Biology, Ecological Impacts, and Management of Japanese Knotweed (*Polygonum cuspidatum* syn. *Fallopia japonica*) in Nova Scotia

by

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FACULTY OF AGRICULTURE

The undersigned hereby certify that they have read and recommend to the Faculty of Graduate Studies for acceptance a thesis entitled “Biology, Ecological Impacts, and Management of Japanese Knotweed (*Polygonum cuspidatum* syn. *Fallopia japonica*) in Nova Scotia” by Todd Larsen in partial fulfilment of the requirements for the degree of Master of Science.

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ABSTRACT

Japanese knotweed is a perennial geophyte from eastern Asia that was originally introduced to Europe and North America in the 19th century as an ornamental. It has since escaped cultivation and establishes in disturbed sites along roads, ravines, and riverbanks. This project assessed the biological characteristics, ecological impacts, and management options of this fascinating plant at study sites in Antigonish and Bible Hill, Nova Scotia. An average growth rate of almost 6 cm per day was recorded for one month in spring until it reached a maximum height of about 2 m in mid June. Knotweed stands contained on average 17 stems and 8.0 kg of fresh biomass per m^2. This dense canopy limits resources for other plant species and eventually a local plant community can become dominated by knotweed. Leaf cover was significantly greater in knotweed patches versus grass and shrub habitats in riparian ecosystems. Plant diversity in knotweed patches was nil, yet invertebrate diversity and abundances from pitfall traps were similar across habitats. Small mammal tracks were more abundant in knotweed than shrub patches, but not as much as grass plots. The insectivorous shrew (*Sorex*) species tracks were just as abundant in knotweed as grass plots, perhaps indicating favourable environmental conditions, protection from predators, or prey abundance. Further, as canopy cover measurements increased in knotweed patches, the abundance of mammal tracks also increased. These ecological benefits may be anomalous due to small, discontinuous patch size and an entire ecosystem dominated by knotweed would possibly yield different results. Herbicides are an effective management option for knotweed, and two herbicides were evaluated at four different dates in 2011. Aminopyralid effectively reduced density and leaf cover for two to eight weeks, but was ineffective one year after treatment. Imazapyr treatments were slower to show damage, but were very successful in reducing knotweed biomass, density, height, and leaf cover the following year. Imazapyr application is recommended at full growth (June) or flowering (August). This project provides new information on a serious invasive weed in eastern Canada, and proposes more research to link this knowledge with other invasive species.

LIST OF ABBREVIATIONS USED

μg	microgram
ae	acid equivalent
AFLP-PCR	Amplified Fragment Length Polymorphism –Polymerase Chain Reaction
ALS	acetolactate synthase
ANOVA	Analysis of variance
AWCP	Antigonish Wetland Compensation Project
CEC	Cation-exchange capacity
cm	centimeter
cmol+	centimol
CO_2	carbon dioxide
D	Simpson diversity index
F	Flowering (growth stage)
g	gram
GIS	Geographic Information System
GPS	Global Positioning System
H	Shannon diversity index
ha	hectare
ISR	Induced Systemic Resistance
kg	kilogram
km	kilometer
L	litre
LAI	Leaf Area Index
M	Maximum height (growth stage)
m	meter
mg	milligram
mL	milliliter
mm	millimeter
MWh	megawatt hour
n	number (sample)
N	number (population)
NS	Nova Scotia
NSAC	Nova Scotia Agricultural College
NSTIR	Nova Scotia Transportation and Infrastructure Renewal
psi	pounds per square inch
PVC	polyvinyl chloride
R^2	coefficient of determination
S	Species richness
S	Senescence (growth stage)
std dev	Standard Deviation
std err	Standard Error
UK	United Kingdom
US/USA	United States of America
VASCAN	Vascular Plants of Canada (database)
WAT	weeks after treatment
YAT	year after treatment

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Thanks to Dr Bob Pett with NSTIR for getting this project going and allowing me to make it my own. Also, Clinton Pinks and Ian Bryson from CBCL for background information and maps of Antigonish which demonstrated to me that this is just a small piece of a larger proposal. To the property owners of the Bible Hill site, Shawn Mooring and Martin Walsh, who always had something interesting to chat about, from guerilla gardening to the inevitable floods.

I appreciate the time that my committee members have dedicated to my work. I would still be floundering without Gord's GIS tips, chats with Vilis, and last minute assistance from Randy. Nathan Boyd helped keep me on track and his thoughtful comments and suggestions really made this project a success.

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Chapter 1: Introduction

Throughout history, humanity's desire to control natural systems has led to some disastrous consequences. Aside from resource overexploitation and rampant pollution, the intentional and/or accidental distribution of species beyond their 'natural' ranges can have a major impact on the earth. While many introduced species have a benign impact in their new environments, some species become pests of varying degrees. In an effort to maximize short-term gains for agriculture, forestry, and energy we have transformed wild ecological systems into homogenized environments, and fight an endless battle against pests to keep it this way. A weed is an anthropocentric term for a plant (native or introduced) growing in an undesirable location. Weeds can be controlled with a variety of management options, but first it is necessary to understand the biology of the weed, and to what extent it is impacting the agricultural or environmental system in order to develop an effective weed management plan.

As the saying goes, "one man's trash is another's treasure", and likewise a weed in one regard may be beneficial to other organisms. Invasive weeds however are in a distinct category due to their rapid growth, range expansion, colonizing success, and difficulty to control which cause negative economic, social, and environmental impacts (Pyšek et al. 2004). Landscapes dominated by an invasive species may be characterized as having lowered plant diversity; however there are examples of natural ecosystems with single dominating species that support healthy ecosystems, such as prairie grasslands or the boreal forest. The difference between a well-established ecosystem and a newly-invaded one is that species within an invaded community must adapt to short-term changes in resource availability, vegetative structure, and possibly biochemical alterations.

Japanese knotweed (*Polygonum cuspidatum* syn. *Fallopia japonica*) is a model example of an invasive weed as it has deleterious impacts on local ecosystems. It grows quickly in the spring and dominates the site with a thick canopy and leaf litter, thereby limiting resources for other plants. It has been reported to have allelopathic properties that inhibit the growth of neighbouring plants (Inoue et al. 1992). Knotweed is a major concern in Europe, and continues to threaten natural environments in North America. Originally introduced for ornamental purposes this plant has escaped cultivation and thrives in disturbed sites along roads, ravines, and river banks.

The overwhelming presence of such a dominating organism has been the focus of several studies investigating possible impacts on local ecosystems. Knotweed invasions have been blamed for changes in plant community structure (Siemens and Blossey 2007), invertebrate species richness (Gerber et al. 2008), and ecosystem functioning (Lecerf et al. 2007). Viewed from another perspective, the presence of an alien plant species actually increases diversity and available micro-niches if populations do not completely dominate an area. A better understanding of how Japanese knotweed interacts with native communities can provide a more balanced view of this debate.

Undeniably there are areas that require drastic measures to prevent a knotweed infestation from interrupting natural ecosystems or human activities. Management options must be carefully evaluated depending on the specific problem to avoid further complications. Cutting down small knotweed stands may be effective if repeated several times a year with constant follow-up visits (Bram 2002). An insect is being tested as a potential biological control (Shaw et al. 2009), but it is not a panacea for the problem. Currently herbicide application is the most commonly used method of management due to its efficacy and ease to employ. Different herbicide types, various spray rates, application methods, and timing of applications must be considered. Herbicide research is constantly improving these options, and additional work is required.

This thesis is an attempt to better understand Japanese knotweed in Nova Scotia and similar regions. There is very little research in Canada on this invasive weed, and although there is a wealth of information in Europe and elsewhere, it is important to learn more about the plant in this environment. By mapping current knotweed populations, we can provide a baseline for future work on range expansion and growth models. Knotweed hybridizes and backcrosses with its congeners, making identification difficult (Zika and Jacobson 2003), and creating different management challenges such as possible viable seed dispersal in North America (Forman and Kesseli 2003). A psyllid insect may be released in Nova Scotia as a potential biological control agent in 2015, so it is important to have a deep knowledge of the local knotweed biology and impact on the ecosystem. If small, contained knotweed populations actually contribute to local biodiversity, then it may not be as imperative to eliminate this ‘weed’. However, if it is a nuisance plant in certain areas then a tested herbicide plan would be greatly beneficial.

The objectives of this thesis are three-fold: (1) Record and describe the biological traits of Japanese knotweed as it grows in Nova Scotia including growth characteristics, population distribution, and phenological stages; (2) Describe its potential impacts on local riparian ecosystems by comparing knotweed patches with shrub and grass habitats, as well as differences in animal communities between these habitats; and (3) Evaluate a herbicide management plan with different application combinations at various times throughout the growing season to determine the optimal treatment for knotweed management. Results from this research will provide much needed information about Japanese knotweed in Nova Scotia for anyone that is confronted with this interesting plant.

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Chapter 2: The Biology of Japanese Knotweed *Polygonum cuspidatum* syn. *Fallopia japonica* in Nova Scotia, Canada

2.1 Abstract

Japanese knotweed is a non-native, perennial geophyte whose presence impacts local ecosystems by out-competing other plant species. There has been a substantial amount of research on the plant's history, biology, description, environmental impact, and management methods, yet questions still remain. Due to a large amount of variation across its range, it is important to get a broad perspective of the plant, as well as understand the characteristics of local varieties. This paper is focused on Japanese knotweed predominantly in the province of Nova Scotia but may be applied to a wider area including the other Atlantic Provinces and the Northeastern United States. Key findings from this paper include a description of knotweed in eastern Canada, distribution in Nova Scotia, climatic conditions, and growth dynamics.

2.2 Species Name and Taxonomic Relationships

Japanese knotweed is a fascinating plant laden with many interesting and sometimes esoteric characteristics. To begin with, even the nomenclature and taxonomic classification of knotweed has not been entirely resolved, with various camps adhering to either *Polygonum cuspidatum* Sieb. & Zucc., *Fallopia japonica* (Houtt.) Ronse Decr., or *Reynoutria japonica* Houtt. (Barney et al. 2006). A majority of research on knotweed is conducted in Europe where the plant is described as *F. japonica*; however the current morphological and molecular data neither confirm nor refute the placement of knotweed in the *Fallopia* genus (Barney et al. 2006). Conversely, North American researchers tend to classify knotweed as *P. cuspidatum* (Zika and Jacobson 2003) and this scientific name is listed in the authoritative list of Canadian weed names by Darbyshire (2003). Japanese knotweed is also known as Japanese bamboo, fleeceflower, elephant ears, wandering sailor, Devil's rhubarb, itadori (Japanese for 'strong plant'), and renouée du Japon

(French), among other less commonly used names (Child and Wade 2000; Darbyshire 2003). The plant is also referred to as simply ‘knotweed’ which gives the false notion that this species is the same as giant knotweed, the hybrid Bohemian knotweed, and several horticultural varieties.

2.3 Description and Account of Variation

Japanese knotweed is a fast-growing, herbaceous, perennial geophyte with an extensive below-ground rhizome structure (Figure 2.1a). Individual stems arise predominantly from perennating buds on the compact clump or ‘crown’, as well as from nodes along the lateral rhizome branches (Figure 2.1b). The crowns remain steadfast in the soil during heavy wind and flooding events, but can become detached from the lateral rhizomes to reveal their spongy, orange cores. The hollow, cylindrical stems are up to 4 cm in diameter at the base, tapering to a point at the end of the branches. The plants maximum canopy height is over 2 m tall. At maturity, the stems are green with reddish-purple streaks. Knotweed’s name refers to the conspicuous nodes along the stem which vary in distance from 30 cm near the base to less than 3 cm at the distal branches –on a 2 m tall plant there may be over 20 nodes. Branches arise alternately from these nodes; the length of branches is often dependent on growing conditions and available space but can reach lengths of 150 cm. The leaves form a distinctive ‘zigzag’ pattern and are found in a variety of shapes and sizes (Figure 2.1c), but are best described as broadly ovate (8-15 cm long by 5-12 cm wide) with a cuspidate apex and flattened base (Barney et al. 2006). Leaf blades are light green, glabrous, coarse-textured, and glaucous with entire margins and short petioles <1-4 cm long (Beerling et al. 1994). The small (2-3 mm diameter) creamy-white flowers have five tepals and eight stamens (Figure 2.1d), and are borne at the leaf axils to form elongated

(4-15 cm long) clusters (Beerling et al. 1994). A single, triangular, dark brown seed (3 mm long) is produced from each flower and is enclosed within a three-winged calyx (Uva et al. 1997) (Figure 2.1e). Knotweed has been described as being dioecious (Bram and McNair 2004), gynodioecious (Beerling et al. 1994), and subdioecious (Forman and Kesseli 2003), although this may be a case of mistaken species identity.

