton, C. (2016, February 21). *Japanese knotweed: The invasive plant that eats the value of your home*. Newsweek. Retrieved May 5, 2019, from https://www.newsweek.com/japanese-knotweed-driving-men-murder-257257

Miller, T. W., Evaluation of knotweed control projects in southwestern Washington (2006). Olympia, Wash.; Washington State Dept. of Agriculture.

Miller, T. W. (2016). Integrated strategies for management of Perennial Weeds. *Invasive Plant Science and Management*, *9*(2), 148–158. https://doi.org/10.1614/ipsm-d-15-00037.1

Monteiro, A., Moreira, I., & Sousa, E. (1999). Effect of prior common reed (phragmites australis) cutting on herbicide efficacy. *Biology, Ecology and Management of Aquatic Plants*, 305–308. https://doi.org/10.1007/978-94-017-0922-4_44

Nice, G. (2007). (publication). *Japanese Knotweed (Polygonum cuspidatum)*. West Lafayette, IN: Purdue University Extension.

Oliver, K. (2011, October). Learning legacy: Lessons learned from the London 2012 Games construction project. London; London Legacy Development Agency.

Panke, B., & Renz, M. (2012). Japanese knotweed Information Sheet. Madison; University of Wisconsin Extension.

Parepa, M., Schaffner, U., & Bossdorf, O. (2012). Sources and modes of action of invasive knotweed allelopathy: The effects of leaf litter and trained soil on the germination and growth of native plants. *NeoBiota*, *13*, 15–30. https://doi.org/10.3897/neobiota.13.3039

Price, E. A., Gamble, R., Williams, G. G., & Marshall, C. (2002). Seasonal patterns of partitioning and remobilization of 14c in the invasive rhizomatous perennial Japanese knotweed (Fallopia japonica (Houtt.) Ronse Decraene). *Ecology and Evolutionary Biology of Clonal Plants*, 125–140. https://doi.org/10.1007/978-94-017-1345-0_7

Renz, M. J., & DiTomaso, J. M. (1998). The Effectiveness of Mowing and Herbicides to Control Perennial Pepperweed in Rangeland & Roadside Habitats. In *Proceedings-Western Society Of Weed Science* (Vol. 51, pp. 129-129). *Western Society of Weed Science.*

Renz, M. J., & Ditomaso, J. M. (2006). Early season mowing improves the effectiveness of chlorsulfuron and glyphosate for control of perennial pepperweed (lepidium latifolium). *Weed Technology*, *20*(1), 32–36. https://doi.org/10.1614/wt-05-007r1.1

Roblin, E. (1988). Chemical control of Japanese knotweed (Reynoutria japonica) on river banks in South Wales. *Aspects Of Applied Biology*, *16*, 201 – 206.

Rudenko, M., & Hulting, A. (2010). Integration of chemical control with restoration techniques for management of Fallopia japonica populations. *Management of Biological Invasions*, *1*(1), 37–49. https://doi.org/10.3391/mbi.2010.1.1.06

Tu, M., Herd, C., & Randall, J. M. (2001, April). Weed Control Methods Handbook: Tools & Techniques for Use in Natural Areas . Logan; Utah State University.

Siemens, T. J., & Blossey, B. (2007). An evaluation of mechanisms preventing growth and survival of two native species in invasive bohemian knotweed (Fallopia xbohemica, Polygonaceae). *American Journal of Botany*, *94*(5), 776–783. https://doi.org/10.3732/ajb.94.5.776

Townsend, A. (1997). Japanese knotweed: a reputation lost. *Arnoldia*, *57*(3), 13-19.

Vanderklein, D. W., Galster, J., & Scherr, R. (2013). The impact of Japanese knotweed on stream baseflow. *Ecohydrology*, *7*(2), 881–886. https://doi.org/10.1002/eco.1430

de Waal, L. C. (1993, September). Treatment of Fallopia japonica near water a case study. Plant invasions: general aspects and special problems. In *Workshop held at Kostelec nad Cernymi lesy, Czech Republic* (pp. 16-19).

Wagner, V., Antunes, P. M., Irvine, M., & Nelson, C. R. (2016). Herbicide usage for invasive non-native plant management in wildland areas of North America. *Journal of Applied Ecology*, *54*(1), 198–204. https://doi.org/10.1111/1365-2664.12711

Wahlers, R. L., Burton, J. D., Maness, E. P., & Skroch, W. A. (1997). A stem cut and blade delivery method of herbicide application for weed control. *Weed Science*, *45*(6), 829–832. https://doi.org/10.1017/s0043174500089037

Ward, J. S., Worthley, T. E., & Williams, S. C. (2009). Controlling Japanese barberry (Berberis thunbergii DC) in Southern New England, USA. *Forest Ecology and Management*, *257*(2), 561–566. https://doi.org/10.1016/j.foreco.2008.09.032

White, S. N., Boyd, N. S., & Van Acker, R. C. (2016). Evaluation of aminocyclopyrachlor applied aloneand in combination with registered herbicides for crop tolerance and weed control in wild blueberry (Vaccinium angustifolium AIT.). *Canadian Journal of Plant Science*, *96*(1), 11–16. https://doi.org/10.1139/cjps-2015-0214

Zanette, M. (2018). Mapping Knotweed Species Along Rivers in the District of North Vancouver. *Ecorestoration: RNS Technical Series*, (1).