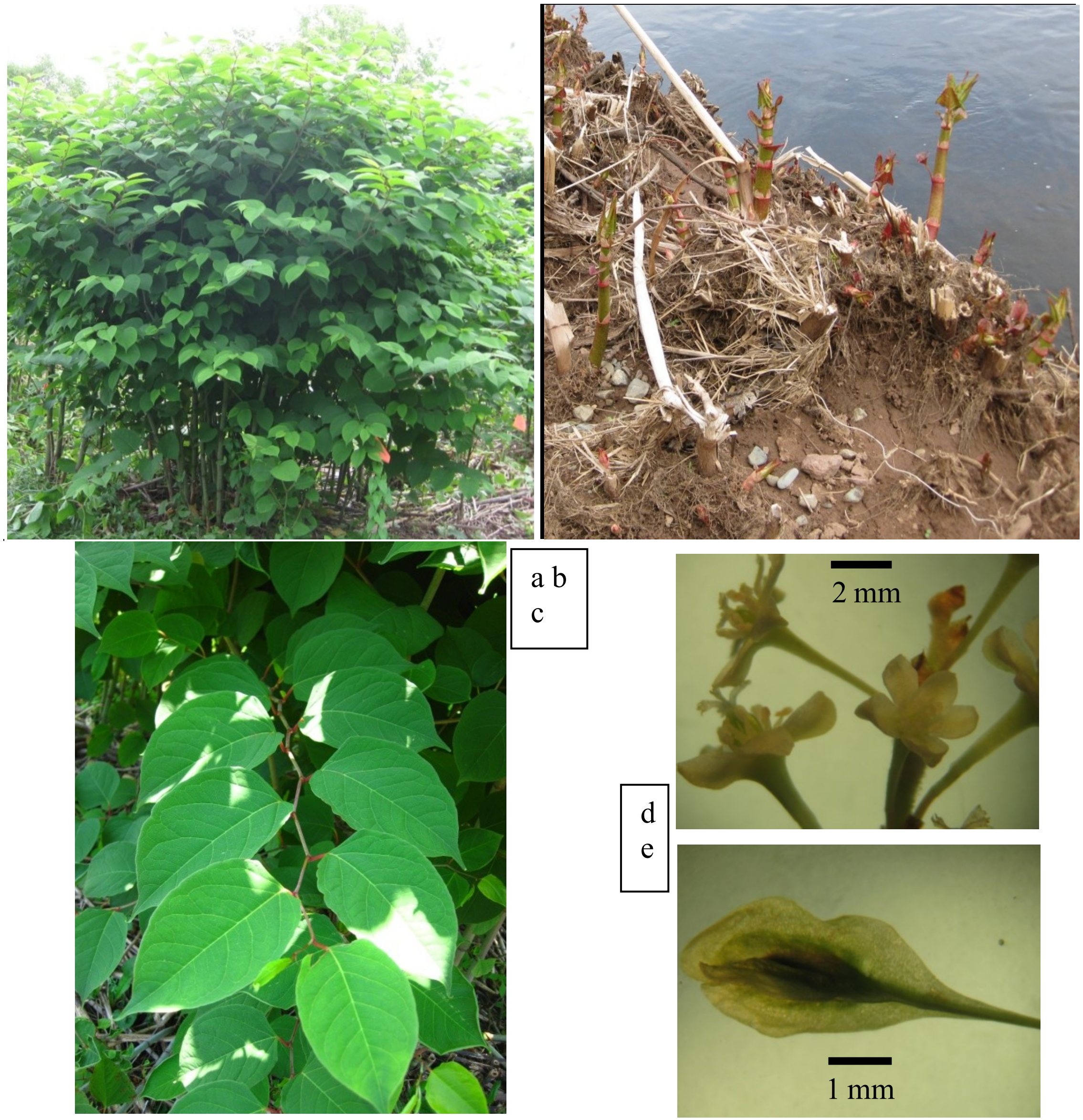

Figure 2.1 Maximum growth habit of knotweed, June 2011 Bible Hill (a). Spring emergence of knotweed shoots May 2011 Antigonish (b). Stem and leaves of knotweed July 2011 Bible Hill (c). Knotweed flowers Sept 2010 Bible Hill (d). Knotweed seed Oct 2010 Bible Hill (e).

In some locations, Japanese knotweed has cross-bred with giant knotweed (*Polygonum sachalinense*) to create the hybrid Bohemian knotweed (*Polygonum* X*bohemicum*) (Zika and Jacobson 2003). This hybrid is known to have viable seeds and even cross-pollinate with Japanese knotweed to create seedlings (Forman and Kesseli 2003). Bohemian knotweed exhibits varying characteristics of both parent species, and can thus be difficult to accurately identify (Zika and Jacobson 2003). *P.* X*bohemicum* has been commonly overlooked and identified as *P. cuspidatum,* and both species are commonly referred to as "Japanese knotweed sensu lato" (Bailey and Conolly 2000; Zika and Jacobson 2003). There are several identification keys with which to differentiate between the three knotweed species (Child and Wade 2000; Zika and Jacobson 2003; Barney et al. 2006; Wilson 2007), although it is impossible to determine if many published or anecdotal reports had accurately identified the species. Furthermore, a horticultural variety known as 'Crimson beauty' or *Polygonum cuspidatum* var. *compactum* is sold in greenhouses and can be found in various locations, including on the Dalhousie University Agricultural Campus in Truro, NS! A chromosomal study by Bailey and Stace (1992) in Britain concluded that the species could be distinguished by chromosomal counts. The diploid number of Japanese knotweed was found to be $2n = 88$, while giant knotweed and the horticultural variety was $2n = 44$, and Bohemian knotweed was $2n = 66$ (Bailey and Stace 1992). Due to the large degree of inter- and intra-specific morphological variation, the most accurate method of species identification appears to be with genetic analyses. Three knotweed samples from Antigonish, NS were sent to Agriculture and Agri-Food Canada in Lethbridge, AB in September 2010 for analysis using AFLP-PCR. They concluded that the specimens were Japanese knotweed *Polygonum cuspidatum.*

2.4 History

The history of Japanese knotweed's introduction to Europe has been well documented by Beerling et al. (1994) and by Bailey and Conolly (2000) in the aptly named article "Prize-winners to pariahs". It is astounding to read that a plant which is now so globally abhorred was once held in high regard. Japanese knotweed was first described and named *Reynoutria japonica* by Houttuyn in 1777 from dried samples of Japanese origin (Bailey and Conolly 2000). About 50 years later, live knotweed plants were shipped from Japan to Holland sometime between the years 1823 and 1829 by the German physician and naturalist Philipp Franz von Siebold. Siebold later established a commercial nursery of exotic plants in Leiden, Netherlands (Beerling et al. 1994), where he and Zuccarini named and described many flora of Japan including *Polygonum cuspidatum* in 1846. They were unaware of the previous description by Houttuyn (Bailey and Conolly 2000). It wasn't until 1901 that Makino discovered that both names described the same species, which he combined to produce *Polygonum reynoutria*, further adding to the list of eponyms for Japanese knotweed. Japanese knotweed became commercially available in 1846 from Von Siebold & Company and it was an instant horticultural sensation, earning the 1847 gold medal as the most interesting new ornamental plant of the year by the Society of Agriculture & Horticulture at Utrecht (Bailey and Conolly 2000). From that time forward, knotweed plants were transported across Europe and it was only a matter of time before they reached the New World.

The earliest herbarium record of knotweed in North America is in Yorkville, NY in 1873, and the first records in Canada include Chilliwack, BC, Longueuil, QC, and Niagara Falls, ON in 1901 (Barney 2006). In Nova Scotia, the earliest herbarium record I found was in 1936 in Wolfville, however Barney's research (2006) discovered evidence of knotweed in Halifax Regional Municipality and Yarmouth County from the early 1900's.

Historical records indicate that knotweed experienced very high initial introduction rates outside of their native range of East Asia, but experienced a lag period of several decades before becoming an invasive pest, as is the case with most invasive species (Crooks and Soulé 1999; Barney 2006). Under ideal climatic conditions and free from natural enemies, knotweed has thrived in its new environments, even though its primary method of reproduction is vegetative and not by seed. It is important to understand to what extent this plant may spread to new habitats if we are to properly prevent new invasions or manage current ones.

2.5 Economic Importance and Environmental Impact

2.5.1 Detrimental By definition, invasive plants have negative environmental, economic, and/or social impacts due to their rapid growth and expansion, ability to out-compete native species, and challenge to control (Pyšek et al. 2004; Canadian Food Inspection Agency 2008). Knotweed is not deemed an agricultural pest but an environmental weed because it "invades natural vegetation, usually adversely affecting native biodiversity and/or ecosystem functioning" (Pyšek et al. 2004). It impacts humans in terms of how much time, money, and effort we use to manage knotweed populations. It is ironic that humans continue to spread this species to new locations, while devoting great expense to eradicate it in others.

Japanese knotweed has an adverse cumulative effect on plant diversity and abundance. It grows remarkably fast in the spring which hinders the growth of native plants, thereby allowing for increased patch size which further reduces the colonization rates of other plants (Siemens and Blossey 2007). Remarkably, there are no known studies that report on the rate of knotweed patch spread, as this information would be valuable in estimating how quickly an area can become infested. Knotweed fragments can be transported by water or humans to establish new patches and eventually an entire ecological area may be dominated by knotweed stands. The resulting decrease in plant diversity (Maerz et al. 2005; Siemens and Blossey 2007; Hejda et al. 2009; Aguilera et al. 2010) has far-reaching effects on the local ecosystem. Various studies suggest that knotweed invasions alter stream communities and ecological functions (Lecerf et al. 2007), decreased invertebrate diversity (Gerber et al. 2008), and cause a decline in frog mass possibly due to a lack of available invertebrates (Maerz et al. 2005). The ground within a knotweed stand is essentially devoid of vegetation so there is nothing to prevent the erosion of topsoil (Figure 2.2), and can lead to increased sediment loads in adjacent watercourses (Child and Wade 2000).

Figure 2.2 Riverbank erosion and sedimentation due to the presence of knotweed along the Rights River, Antigonish.

Knotweed patches are commonly found along roads and near vacant lots, likely due to the transportation and disposal of contaminated soil and plant material, as well as the presence of moist, disturbed soil. As the patch expands there may be hazards for motorists as the line of sight is blocked, and it becomes an added expense for road crews to contend with. Knotweed is infamous for its ability to grow through asphalt and concrete. A study by Beerling (1991) tested seven commercially available revetment blocks used as erosion-reducing riverbank armour. He concluded that all of the blocks failed to prevent displacement and penetration by knotweed in Wales. Likewise, in Antigonish, NSTIR (Nova Scotia Transportation and Infrastructure Renewal) used riprap rock armour along the banks of the Rights River to decrease river scouring along a bend, and in the following summer knotweed was growing in between the rocks. In the UK, it is an offence to "plant or otherwise cause Japanese knotweed to grow in the wild" (Cornwall Council 2012). The plant is also deemed a controlled waste which must be disposed of according to government approved regulations (Environment Agency 2012). Along watercourses, dense stands of knotweed greatly restrict water access for recreationalists such as anglers, boaters, and nature enthusiasts. Furthermore, there are exacerbated flooding risks due to the inhibition of water flow and blocked drains by the profusion of vegetative material (Child and Wade 2000).

2.5.2 Beneficial Similar to several other weeds in Canada, Japanese knotweed was intentionally introduced as an ornamental plant. It was coveted for its unique, exotic growth form which resembles that of bamboo. Knotweed's rapid growth made it an excellent specimen for hedges and privacy screens. The profusion of fragrant flower clusters in autumn are also admired as there are not many other plant species which flower in that season. Knotweed stands serve as a late season nectar source for bees and other palynivores, and is thus valued by apiarists (Ferrazzi and Marletto 1990). At my wedding in September, knotweed flower clusters were used in flower bouquets, on table arrangements, and in a boutonniere. Japanese knotweed specimens can occasionally by found for sale at greenhouses, but usually it is in the less invasive, compact variety ‘Crimson Beauty’ (Forman and Kesseli 2003).

Aside from horticultural uses, knotweed was also originally used as fodder for livestock, and as erosion-control on sand dunes, but was abandoned because it out-competed desired vegetation and was not as effective as native grasses (Bailey and Conolly 2000). In consideration of the massive amounts of plant material that can be produced annually, there is much discussion regarding the use of knotweed as a biofuel source, yet there is not much research in this area. One study by Lehtomäki et al. (2008) evaluated the potential methane and energy produced from a variety of grasses, legumes, leafy crops, and straws in Finland. They found that giant knotweed when harvested in late summer could yield 3800 m^3 of methane per hectare, which corresponds to a gross energy yield of 36 MWh per hectare or passenger car transportation of 47 000 km. These values were within what they regarded as highly potential (along with reed canary grass, timothy-clover grass, and Jerusalem artichoke), although the high lignin content and reality of cultivating knotweed crops limited its acceptance as a realistic biogas crop (Lehtomäki et al. 2008). Another interesting use of giant knotweed is for disease suppression on edible crops and ornamentals. Marrone® Bio Innovations (Davis, CA, USA) recently registered the product Regalia® with Canada’s Pest Management Regulatory Agency as a biofungicide containing giant knotweed extract which “activates Induced Systemic

Resistance (ISR), an internal defence mechanism in plants that prevents growth of certain plant pathogens" (Pest Management Regulatory Agency 2011).

In its native Japan, and within its expanded range, knotweed is consumed by humans; there are many recipes on the internet ranging from wine to pie. The young shoots can be trimmed of leaves and steamed or stewed to make them more palatable as the uncooked stem contains oxalic acid. Knotweed is also a major source of resveratrol. This compound is a phytoalexin also found in grapes that is a cardiovascular protector (Bradamante et al. 2004), antioxidant, antimutagen, and a cancer chemopreventive agent for humans (Jang et al. 1997). Resveratrol is frequently supplied as a dietary supplement in pill form and the main ingredient is generally *Polygonum cuspidatum*. While grapes contain between 1.5 and 7.8 µg g^{-1} of resveratrol, knotweed contains 497, 867, and 2170 µg g^{-1} within samples of fresh stem, leaf, and root, respectively (Burns et al. 2002). A patent application by Kuhrts (2002) proposed to use extracts of knotweed root as a source of resveratrol to create a weight loss supplement, along with the many other beneficial attributes of the stilbenoid.

2.6 Geographical Distribution

Knotweed species are found across North America, but are less concentrated in the interior of the continent. The Database of Vascular Plants of Canada (VASCAN) indicates Japanese knotweed presence in every province except for Alberta and Saskatchewan, and none of the Territories (VASCAN 2012). Barney (2006) canvassed herbariums in Canada and the US for records of knotweed and compiled a map of counties and municipalities indicating presence and duration of occupation. The greatest majority of knotweed locations were in Eastern North America, roughly east of the Mississippi River (Barney

2006) and from the Carolinas in the south to the Saguenay region of Quebec in the north. On the west coast, the map indicates knotweed locations from California up to British Columbia. Of particular interest to this paper is Barney's (2006) account of knotweed distribution in Nova Scotia. His investigation concluded that knotweed has been present in two Nova Scotia counties (Halifax Regional Municipality and Yarmouth) for at least ten decades. Of the eighteen counties of Nova Scotia, Barney (2006) found no record of knotweed in six of them –Cumberland, Colchester, Pictou, Queens, Lunenburg, and Victoria. Knotweed was present in the remaining ten counties for at least four decades as of 2003, and in some cases substantially longer. To augment the known species distribution in Nova Scotia, I recorded knotweed sightings while traveling over the province between the fall of 2010 and 2012 (Figure 2.3). This map indicates that knotweed is wide spread across the province of Nova Scotia, but is not intended to suggest entire provincial distribution. Over 180 Japanese knotweed stands were recorded, as well as five patches of giant knotweed, and one possible Bohemian knotweed in the community of Glen Margaret. As the map shows, knotweed is distributed across the province, although I spent limited time driving in the interior of the province, along the Northumberland shore, and parts of Cape Breton. The only county where knotweed was not sighted was Richmond County, which I did not visit during daylight hours. The majority of knotweed stands were located along older roads, abandoned homesteads, and well-established communities.

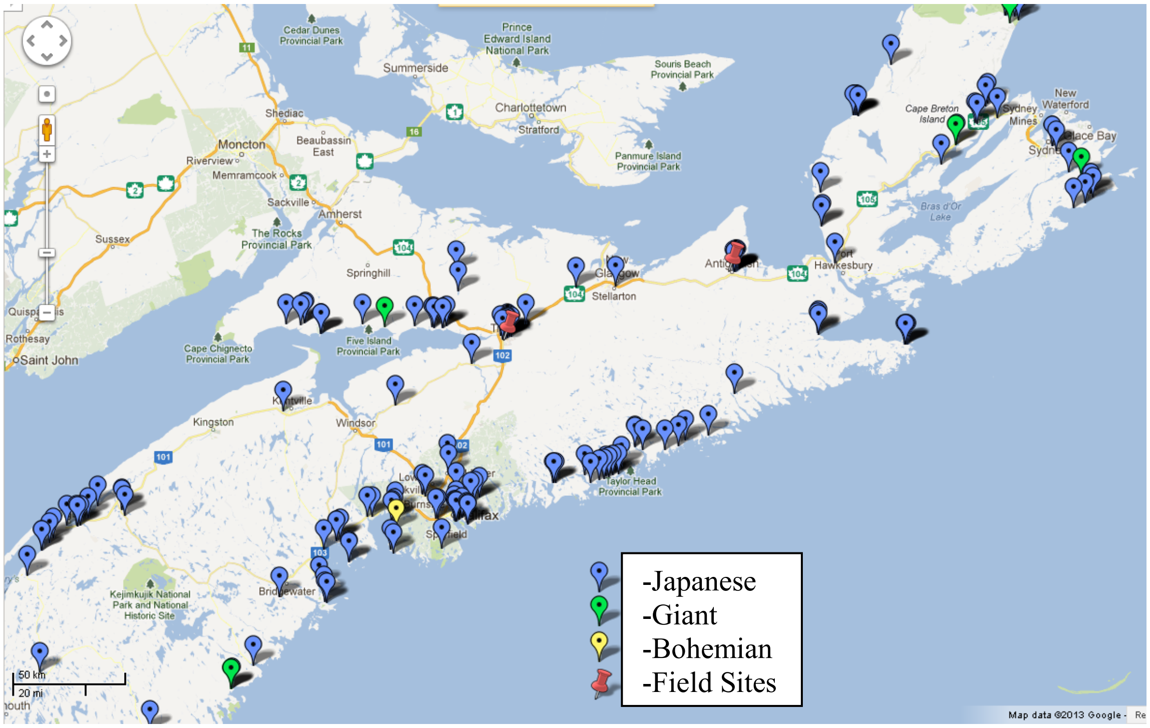

Figure 2.3 Knotweed locations in Nova Scotia: passive observations along motorways from fall 2010 to fall 2012. Tags indicate patches of knotweed species and red pins indicate field sites in Bible Hill and Antigonish.

I also searched for knotweed samples in four University Herbariums in the fall of 2010 including the former Nova Scotia Agricultural College (currently Dalhousie University, Agricultural Campus); St Francis Xavier University; and Acadia University (online catalogue includes Cape Breton University). A total of 48 Japanese knotweed, four giant knotweed, and one Crimson Beauty samples were found in the collections, with locations ranging across the province in many habitat types. The earliest sample was collected in Wolfville, Kings County by AE Roland in 1936.

Apparently, knotweed's range expansion has not dramatically increased in Canada since the 1970's with presence in 93 out of 288 municipalities (Barney 2006). Conversely, Bourchier and Van Hezewijk (2010) suggested that climatic conditions were ideal for knotweed range expansion by at least 100% in BC and Ontario. The combination of both studies suggests that knotweed is currently limited to the broad regions of eastern Canada and BC, but there is a great potential for the plant to dominate on the local scale where growing conditions permit. Bourchier and Van Hezewijk (2010) analyzed the present distribution of knotweed in British Columbia and Ontario to determine optimum growing conditions for potential invasion. They then modeled annual provincial climate data with suitable growing thresholds of minimum temperature (-30.2 C), growing degree days (2505), and precipitation (735 mm) to hypothesize the extent of range expansion. They determined that knotweed is currently only occupying half of the sites that provide suitable growing conditions, and there is risk of further invasion. These current potential knotweed locations are further augmented by a warming climatic trend which could result in an increase of 53% of viable conditions in Ontario (Bourchier and Van Hezewijk 2010). Their findings suggest the use of climatic thresholds for distribution predictions to delineate an area of risk in which to concentrate invasion preventative and/or control measures (Bourchier and Van Hezewijk 2010). By analyzing climate data of other regions it may be possible to map potential knotweed distribution, as well as predict further range expansion due to climate change.

The combination of previous research papers, road sightings, and herbarium records imply that Japanese knotweed is widespread across the province of Nova Scotia, and has been documented for approximately one hundred years (Barney 2006; personal observations).

2.7 Habitat

2.7.1 Climatic Requirements Nova Scotia is essentially a peninsula that juts into the Atlantic Ocean, encircles the Bay of Fundy, and lies below the Northumberland Strait. Although it is considered a modified continental climate, much of its weather is affected by the ocean. Average annual precipitation is between 900 mm and 1500 mm, and average annual temperature is around 6°C. The provincial growing season (average temperature above 5 °C) is between 170 and 210 days long from April to November (NS Museum of Natural History). Most of the province experiences an average of 1400 to 1800 growing degree-days above 5 °C (Gordon and Bootsma 1993). These climatic averages are within the thresholds for suitable knotweed conditions (Bourchier and Van Hezewijk 2010), which suggests that Nova Scotia provides adequate growing requirements for knotweed.

Knotweed will begin to send up shoots around mid-April, but is very frost-susceptible and any freezing periods in the spring will cause the shoots to die, which are rapidly replaced by new growth. In the fall, the first major frost will generally cause the plant to lose its leaves and go into dormancy, with a large percentage of photoassimilates mobilized to the rhizome for storage (personal observations; Price et al. 2002). Agriculture and Agri-Food Canada (1997) reports the 30 year average from 1961-1990 of the growing season when the mean daily air temperature equals or exceeds 5 °C. Seven communities in distinct ecodistricts of Nova Scotia were chosen for variation in growing season length (Nova Scotia Department of Natural Resources 2007) (Table 2.1). Knotweed is reported in all selected ecodistricts, and its growing season can be expected to start and end within the dates shown. With the exception of the harsh climate of the Cape Breton Highlands, knotweed may begin growing in late April and experience a killing frost in mid October.

Table 2.1 Growing season length (1961-1990 average) in various locations across Nova Scotia, from Agriculture and Agri-Food Canada 1997.

		Growing Season		
Community	**Ecodistrict**	**Start**	**End**	**Length (days)**
Antigonish	St Georges Bay	Apr 25	Nov 6	196
Bible Hill	Minas Lowlands	Apr 24	Oct 10	170
Halifax	Eastern Interior	Apr 30	Oct 16	170
Kentville	Annapolis Valley	Apr 22	Oct 15	177
Meat Cove	Cape Breton Highlands	May 27	Sep 14	111
Sydney	Bars d'Or Lowlands	Apr 21	Nov 9	203
Yarmouth	Tusket Islands	Apr 25	Oct 18	177

2.7.2 Substratum Japanese knotweed is distributed across the province and occurs in a large variety of soil types that are mainly characterized as being low in organic matter, fertility, and pH levels (Sangster et al. 2010). Soil was tested in Antigonish and Bible Hill, within three habitats at each site: grassland, knotweed, and shrub. Four samples were taken in July 2012 at each habitat using a 10 cm deep soil core, and then were combined to create a homogenous sample for analysis at the Nova Scotia Department of Agriculture soil testing lab (Harlow Institute, Truro, NS). Various soil characteristics were analyzed, including pH, organic matter, plant available nutrients, cation exchange capacity (CEC), and base saturation (Table 2.2). In general, all samples showed low pH levels (<7), good organic matter levels (>3.5 %), low in S (<40 kg/ha), high in Fe (>100 ppm), high in Mg (>60 ppm), high in Cu (>3.0 ppm), and high CEC (>11 cmol[+] kg^{-1} soil). These values are all within recommended levels. When comparing soil within knotweed patches versus soil in grass and shrub habitats, there are a few obvious trends. Values are higher in knotweed soil at both sites for organic matter, Na, Fe, Zn, and base saturation of Na. Values are lower only with base saturation of K. The biggest disparity between values is that the amount of Cu in Bible Hill knotweed soil is over five times higher than the two other habitats.

Table 2.2 Soil test report from NS Department of Agriculture for three riparian habitats at two locations in Nova Scotia in July 2012.

		Antigonish			Bible Hill		
Soil Characteristic		Grass	Knotweed	Shrub	Grass	Knotweed	Shrub
pH		5.6	5.8	5.7	6.2	5.7	5.5
Organic Matter (%)		4.8	5.3	4.5	4.0	4.5	4.1
P_2O_5 (kg/ha)		90	168	106	374	202	316
K_2O (kg/ha)		224	232	273	228	131	288
Ca (kg/ha)		2668	3187	2998	4162	3219	3199
Mg (kg/ha)		258	308	263	282	249	237
Na (kg/ha)		100	123	97	69	77	58
S (kg/ha)		28	26	27	28	21	28
Al (ppm)		1074.25	963.53	1061.44	788.46	1016.69	973.50
Fe (ppm)		280	289	265	289	312	294
Mn (ppm)		133	152	119	237	160	177
Cu (ppm)		3.82	3.56	3.55	4.55	20.14	4.59
Zn (ppm)		4.6	5.2	4.8	64.7	71.1	63.8
B (ppm)		≤ 0.50	≤ 0.50	≤ 0.50	0.69	≤ 0.50	0.54
CEC (cmol[+] kg^{-1} soil)		11.9	13.7	12.8	14.6	13.0	13.3
Base sat.	K (%)	2.0	1.8	2.3	1.7	1.1	2.3
	Ca (%)	56.1	58.2	58.7	71.2	61.9	60.0
	Mg (%)	9.0	9.4	8.6	8.0	8.0	7.4
	Na (%)	1.8	2.0	1.7	1.0	1.3	0.9
	H (%)	31.0	28.6	28.8	18.1	27.7	29.4

A soil analysis of particle size distribution was conducted in May 2011, using methods as described by Sheldrick and Wang (1993) and Brewster (2001). Soil samples were taken with a 10 cm deep soil core from three locations within a large knotweed stand at two sites: Antigonish and Bible Hill. The analysis revealed a composition of 62% sand, 13% clay, and 25% silt in Antigonish and a composition of 70% sand, 15% clay, and 15% silt in Bible Hill. The high sand content can be attributed to the site locations which experience periodic flooding from the adjacent rivers.

2.7.3 Communities in Which the Species Occurs As suggested in the provincial distribution of knotweed (Figure 2.3), the plant is commonly found in close proximity to human-disturbed sites such as roads, dump sites, vacant lots, abandoned homesteads, and yard waste piles. This is possibly due to the improper or careless transportation and/or disposal of unwanted plant matter. In more natural settings, knotweed is found in areas associated with water such as ditches, ravines, and riverbanks (Barney 2006). Although knotweed is quite tolerant of dry soil conditions, it is often found in wet habitats due to the transport of plant fragments to downstream sites during flooding events (Beerling et al. 1994). When floodwaters recede, knotweed stems are often deposited in back-eddies or along the riverbank where a new stand can become established. At two knotweed invasion sites in Antigonish and Bible Hill, there were several other plant species within the riparian ecosystem including reed canary grass*[*] (*Phalaris arundinacea*), foxtail, goldenrod (*Solidago* spp.), jewelweed (*Impatiens capensis*), dame's rocket* (*Hesperis matronalis*), Jerusalem artichoke (*Helianthus tuberosus*), cow parsnip (*Heracleum maximum*), wild parsnip* (*Pastinaca sativa*), blackberry (*Rubus* spp.), dogwood (*Cornus alternifolia*), alder (*Alnus* spp.), cherry (*Prunus* spp.), hawthorn** (*Craetagus* spp.), along with several other species, as documented in Chapter 3. Japanese knotweed has the potential to drastically disrupt the plant diversity by dominating the ecosystem (Figure 2.4).

Figure 2.4 Japanese knotweed invasion along the Rights River, Antigonish, NS 2011.

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[*] = not native to NA; ** = *possibly* not native to NA

2.7.4 Mapping of Japanese Knotweed Stands at Two Sites in Nova Scotia Knotweed stands were mapped at Antigonish and Bible Hill, NS because they both had an extensive knotweed infestation, and had similar site characteristics in terms of the riparian ecosystem, hydrology, and proximity to urban areas. Antigonish is approximately 100 km east of Bible Hill. At each site, knotweed patches as well as shrub patches were located and mapped using a Trimble® GeoXT™ handheld GPS unit. This was done in the fall of 2011 and 2012 at both sites, as well as in the fall of 2010 in Antigonish, to evaluate any changes in population area. GPS data were later uploaded to Terrasync™ Pathfinder software and processed in ArcMap 10.1 (Esri, Redlands, CA, USA), overlaid on ortho-rectified aerial photographs of the regions (Figure 2.5).

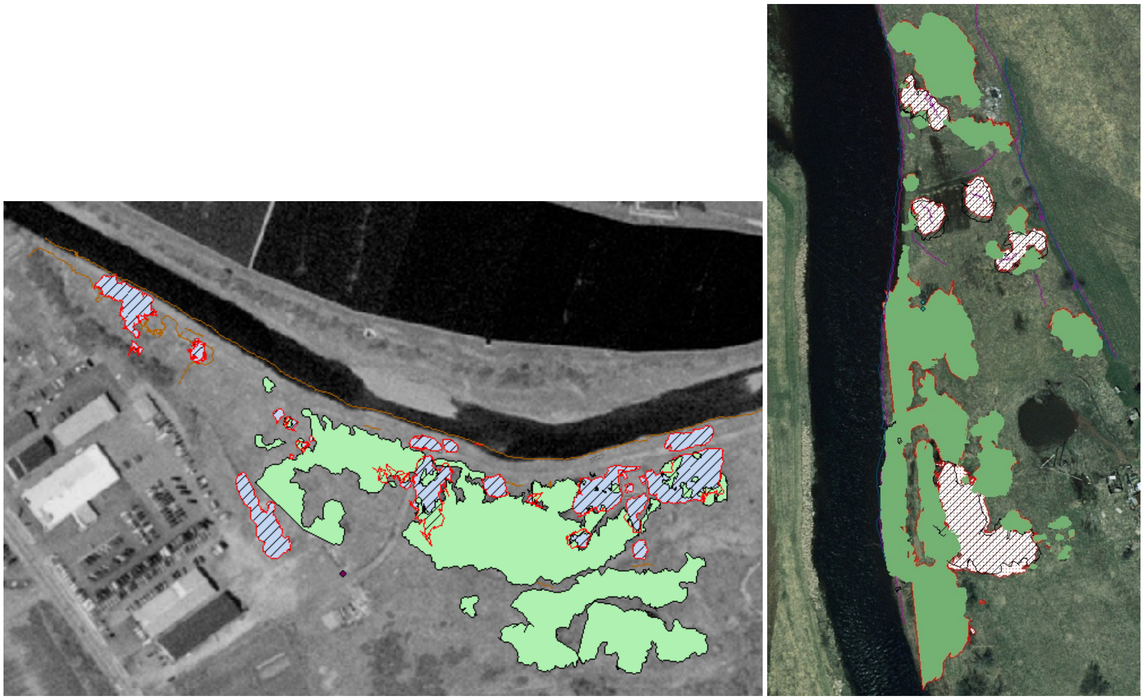

Figure 2.5 Distribution of Japanese knotweed (red hatch marks) along the Rights River in Antigonish (left) and the Salmon River in Bible Hill, NS (right). Green polygons indicate shrub patches in a grass-dominated floodplain.

2.8 Growth and Development

2.8.1 Plant Growth The rapid growth of Japanese knotweed is legendary, yet there is a paucity of data in regards to individual stem growth patterns, as well as the rate of patch spread. In order to quantify some aspects of knotweed growth, I collected numerous height measurements of knotweed plants throughout the 2011 growing season at two sites: Antigonish and Bible Hill. Height was measured using a tape measure from the ground to the highest point of the plant, but not including the terminal leaf. By late June, the knotweed ramets began to bend over due to their height and weight, so the height was measured to the highest point of the arch. Five ramets were systematically selected for measurement to get a good representation of plant height within each 2 x 2 m plot over time. One side of the plot was separated into five, 40 cm wide rows and the first ramet was picked from each row. This was repeated at two other plots for a total of 5 x 3 = 15 measurements per time period at each site. Data were recorded approximately every two weeks for five months from late May to late October (Figure 2.6).

As the data suggest, the time of maximum plant height were reached at different times, even though the sites were only 100 km distant from each other. In Antigonish this was late June (28th), while it was two weeks earlier in Bible Hill in early June (10th). This observation shows how large an influence that local climatic conditions played on plant growth. Unfortunately there were no data from May in Antigonish to illustrate the rate of growth in the spring. The Bible Hill site showed how the plant's average height increased from 37 cm on May 11th to 210 cm on June 10th. A mean growth rate of 173 cm in 30 days is quite remarkable. The maximum average height remained around 190 cm for the rest of the growing season. The largest individual stem measured was 164 cm tall.

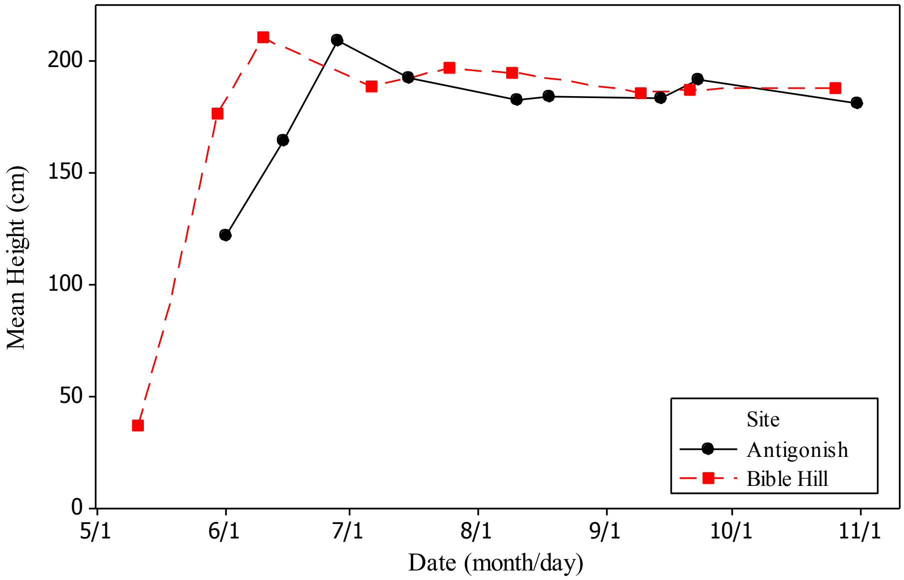

Figure 2.6 Average height of Japanese knotweed (*n* = 15) in 2011 at Antigonish and Bible Hill, NS.

Along with height, knotweed density was also determined by counting all stems over 10 cm tall within each 4 m^2 plot. This was done at three replicates on six occasions at both sites between May and October, 2011 to give an average density of knotweed stems per m^2. Although the density per plot did not change much over time, there was some degree of variance between replicates as well as sites. The Antigonish site had an average of 18 stems m^{-2} (std dev = 3), and Bible Hill had 15 stems m^{-2} (std dev = 8).

Finally, knotweed biomass was measured in late June 2012 by cutting down all knotweed ramets over 10 cm tall per plot and weighing them to determine fresh weight. We found 8.59 and 7.98 kg per m^2 of fresh biomass in Antigonish and Bible Hill, respectively. Knotweeds dominance over the ecosystem cannot be refuted when these figures are applied to a large knotweed infestation. In a 1 ha stand there could be 170 000 knotweed ramets, each around 2 m tall, and a total aboveground mass of over 80 000 kg!

2.8.2 Phenology The form of knotweed changes drastically over a season in an incredible display of rapid plant growth, as shown in Figure 2.7 and described below. Knotweed shoots emerge in the early spring (April to May) from perennating buds on the crown or from lateral belowground rhizome nodes. These initial shoots grow rapidly and are sensitive to any frosts, which often results in ramet death but is succeeded by new growth. The majority of the stand emerges simultaneously, with more stems arising during disturbances, to create a homogenous patch of straight, erect, reddish green ramets with fluorescent-green leaves. Within four to six weeks (mid to late June), the knotweed stems have reached their maximum height of approximately 210 cm, although the plant is significantly taller if measured along the arc from base to tip. The plants begin to bend over while they grow, causing their maximum height (from ground to highest point in the arc) to decrease to about 190 cm. By this stage, lower branches have grown out to fill any gaps in the canopy in order to maximize solar capture, and leaves are a lime green colour. Stems within a dense stand generally have less lateral branches than those on the edge. Flower clusters begin to emerge in late August as erect and/or drooping panicles at the base of most leaves to create a profusion of tiny, creamy-white flowers mixed with dark green leaves. Each flower may produce a small seed in September or October which falls to the ground in late autumn. The first killing frost in October generally causes the plant to begin senescence, and the leaves turn yellow before dropping off of the branches. The dead, reddish-brown knotweed stems stay standing throughout the winter months unless there is a major disturbance such as wind, water, or snow. Again, in the spring new shoots emerge and eventually displace the previous season's dead stalks.

Figure 2.7 Japanese knotweed growth habits in Nova Scotia, 2011-2012. Newly emerging buds from crown (top left), or shoots from rhizome nodes (top right) give rise to a consistent early growth habit (centre left). Flowers emerge in late autumn (centre right), and leaves drop after a frost (bottom left), leaving dead knotweed stems in winter (bottom right).

2.9 Reproduction

2.9.1 Floral Biology The floral biology of Japanese knotweed and its congeners has been under much discussion due to the degree of cross-hybridization, inaccurate species identification, and incomplete seed viability studies (Beerling et al. 1994; Bram and McNair 2004; Barney et al. 2006). However, it is generally agreed that the predominant species in the UK, *Fallopia japonica* var. *japonica*, is a male-sterile, female clone (Hollingsworth and Bailey 2000), therefore incapable of producing viable seeds (Beerling et al. 1994). Furthermore, it appears that Japanese knotweed can be pollinated by giant knotweed to create the hybrid Bohemian knotweed (Zika and Jacobson 2003).

Studies in the US on seed viability and germinability seem to contradict those from the UK, with positive germination rates. Bram and McNair (2004) collected Japanese knotweed seeds in Pennsylvania in late October, buried the seeds in mesh bags, and observed a mean germination rate of 82% the following March. They also estimated the number of seeds per stem to be over 127 000 (Bram and McNair 2004), which indicates that sexual reproduction in this knotweed population could add a new challenge when considering knotweed management plans. A similar germination study in Massachusetts by Forman and Kesseli (2003) confirmed that Japanese knotweed seeds had high germination rates (83%), and many of them survived to adulthood and produced flowers within the first growing season in a greenhouse. Mean days to germination for seeds from a variety of parents was 9 days, with a range from 7 to 36 days. Seedlings in the wild were more likely to survive when dispersed in an open area, away from the dense knotweed canopy (Forman and Kesseli 2003). The authors from both of these studies acknowledged the isolated locations of their specimens, as well as the difficulty in identifying pure

Japanese knotweed plants that have not been cross-bred with other congeners (Forman and Kesseli 2003; Bram and McNair 2004).

I also tested the seed viability of knotweed samples at two sites in Nova Scotia: Antigonish and Bible Hill. I collected seeds from approximately ten different Japanese knotweed plants in the fall of 2011 (September 21st in Bible Hill and September 23rd in Antigonish). These were left in brown paper bags for six months and removed on March 26th 2012. For each site, eight Petri dishes were prepared using two filter papers covered with 5 mL water and 20 knotweed seeds were placed in each dish, and then covered with Parafilm. Half of the samples were put in a cardboard box away from light and the rest were left on a laboratory bench at room temperature (20 °C). These were checked after seven days and every day afterwards for 30 days. Of the 320 seeds, not one germinated. This could be from unsuitable conditions or improper methodology, or simply that these knotweed samples do not have viable seeds. It remains plausible that Japanese knotweed stands which are located in close proximity of giant knotweed patches (as is the case in multiple NS locations) may be cross-pollinated to produce the hybrid Bohemian knotweed from viable seeds. In the late fall, knotweed patches are literally abuzz with pollinating insects such as bees which take advantage of the late-season nectar source (Figure 2.8).

Figure 2.8 Knotweed flower cluster in early September 2011 with bees.

2.9.2 Vegetative Reproduction The belowground portion of the plant is capable of storing a vast amount of energy for future use. The main crown of a plant can have dozens of quiescent buds that have the potential to send up a new shoot if necessary. As well, lateral rhizomes extend away from the crown and can produce vertical shoots from nodes along its length. An interesting function of the rhizome complex is the ability to produce new shoots from very small (> 0.7 g) disjunct plant fragments, as long as a node or bud is present (Brock and Wade 1992). This greatly aids in population dispersal as the fragments are transported by humans or water to new sites. There are several features of the rhizome complex that are not clearly understood, such as the amount of belowground biomass, rate and extent of rhizome spread, and interaction amongst individual roots, among others. Considering the huge role this structure plays in plant dispersal and function, it would be useful to have a better understanding of its biology.

2.10 Hybrids

The most recognized knotweed species in North America include Japanese knotweed (*P. cuspidatum*) and giant knotweed (*P. sachalinense*) (Figure 2.9) which have characteristics that make them easy to differentiate from each other such as size, leaf hairs, and flower arrangement (Child and Wade 2000; Wilson 2007). However, the hybrid Bohemian knotweed (*P.* X*bohemicum*) can possess intermediate characteristics between these two species (Zika and Jacobson 2003), and additional back-crosses of Bohemian with other congeners further confound identification (Bailey et al. 2009). In comparison with its parents, Bohemian knotweed has intermediate-sized and variable-shaped leaves, and its height also varies between that of the parents (Zika and Jacobson 2003; Wilson 2007). The most defining characteristic is the amount of pubescence on the underside veins of leaves; Bohemian has broad-based stout single-celled hairs (Zika and Jacobson 2003). Bohemian knotweed has been identified in North America in British Columbia (Wilson 2007), northeastern USA, and the western states, including Washington where it is considered to be more populous than the parent species (Zika and Jacobson 2003). Aside from BC, VASCAN (2012) also lists Bohemian knotweed in all of the eastern provinces except for Nova Scotia (ON, QC, NB, PE, and NL). A potential Bohemian knotweed stand was located in Glen Margaret, NS along the roadside, adjacent to Woodens River, although it has not been genetically identified.

Figure 2.9 Giant knotweed near Economy, NS in 2011.

Other knotweed species in North America include Himalayan knotweed (*Polygonum polystachum*) and the horticultural variety 'Crimson Beauty' (*Polygonum cuspidatum* var. *compactum*). Although they are not known to hybridize with other knotweed species in North America (Barney et al. 2006), their invasiveness is worth noting especially in BC (Wilson 2007).

2.11 Population Dynamics

The rate of patch expansion or spread is not currently well understood, although it would be very useful for determining how quickly an area may become entirely infested with knotweed. Many established stands in Nova Scotia appear to have been independently colonized by floating fragments along rivers or via human transportation along roadways and at dump sites. Over time these patches expand in area and eventually coalesce with adjacent patches to form larger uninterrupted knotweed stands. A local ecosystem may become dominated by knotweed if there are suitable growing conditions and protection from major disturbances.

Another fascinating attribute of knotweed is its allelopathic properties, which is a natural defense system produced within the plant and released into the surrounding environment to inhibit growth and germination of other plants (Inoue et al. 1992). A study by Inoue et al. (1992) used giant knotweed extracts from rhizome, stem, and fallen leaves to evaluate and identify the allelochemicals utilized by the plant. They found that anthraquinone compounds are produced in the form of emodin and physcion which effectively inhibited hypocotyl and root length in lettuce, amaranth, and timothy. Interestingly, the highest levels of these compounds were detected in leaves that had fallen four months previously, followed by fresh rhizome and stem fragments, as well as some levels in the soil (213, 158, 72, and 55 mg of emodin per kg of sample, respectively) (Inoue et al. 1992). Vrchotova and Sera (2008) also found that rhizome extract did not significantly decrease germination of white mustard seeds, but did decrease radical and hypocotyl length, as well as root/shoot ratio. Furthermore, they found no difference in allelopathic effects from rhizome extracts between Japanese, giant, and Bohemian knotweed species (Vrchotova and Sera 2008). The results from these studies indicate that knotweed species utilize a powerful weapon in order to inhibit the growth of neighbouring plants, and that fallen dead material may be more important than root exudates.

2.12 Responses to Human Manipulations

There are several alternatives to herbicide use for management of knotweed stands, such as cutting, pulling, burning, and covering with fabric or wire grid. Cutting the plant down over multiple periods of the growing season often results in shorter but more numerous ramets. Seiger and Merchant (1997) suggested that knotweed is unlikely to be eradicated by cutting alone, even with a decrease of 87% in rhizome biomass after three cuttings in a season. Pulling up the plant is not very effective since it is improbable that no plant fragments will remain at the site and subsequently grow. Additionally, these methods of mechanical control may further spread the knotweed infestation due to the movement of contaminated soil, plant fragmentation, and an increase in disturbed ground for further invasion. Due to the high water content of knotweed, it would be difficult to burn the fresh plant matter, but the Environment Agency (2012) in the UK suggests burning to dispose of dried cut material. Another occasionally recommended control method involves cutting down knotweed stems and covering the area with a large tarp, carpet, or geotextile fabric and weighing it all down with rocks. Not only is this method unattractive, it also has limitations in terms of the size of infestation, presence of desirable vegetation, evenness of ground, gaps between materials through which knotweed will grow, and the need to continuously maintain. The cover may be subjected to wind, flooding, and vandalism, and must be left down for a long period of time, after which the barren ground is ripe for weed colonization. This method was employed in Point Pleasant Park, a large urban park in Halifax NS. The Supervisor stated that the fabric was down for three years, required regular maintenance, and did not show promising results (Steve Rice, personal communication). Finally, a novel method is currently being tested by Dr. Nathan Boyd utilizing a wire mesh. It also involves cutting down knotweed stems, and a wire grid with 2 cm spacing is placed over the patch (Figure 2.10). As the new shoots grow through the mesh they increase in height and diameter until they are girdled and die. Other forbs and grasses can grow through the mesh which anchors it to the site to prevent movement. A disadvantage of this strategy is the difficulty in removing the mesh when desired. When choosing a control method, it is important to properly assess the problem and evaluate any possible solutions, and often it is necessary to combine several control strategies into an integrated management plan.

Figure 2.10 Knotweed girdled by wire mesh in Antigonish, NS, 2012.

2.13 Response to Herbivory, Disease and Higher Plant Parasites

2.13.1 Herbivory Knotweed was originally introduced as a forage crop for livestock. Some goat shepherds will rent out their herd for knotweed control within a fenced area –although they will eat all other plants as well. There are no reports of birds eating knotweed seeds or plant material. Several species were observed nesting within knotweed stands, including Dark-eyed Juncos and Cedar Waxwings in Bible Hill and Antigonish, respectively.

There are many invertebrates associated with knotweed stands in NS, but there were few incidents of negative impacts from herbivory. At two locations, some newly emerged caterpillars were observed on leaves in August, but they did not have long-lasting effects on the plant (Figure 2.11). A sap-sucking psyllid (*Aphalara itadori*) has been rigorously evaluated for use as a biocontrol agent in the UK (Shaw et al. 2009). This arthropod was chosen amongst over 180 natural insect enemies to knotweed (Shaw et al. 2009), and in 2010 became the first released biocontrol agent against a weed in the European Union (CABI 2011). This insect is also currently being evaluated as a potential biocontrol agent for release in Canada, at an Agriculture and Agri-Food Canada testing facility in Lethbridge, AB. While biocontrol options do not eliminate invasive species, they do provide an additional tool for management plans.

Figure 2.11 Lepidoptera species on knotweed leaves in August 2011 in Antigonish (left) and Bible Hill (right).

2.13.2 Diseases There are no apparent instances of bacterial or viral diseases that afflict knotweed plants. Research conducted by Kurose et al. (2009) identified *Mycosphaerella polygoni-cuspidati*, a fungus which causes a leaf-spot disease in knotweed's native range. As further research develops, teams may also consider this fungus as a biocontrol agent.

2.14 Conclusion

It is apparent that Japanese knotweed has been well studied, yet many characteristics remain enigmatic. Foremost, an accurate genetic identification of the plant is necessary in order to differentiate between species and hybrids. A large population in Antigonish is known to be male-sterile *Polygonum cuspidatum*, and morphological similarities suggest that many other stands across the province are also Japanese knotweed. A distribution map across Nova Scotia indicates Japanese knotweed locations, as well as several giant knotweed locations, and a possible Bohemian knotweed specimen, which could potentially influence knotweed hybridization. The site-specific benefits and detriments of knotweed’s presence need to be evaluated in detail to determine what type of management plan (if any) must be implemented. Local growing conditions indicate which climes are optimal for knotweed growth in Nova Scotia. Also, current GPS mapping of several knotweed stands provide an excellent baseline with which to compare future range expansion. Plant growth and development data illustrate potentially ideal timeframes for herbicide treatments, such as time of maximum growth in mid-June, flower emergence in late August, and senescence in mid-October. Other characteristics such as maximum canopy height (190 cm), density (17 stems per m^2) and biomass (8 kg per m^2) also allow for a better understanding of Japanese knotweed life history. Perhaps just as important is what questions remain to be answered so that future research can positively contribute to the growing knowledge of this remarkable species.

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Chapter 3: An Ecological Impact Assessment of a Japanese Knotweed (*Polygonum cuspidatum* syn. *Fallopia japonica*) Invasion in Riparian Environments

3.1 Abstract

Japanese knotweed is a fast-growing invasive weed which outcompetes other plants and has the potential to dominate an area with far-reaching ecological impacts. This study compared the characteristics of knotweed, shrub, and grass habitats at two sites in Nova Scotia in 2011 and 2012. Plant, invertebrate and small mammal surveys were conducted in each habitat to estimate the impact of knotweed stands on these communities. Knotweed stands had significantly less plant diversity than shrub and grass habitats but provide more ground cover in terms of leaf area index. There was no statistical difference between habitats in invertebrate diversity or abundance. Small mammal tracks were more abundant in grass than shrub habitats, and knotweed patches were intermediary with no statistical difference between knotweed and the two other habitats. *Sorex* species were present more than twice as often in knotweed versus shrub habitats possibly due to the increased cover or prey abundance. Overall, small, discontinuous knotweed patches may not have a large impact on a riparian ecosystem, but if significant range expansion occurs over time knotweed is likely to become the dominant plant species and greatly reduce diversity.

3.2 Introduction

Ecosystems are constantly changing with a wide assortment of mobile and sessile organisms characterizing specific assemblages in a dynamic rhythm that is ever evolving. Populations of various plant species co-exist with each other to form niches and habitats that support food and shelter for a gamut of animal species such as invertebrates, fish, and mammals. This diversity of life is magnificent in its complexity but also susceptible to disturbances which can significantly alter an entire ecosystem.

Invasive species are thought to have a crucial impact on biodiversity loss in the world, second only to habitat loss (Pimentel et al. 2005, United Nations Environment Programme 2012). All organisms have a natural distribution that is primarily altered by anthropogenic processes which results in the introduction of species to areas beyond their natural range. Invasive species belong to many different taxons including fungi, bacteria, invertebrates, and mammals; however this paper will focus solely on an invasive plant: Japanese knotweed.

Introduced species are considered 'invasive' if they have negative impacts on the environment and economy and have the potential to spread over a large area (Pysek et al. 2004). The Canadian Food Inspection Agency (2008) reports that Canada hosts approximately 3858 native vascular plant species and an additional 1229 non-native species which is over 30% of the described vascular flora. An estimated 40% or 486 of these alien species are considered invasive across the country. A review of invasive species in the US by Pimentel et al. (2005) references many examples of environmental damage and high cost associated with weedy and invasive plants, with an estimated cost of $35 billion US dollars per year.

Japanese knotweed (*Polygonum cuspidatum* syn. *Fallopia japonica* s.l.) is an herbaceous perennial geophyte. Originally introduced from eastern Asia as an ornamental specimen, it has been present in the Maritimes since the early 1900's, and experienced a lag period of several decades before becoming an invasive pest (Barney 2006). Knotweed grows rapidly in the early spring reaching heights in excess of 2 m. This allows it to successfully compete with other plants for space, light, and nutrients (Siemens and Blossey 2007; Aguilera et al. 2010). Over time, knotweed patches can extend their size primarily through vegetative reproduction to become the dominant plant in an area. While mostly associated with ruderal sites due to human development such as roadsides, ditches, and vacant lots, knotweed fragments can travel along watercourses and establish new stands along riverbanks in less disturbed or natural areas.

Various studies associate the presence of knotweed patches with negative environmental impacts such as decreased plant diversity (Maerz et al. 2005; Siemens and Blossey 2007; Aguilera et al. 2010), altered stream ecosystem function due to leaf litter (Lecerf et al. 2007), and decreased invertebrate diversity (Gerber et al. 2008). Few articles report research involving knotweed infestations and how the entire ecosystem is affected. A majority of knotweed research is conducted in the UK and northern Europe where it has been designated a 'hazardous waste' that must be properly disposed of (Child and Wade 2000; Cornwall Council 2012). In North America, Japanese knotweed is found almost everywhere, and most research is conducted in the western and northeastern US, with few projects based in Canada.

Several management options are recommended for knotweed control including cutting, pulling, and covering with fabric. These mechanical options are labour- and time-intensive and do not yield great results (Child and Wade 2000). Herbicides are effective at long-term control but difficult to apply in large patches or may not be desirable in natural or urban environments. An insect was recently released to be used as a biological control in the UK (Shaw et al. 2009) and is also being considered in Canada. A long-term, integrated management plan is often recommended to control knotweed.

Gerber et al. (2008) found that the replacement of native plant species by exotic knotweed stands resulted in a reduction of insect abundance and species richness. Maerz et al. (2005) took this a step further by retaining green frogs in cages along a transect line from a native plant community into a knotweed stand. Besides the obvious differences in plant compositions, they found that the frogs tended to have a decreased mass after 38 hours in the knotweed stand as opposed to an increase in the more diverse habitat. The authors attribute this to reduced foraging success due to a reduction of invertebrates in the knotweed habitat (Maerz et al. (2005).

Several species of small mammals (< 1 kg in weight) are distributed across Nova Scotia, including shrews (six *Sorex* species and *Blarina brevicauda*), a mole (*Condylora cristata*), squirrels (*Tamias striatus*, *Sciurus carolensis*, *Tamiasciurus hudsonicus*, and *Glaucomys sabrinus*), mice (*Peromyscus maniculatus*, *P. leucopus, Zapus hudsonicus*, and *Napaeozapus insignis*), and three species of voles (*Microtus pennsylvanicus*, *Synaptomys cooperi*, and *Clethrionomys gapperi*) (Banfield 1974). These species occupy various habitats and eat different diets. Shrews are insectivores that eat a variety of invertebrates including coleopterans, dipterans, lepidopterans, earthworms, gastropods, arachnids, and centipedes (McCay and Storm 1997). McCay and Storm (1997) found that moist environments had higher numbers of invertebrates and correlated this with higher shrew abundances. *Peromyscus* species are generalists which are found in a variety of habitats and one study suggested their diet is comprised of about 50% plant material and 50% invertebrates such as coleopterans, lepidopterans, orthopterans, and arachnids (ONeill and Robel 1985). Small mammals are good measures of biodiversity and ecosystem health due to their relative specificity to certain prey and habitats as well as their abundance and ability to be tracked (Delany 1974).

The objective of this study was to estimate the impact of Japanese knotweed on riparian ecosystems. The plant diversity, species richness, and leaf cover within knotweed plots were compared with that in shrub and grass plots. Small mammal tracks in each habitat type were recorded to assess any differences of mammal abundance or the presence of particular species between habitats. Invertebrate catches from pitfall traps were also analyzed for differences of species richness and overall abundance between habitats as well. It was hypothesized that knotweed stands had very low plant diversity which could be related to reduced abundances of invertebrates and small mammals when compared with adjacent riparian habitats.

3.3 Materials and Methods

3.3.1 Site Description Biodiversity assessments were conducted at two sites in Nova Scotia, Canada: Antigonish and Bible Hill. Habitat boundaries and transects were mapped using a Trimble® GeoXT™ handheld GPS unit in the fall of 2011, uploaded to Terrasync™ Pathfinder software, processed in ArcMap 10.1 (Esri, Redlands, CA, USA), and overlaid on ortho-rectified aerial photographs of the regions (Figure 3.1).

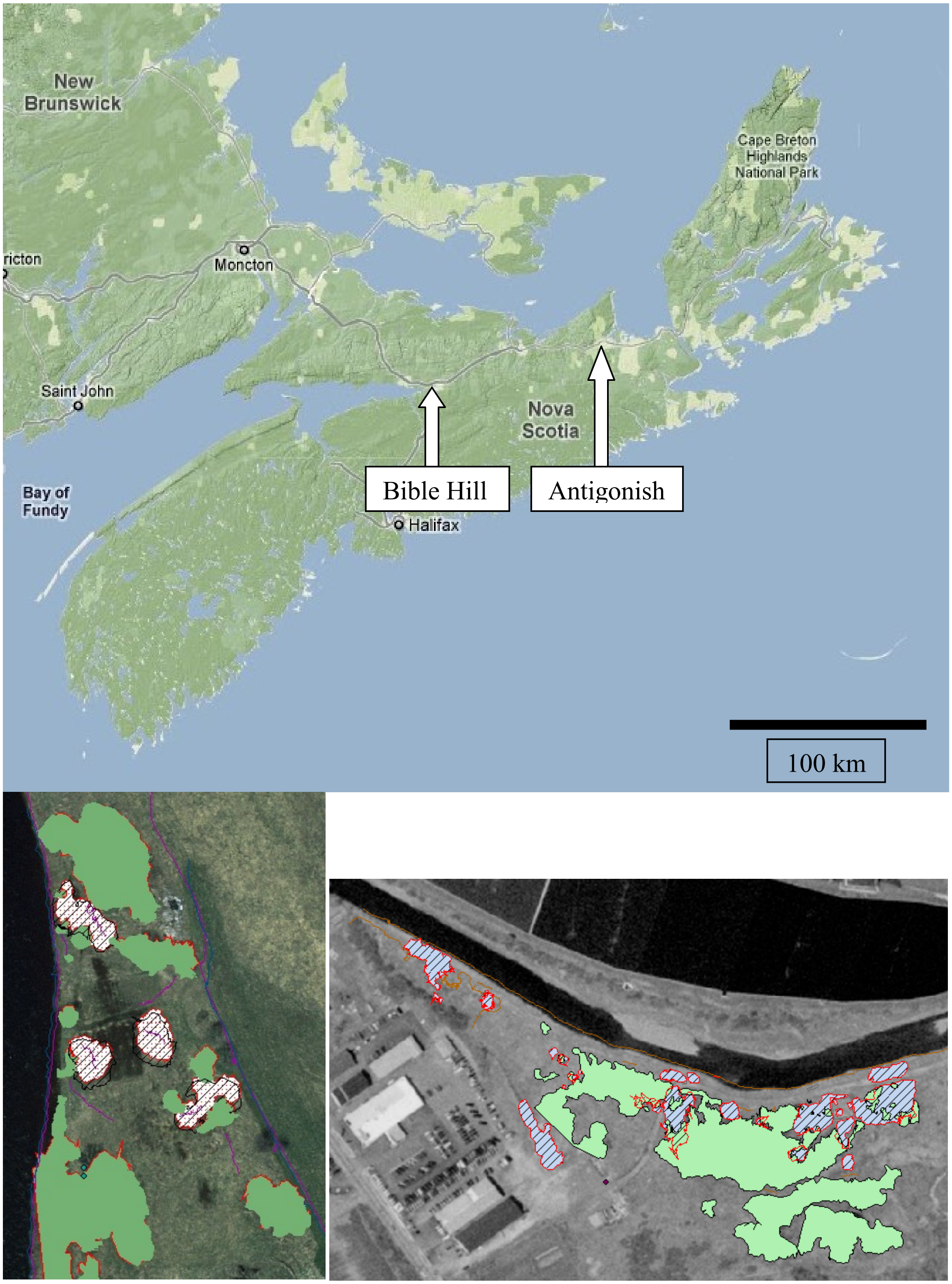

Figure 3.1 Map of sites in Nova Scotia, Canada where Japanese knotweed stands were evaluated (top). GIS map of Bible Hill (left) and Antigonish (right) study sites with shrub patches (green), knotweed stands (hatch) and surrounding grasslands.

The Antigonish site was located along the south side of the Rights River (45.622°N, 61.972°W), which flows through the town of Antigonish and empties into Antigonish Harbour. In the spring and fall the area is commonly subjected to flooding and as a result, parts of the river have been channelized and armoured. Historically, the floodplain supported freshwater wetlands but was subsequently cleared and drained for agriculture. Japanese knotweed is found in recurring, persistent patches along both sides of the river for about 2 km. It likely originated from the dumping of yard waste and the successive movement of knotweed fragments by ice and water. Although it is difficult to estimate patch age, the oldest knotweed specimen from the site preserved in the herbarium of the local St Francis Xavier University was collected in 1986. A soil analysis of particle size distribution was conducted in May 2011, using methods as described by Sheldrick and Wang (1993) and Brewster (2001). A 2.5 cm diameter soil core was taken 10 cm deep from three knotweed stands and revealed a composition of 62% sand, 13% clay, and 25% silt.

The Bible Hill site was located along the Salmon River which separates the towns of Bible Hill and Truro as it flows into the Bay of Fundy (45.371°N, 63.277°W). The ecological assessment was on the east side of the river and also experiences periodic flooding. Part of the site had historically been used for pastureland and crops but has been left fallow for several years. The age of the well-established knotweed patches are unknown but they were likely introduced to the site from flooding events. A soil analysis of particle size distribution in May 2011 revealed a composition of 70% sand, 15% clay, and 15% silt.

The dominant plant communities at both sites were characterized by herbaceous (non-woody) graminoids and forbs such as grasses, goldenrod (*Solidago* spp.), dame's rocket (*Hesperis matronalis*), and cow parsnip (*Heracleum maximum*) among others. This ‘floodplain grassland’ was punctuated by patches or stands of woody shrubs and trees, which consisted of a canopy of alder (*Alnus* spp.), cherry (*Prunus* spp.), and/or hawthorn (*Craetagus* spp.), with an understory comprised mostly of jewelweed (*Impatiens capensis*) and dame’s rocket (*Hesperis matronalis*). Japanese knotweed was also prevalent at both sites in patches of varying sizes with no other plant species typically occurring within the stands.

3.3.2 Experimental Design A biodiversity assessment was conducted to compare the differences in three riparian habitats: (i) knotweed patches, (ii) shrub-dominated stands, and (iii) grassland/non-woody forbs, similar to the work by Gerber et al. (2008). These three habitat types were each at least 100 m^2 in size and arranged into groups (blocks) according to their proximity of one another. All habitats within one group were not spaced farther than 50 m apart from each other. At the Antigonish site there were five blocks consisting of each habitat and four blocks at the Bible Hill site.

Within each habitat type a 12.5 m^2 circular plot was created by placing a stake in the centre of the habitat with a rope to form a 2 m radius. Also in each habitat a transect line was delineated with five flags approximately 3 m from each other with the middle flag beside the central stake. No flags were within 2 m from the edge of a habitat.

3.3.3 Habitat Characterization The objective was to determine the differences between the three habitats in terms of species richness, diversity, and canopy leaf area cover at different heights. Within each plot all plant species were identified using various identification guide books (Peterson and McKenny 1996; Zinc 1998). A 1 m^2 quadrat was placed at the centre of each plot and the numbers of each species were counted. The leaf area index (LAI) was measured using the optical instrument LAI-2000 (LI-COR® Lincoln, NE) at two heights: shrub layer (1.5 m from the ground) and ground level (0.05 m). Plant diversity was calculated for each plot using the Shannon Index for species richness and evenness, and Simpson's Index for species dominance (Magurran 2004).

Shannon (H): $$H = -\sum_{i=1}^{S} p_i \ln(p_i)$$

where p_i represents the proportion of individual species comprised of ith species.

Simpson (D): $$D = \sum_{i=1}^{S} \frac{n_i(n_i - 1)}{N(N - 1)}$$

where n_i represents the number of organisms of species i and N represents the total number of all organisms of all species. This index represents decreasing diversity from 0 to 1, and therefore will be reported as 1-D to simplify comparisons.

3.3.4 Additional Biodiversity Assessments Several assemblages of animals were surveyed within each habitat type: invertebrates, birds, and small mammals. Due to the nature of these respective creature's habits and movements, various tracking methods were employed.

3.3.4.1 Invertebrates Three pitfall traps were placed along the transect line in each habitat type. One was placed at the central stake, and the other two were at flagged locations approximately 3 m away in opposite directions. At each trap location a hole was excavated 12 cm deep by 10 cm wide using a soil corer and trowel, then two nested plastic cups were placed in the hole flush with the ground surface and partially filled (5 cm depth) with a mixture of water, soap, and salt. At the Antigonish site, 45 traps (3 traps x 3 habitats x 5 blocks) were set July 13th 2011 and retrieved July 17th for a total of 180 pitfall trap days. In Bible Hill, 36 traps (3 traps x 3 habitats x 4 blocks) were set on July 16th 2011 and retrieved July 19th for a total of 108 pitfall trap days. The contents of the traps were transferred to sealed containers with 70% ethanol and stored in a cold room at 4°C for later identification. Each organism was identified to morphospecies as surrogates for taxonomic species (Derraik et al. 2002; 2010). Only specimens from the Bible Hill site were identified and analyzed. Data were analyzed with all three sampling points pooled together to represent the habitat. For each habitat it was possible to calculate the number of species (S = species richness), the total number of organisms (N), and species diversity using the Shannon index (H) and the Simpson index (1-D). Rank/abundance plots were created using the relative proportion (p_i) of each species present per habitat in order of abundance to show patterns of species richness, as reviewed by Magurran (2004).

3.3.4.2 Birds Birds were observed in each habitat type at both locations during site visits throughout the 2011 and 2012 research seasons, as well as from monitoring platforms during the breeding season of June 2012. Species were identified by sight using reference books by Tufts (1986) and Sibley (2000). A bird observation platform was created in Antigonish at a height of 8.5 m in a willow tree, and 6.5 m high in a Bible Hill elm tree. From these heights it was possible to oversee all three representative habitat types of approximately equal sizes within a 50 m distance and 120° arc from the platform. This area of just over 2600 m^2 could be continually scanned for bird movement, which were identified and recorded according to the type of associated habitat. Monitoring periods typically lasted one hour at various times and weather conditions on four occasions in Antigonish and six in Bible Hill during the peak breeding season in June 2012. These limited observational studies were not conducted for a long enough period to merit statistical analyses. They do however provide some interesting notes on bird presence in distinct habitats.

3.3.4.3 Small Mammals Preliminary tracking investigations as well as reference material (Banfield 1974; Burt and Grossenheider 1976) suggested that several species of small mammals inhabited the diverse micro-habitats within the riparian grassland/shrub ecosystem at both sites. To conduct a population survey of the resident small mammals, tracking tubes were employed to determine track presence and abundance of species in each habitat in 2011 and 2012. Tracking tubes were 35 cm long, 3.75 cm diameter PVC tubes with an inkpad on a strip of white paper in the center of the tube for recording the tracks (Nams and Gillis, 2003). Five tracking tubes were placed along a transect line in each habitat type approximately three meters apart. The tubes were in place for one week in mid- to late-summer during various lunar cycles and weather conditions (Table 3.1). In 2012 at Antigonish, tubes were also put out in early July, however upon collection the papers had been almost entirely consumed by slugs and were unreadable. Two weeks later more tracking strips were placed in each tube and a slug deterrent with 6% ferric sodium pellets (Safer's® Slug & Snail Killer by Woodstream Canada Corporation, Brampton ON) was sprinkled nearby to dissuade their encroachment. Tracks were counted and identified to species groups using a key by Nams and Gillis (2002) and a guide book (Burt and Grossenheider 1976).

Table 3.1 Timeline of tracking tube placement for small mammal surveys at two Nova Scotian locations in 2011 and 2012.

		No. of tubes	**Tubes out**	**Tubes collected**	**Lunar cycle**	**Tracking days**
2011	Antigonish	75	Aug 24	Aug 30	Crescent to new	450
	Bible Hill	60	Aug 23	Aug 30	Crescent to new	420
2012	Antigonish	75	Jul 27	Aug 3	Gibbous to full	525
	Bible Hill	60	Jul 9	Jul 16	Waning crescent	420
		270				**1815**

In order to cross-reference the tracks some live traps were also set in the research site in 2011 (Delany 1974). After evaluating the tracking strips to see which locations yielded high numbers of tracks, a wire-mesh cage trap (10 x 10 x 30 cm) was placed at one point in each transect with oats, an apple slice, and polyester stuffing for bedding. In Antigonish, 17 traps were set at noon on September 23rd 2011, checked at 1830 and 0700, then removed at noon on the 24th. In Bible Hill, 13 traps were set on September 27th at noon, checked at 1800 and 0645, then removed at noon on the 28th. Any captured animals were identified, photographed, and a track sample was taken prior to release. All animals were treated with care as outlined by the Canadian Council for Animal Care, and submitted to the NSAC Animal Care and Use Committee, file number 2011-014.

3.3.5 Data Analysis ANOVA of all data was completed using PROC MIXED in SAS ver. 9.3 (SAS Institute Inc., Cary, NC) to determine the effects of site and habitat. For this study, a two-stage nested design, with either four or five blocks, was used with habitat nested in site. The three habitat types (Knotweed, Shrub, and Grass) were the levels at two sites (Antigonish and Bible Hill). The two years of data collection (2011, 2012) were used as blocks in combination with the actual blocks in the field to create 10 blocks in Antigonish (2 x 5) and 8 blocks in Bible Hill (2 x 4).

The SAS model was: y = site habitat(site) block

Normality and constant variance assumptions were verified. In some cases, transformations were used to achieve normality and back-transformed means are presented. For significant effects, the least squares means were compared with Tukey's adjustment ($p \leq 0.05$). Then the subroutine pdmix800.sas (Saxton 1998) was utilized to provide letter groups.

Some of the plant and animal variables were compared with each other using scatterplots and separated by habitat group in Minitab® Statistical Software ver. 16 (State College, PA).

3.4 Results and Discussion

3.4.1 Habitat Characterization A total of 6338 plants from 52 species were identified and counted from both sites over two years (Appendix A). All variables for habitat characteristics were analyzed to determine the significance levels of site and habitat. Normality assumptions were met for all variables except for Simpson Index (1-D), but the data were normal when separated by site. For three of the variables (Simpson, Shannon, and $\mathrm{LAI}_{\mathrm{ground}}$), site was significantly different ($p \leq 0.05$). Site effects for Species richness and $\mathrm{LAI}_{\mathrm{shrub}}$ were not significantly different ($p \geq 0.05$). For all variables, the interaction effect of habitat nested in site was significantly different ($p \leq 0.05$), so sites were analyzed separately (Table 3.2).

Table 3.2 Significance levels (*p*-values) for the effects of site and habitat on plant communities in Antigonish and Bible Hill, NS in 2011 and 2012.

Effect [a]	Simpson Diversity (1-D)	Shannon Diversity (H)	Species Richness (S)	Leaf Area Index (ground)	Leaf Area Index (1.5 m)
site	0.0017	0.0023	0.4229	0.0314	0.2005
habitat(site)	0.0001	0.0001	0.0001	0.0001	0.0001
block	0.8445	0.1495	0.7273	0.5004	0.0026

[a] Effects were: site (Antigonish, Bible Hill); interaction with habitat (Knotweed, Shrub, Grass) nested in site; block (2011, 2012 + blocks in the field).

Diversity data were analyzed with a means comparison for each site (Table 3.3). Grass was the most plant-diverse habitat at both sites for each index. Knotweed was the least diverse, and shrub habitats were intermediate between the others. In general, Antigonish plots tended to have higher diversity values than at Bible Hill.

Table 3.3 Plant diversity indices in 1 m^2 quadrats from three habitats at two Nova Scotian sites in 2011 and 2012.

	Simpson Index (1-D) [a]		**Shannon Index (H)**	
Habitat	Antigonish	Bible Hill	Antigonish	Bible Hill
Knotweed	0.00 a	0.00 a	0.00 a	0.00 a
Shrub	0.58 b	0.29 b	1.10 b	0.54 b
Grass	0.72 c	0.64 c	1.54 c	1.41 c
Std Err [b]	0.02	0.07	0.06	0.12
***p*-value**	0.0001	0.0001	0.0001	0.0001

[a] Values for a given index with the same letter were not significantly different between habitats in each site using Tukey's test at $p \leq 0.05$.

[b] Standard Error used for means comparisons.

Species richness followed the same trend as diversity with grass habitats consisting of the greatest number of species at each site, followed by shrub and knotweed (Table 3.4). These results are similar to those found in a similar study by Gerber et al. (2008), who found approximately 13, 9, and 6 plant species in 2 x 2 m plots of grassland, bush, and knotweed, respectively, at several locations in Europe over two years. The species richness table presents an erroneous idea that these habitats were limited to such low numbers of species over the entire site. Due to the relatively small sample plots for species richness (12.5 m^2) many other species were present at the site, but were not measured in the sample plots. Appendix A lists all plant species located in sample plots at both sites over two years.

Leaf Area Index (LAI) data at both heights (ground and shrub layer) also met normality assumptions (Table 3.4). The knotweed habitat had significantly higher LAI values at the ground and shrub levels than the other habitats, indicating a greater amount of leaf area. There was not a significant difference at the ground level in Bible Hill between knotweed and shrub habitats. Although it is understood that this method underestimates actual leaf area due to overlap of leaves (Smith et al. 1993; Chen et al. 1997), it is a good indicator of the amount of light penetration through the canopy. It is possible that the high LAI values within knotweed stands prevents many plants from receiving enough sunlight to become established and survive. At the shrub layer within shrub habitats, the LAI is low enough to allow for the growth of understory plants within this habitat, but this value is approximately 65% higher within knotweed stands and may be a reason for reduced species richness.

Table 3.4 Species richness (S) and Leaf Area Index (LAI) at ground and shrub layer levels in 12.5 m^2 plots from three habitats at Antigonish and Bible Hill sites in 2011 and 2012.

	Species Richness [a]		**$\text{LAI}_{\text{ground}}$**		**$\text{LAI}_{\text{shrub}}$**	
Habitat	Antigonish	Bible Hill	Antigonish	Bible Hill	Antigonish	Bible Hill
Knotweed	1.80 a	1.50 a	4.58 a	4.93 a	4.19 a	4.62 a
Shrub	7.50 b	4.88 b	3.35 b	4.15 ab	2.52 b	2.83 b
Grass	11.00 c	12.75 c	3.53 b	3.73 b	0.22 c	0.40 c
Std Err [b]	0.74	0.63	0.13	0.31	0.22	0.24
***p*-value**	0.0001	0.0001	0.0001		0.0001	0.0001

[a] Values for a given measurement with the same letter were not significantly different between habitats in each site using Tukey’s test at $p \leq 0.05$.

[b] Standard Error used for means comparisons.

While these measurements of habitat characteristics do not illustrate the full complexity of vegetation structure, intra-specific relationships, phenologies, and other plant community traits, they do provide a means to simply compare the diversity, abundance, and density between habitats. It is obvious that knotweed plots are dense, mono-specific stands that are quite different from the more complex shrub patches and contiguous grasslands of riparian environments. If a knotweed population were to extend its range over a large area, the resulting decline in plant diversity would have far-reaching impacts on other organisms and the entire ecosystem.

3.4.2 Invertebrate Survey Pitfall traps do not capture all individuals within a trapping section, but they are a good representation of terrestrial activity and are commonly used in biological inventories such as this one (Antvogel and Bonn 2001; Topp et al. 2008). When interpreting pitfall data, it is important to consider that they record activity densities, therefore the abundance of species that are more mobile or active may be overestimated, and other species within the area may not be captured (Luff 1975; Topp et al. 2008).

Results were analyzed from the Bible Hill site using four blocks of habitat groups to determine invertebrate diversity indices (Simpson (1-D) and Shannon (H)), abundance (N), and species richness (S). Data were normally distributed for all variables except species richness, which was square-transformed to achieve normal distribution. There were no significant differences between habitats in terms of these measures of invertebrate presence at Bible Hill in 2012 (Table 3.5). This suggests that small knotweed patches may not be as species-poor as previously noted by Gerber et al. (2008). While the habitats are not statistically different, there are some trends such as greater abundance and species richness in grass plots. Interestingly, the diversity indices tend to have higher values in knotweed and shrub habitats than grass which may be due to the constraints of each index equation: Simpson’s index (1-D) places more emphasis on the most abundant species, and less on species richness (Magurran 2004); whereas the Shannon index (H) can have confounding effects from both species richness and evenness (Magurran 2004).

Table 3.5 Invertebrate diversity indices (Simpson and Shannon), mean abundance of organisms (N), and mean number of species (richness) from pitfall traps in three habitats in Bible Hill, NS July 2012.

Habitat	**Simpson (1-D)**[a]	**Shannon (H)**	**Abundance**	**Sp. Richness**[c]
Knotweed	0.90 a	2.73 a	84 a	24.6 a
Shrub	0.91 a	2.61 a	79 a	23.3 a
Grass	0.82 a	2.48 a	153 a	29.1 a
Std Err[b]	0.04	0.17	21.37	9.7
***p*-value**	0.4193	0.6120	0.0870	0.1305

[a] Values for a given measurement with the same letter were not significantly different between habitats in each site using Tukey's test at $p \leq 0.05$.

[b] Standard Error used for means comparisons.

[c] Back-transformed means; standard error values were square-transformed.

Rank/abundance plots were created for each habitat to better visualize any contrasting patterns of species richness (Figure 3.2). All three habitats have similarly-shaped curves, although knotweed and grass seem to have a greater proportion of 'rare' or single-sampled species than in shrub.

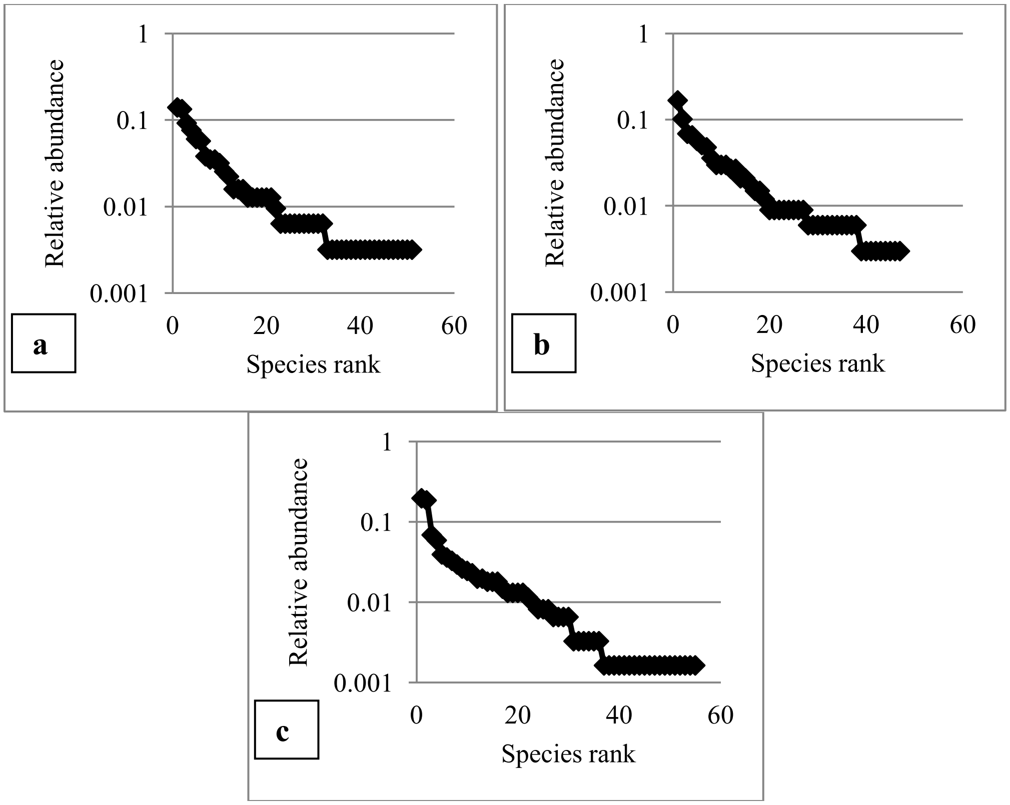

Figure 3.2 Rank/abundance plots of invertebrate species in knotweed (a), shrub (b), and grass (c) habitats in Bible Hill, NS July 2012.

A summary of invertebrate species recorded from pitfall traps can be found in Appendix B. A total of 1265 organisms, representing 81 morphospecies were collected and identified from the Bible Hill site in July 2012, and while the sample size is relatively small, the data suggest a few trends. Within knotweed habitats, worms, Neuroptera larvae, and two carabid species were found in higher abundances than elsewhere, whereas the abundances of woodlice and an otherwise common carabid species were much lower. In shrub habitat there was an abundance of millipedes, slugs, a weevil, and several fly species, and a relative lack of centipedes. Finally the grass habitat had an abundance of spiders, centipedes, several carabid species, some flies, leaf hoppers, ants, toads, and shrews, and a lack of millipedes. These comparisons within smaller assemblages can reveal certain habitat requirements and preferences. For example, the leaf litter and lack of ground covering plants within knotweed patches may provide a better niche for earthworms, but not enough decomposing plant matter for the detrivorous woodlouse. Gerber et al. (2008) also found a lack of woodlice in knotweed habitats. Similarly, they also recorded relative abundances in knotweed patches of carabids, in shrub plots of flies, and in grass habitats of spiders, carabids, ants, and flies (Gerber et al. 2008).

Additional invertebrate surveys at several locations and at various times of year would allow for a more robust data set for determining differences amongst invertebrate assemblages from distinct habitats.

3.4.3 Ornithology Observations Not enough data were collected to make statistical inferences regarding bird preferences amongst habitats, although these observations provide some information about bird activity at two riparian sites in Nova Scotia. Birds are highly mobile animals, however many species have specific diets and nesting requirements. While conducting various site visits in Antigonish and Bible Hill, NS from September 2010 to summer 2012, I observed several bird species near Japanese knotweed patches and the surrounding riparian environment. Several nests were found among the dense canopy of knotweed branches which would only be used for one season as the dead stalks fall down each winter. I identified young Cedar Waxwings in an Antigonish knotweed stand in August 2011, as well as Dark-eyed Junco chicks in a ground nest at the base of a knotweed clump in Bible Hill in mid June 2011 (Figure 3.3). Results from bird observations in Antigonish and Bible Hill over four and six respective one-hour periods in June 2012 are shown in Appendix C. There are no noticeable differences between habitats regarding the species richness at either site, and the number of bird sightings are similar across habitats, except for a decreased number in Bible Hill knotweed habitats. The most common species at both sites were Yellow Warbler and Song Sparrow. In Antigonish, the most commonly observed species in knotweed patches was Cedar Waxwing and a nest was located in one of the stands (in addition to the one photographed in Figure 3.3). Grass habitats were visited primarily by warblers and sparrows at both sites, and shrub patches were visited by a variety of species including warblers, sparrows, flycatchers, blackbirds, and goldfinches.

These results, though sparse, can be verified with bird distribution ranges and known habitat preferences (Tufts 1986; Sibley 2000). Also, a desktop ornithology report by John Kearney (2010) for the Antigonish Wetland Compensation Project confirms the presence of these species in Antigonish, but it does not record any species within knotweed habitats. Indeed, there are no known studies that show an impact of invasive plant invasions on bird presence. Perhaps a more detailed ornithology study, as recommended by Kearney (2010) for species monitoring, could determine if a mono-specific plant invasion would affect bird behaviour, or breeding and feeding habits.

Figure 3.3 Dark-eyed Junco chicks Bible Hill June 2011 (left), and Cedar Waxwing nestlings Antigonish August 2011 (right).

3.4.4 Small Mammal Survey Small mammal tracks from both sites over one week in 2011 and 2012 were identified to Genus or species in six categories: *Sorex* spp., *Blarina*, *Peromyscus*, Vole spp. (three species of voles), *Zapus*, and Unknown (tracks that could not be identified, but likely belonged in one of the other categories). A total of 489 tracks were analyzed (Appendix D). *Sorex* spp. had the most common tracks (238) and *Zapus* had the least common (5). All six track categories were analyzed individually, as well as lumped together to assess the total presence and the abundance of small mammal tracks. Track presence indicated if a track of a certain category was observed so it was possible for several mammal categories to be present on one tracking sheet. Track abundance was an indication of the number of tracks of each category per tracking sheet and can suggest the level of mammal activity at each tracking station.

All track categories were analyzed to determine the significance levels of site and habitat (Table 3.6). Track data did not meet normality assumptions for *Blarina* species or for the abundance of *Peromyscus* species. *Zapus* and unknown categories did not have sufficient numbers to merit statistical analysis, but they were included in the total. Site was significantly different ($p \leq 0.05$) for the combined track category and voles. Site effects for *Sorex* and *Peromyscus* presence were not significantly different ($p \geq 0.05$). For all categories except for *Peromyscus* presence, the interaction effect of habitat nested in site was significantly different ($p \leq 0.05$), so sites were analyzed separately for that species.

Table 3.6 Significance levels (*p*-values) for the effects of site and habitat on small mammal track presence and abundance in Antigonish and Bible Hill, NS in 2011 and 2012.

	All mammal species		*Sorex*		*Peromyscus*	Vole	
Effect[a]	Presence	Abundance	Presence	Abundance	Presence	Presence	Abundance
site	0.0034	0.0005	0.0714	0.3409	0.0669	0.0427	0.0032
habitat(site)	0.0053	0.0004	0.0012	0.0076	0.5269	0.0011	0.0001
block	0.1173	0.0034	0.2289	0.1069	0.0871	0.6457	0.5887

[a] Effects were: site (Antigonish, Bible Hill); interaction with habitat (Knotweed, Shrub, Gras) nested in site; block (2011, 2012 + blocks in the field).

Sites were analyzed separately for the total number of species present and abundant in each habitat. Both sites met normality assumptions and showed differences between habitats (Table 3.7). Although the sites were different from each other, they exhibit the same pattern with grass habitats recording the highest number of mammal species, shrub stands having the lowest, and knotweed patches in between the two. Mammal track abundance in Bible Hill knotweed patches is significantly different from grass. These results suggest that the two ‘native’ habitats (grass and shrub) are distinct from each other in terms of mammal presence and abundance, but knotweed patches share characteristics of both. By breaking down all small mammals into smaller groups it may be possible to discern differences between taxa.

Table 3.7 Presence and abundance of six small mammal track types (*Sorex* spp., *Blarina*, *Peromyscus*, Vole spp., *Zapus*, and Unknown) in different habitats at Antigonish and Bible Hill, NS sites together in 2011 and 2012.

	Mammal Track Presence [a]		**Mammal Track Abundance**	
Habitat	Antigonish	Bible Hill	Antigonish	Bible Hill
Knotweed	2.0 ab	1.4 ab	11.9 ab	6.3 a
Shrub	1.4 a	0.8 a	4.4 a	3.3 a
Grass	2.6 b	2.0 b	18.6 b	8.0 b
Std Err [b]	0.27	0.31	2.38	1.25
***p*-value**	0.0212	0.0373	0.0021	0.0520

[a] Values for a given site with the same letter were not significantly different between habitats in each site using Tukey's test at $p \leq 0.05$.
[b] Standard Error used for means comparisons.

Sorex species tracks had significantly lower presence in the shrub compared to the knotweed and grass habitats in Antigonish. *Sorex* track abundance data in Antigonish were square-root transformed to meet normality assumptions. Similarly, these tracks were less abundant in shrub compared to grass habitats, but tracks did not have significantly different abundances in knotweed versus both 'native' habitats (Table 3.8). These differences were not statistically different at Bible Hill, but follow a similar trend. Due to their extraordinarily high metabolism rates, shrews require high prey abundance, as well as moist habitat conditions (McCay and Storm 1997). These results suggest that the knotweed habitats may provide these conditions which are desirable *Sorex* requirements, as well as the observation of no significant difference in prey abundance between habitats.

Peromyscus track presence was similar at both sites and could be combined. Track abundance data did not meet normality assumptions and were not included in analysis. Habitats were not significantly different and this species was found in all three habitats nearly equally (Table 3.8). These results are not surprising as *Peromyscus* are known generalists in terms of habitat selection, occupying forests, grasslands, and combinations of both (Burt and Grossenheider 1976).

Table 3.8 Presence and abundance of *Sorex* and *Peromyscus* species in different habitats at Antigonish and Bible Hill, NS sites in 2011 and 2012.

	***Sorex* Tracks**[a]				***Peromyscus* Tracks**
	Presence		Abundance		Presence
Habitat	Antigonish	Bible Hill	Antigonish[c]	Bible Hill	Both sites
Knotweed	0.96 b	0.75 a	2.98 ab	5.00 a	0.36 a
Shrub	0.40 a	0.25 a	0.56 a	1.75 a	0.47 a
Grass	1.00 b	0.75 a	8.0 b	4.75 a	0.30 a
Std Err[b]	0.10	0.15	0.31	1.54	0.11
***p*-value**	0.0014	0.0585	0.0007	0.2832	0.5461

[a] Values for a given species with the same letter were not significantly different between habitats in each site using Tukey's test at $p \leq 0.05$.

[b] Standard Error used for means comparisons.

[c] Back-transformed means; standard error values were square-root transformed.

Vole tracks likely belonged to the meadow vole (*Microtus pennsylvanicus*) due to habitat type, but could not be distinguished from those of the red-backed vole (*Clethrionomys gapperi*) or the Southern bog lemming (*Synaptomys cooperi*), so were grouped as "vole species" (Nams and Gillis 2002). Vole track data were not normally distributed for the Bible Hill site, so only results from Antigonish are reported. Vole track abundance was square-root transformed to meet normality assumptions. In Antigonish, voles were most common and abundant in grass habitats, with no difference between knotweed and shrub habitats (Table 3.9). These results correspond with reported observations that *Microtus* voles prefer this type of non-woody, 'meadow' habitat (Grant 1971, Burt and Grossenheider 1976).

Table 3.9 Presence and abundance of voles in different habitats at Antigonish, NS in 2011 and 2012.

	Vole Tracks [a]	
Habitat	Presence	Abundance
Knotweed	0.2 a	0.07 a
Shrub	0.0 a	0.00 a
Grass	0.7 b	4.53 b
Std Err [b]	0.12	0.26
***p*-value**	0.0024	0.0001

[a] Values for a given species with the same letter were not significantly different between habitats in each site using Tukey's test at $p \leq 0.05$.

[b] Standard Error used for means comparisons.

Live-trapping in September 2011 confirmed the presence of several species including *Sorex* species (Antigonish, knotweed and grass habitats), *Peromyscus maniculatus* (Bible Hill knotweed patch), and *Blarina brevicauda* (Antigonish knotweed and grass habitats) (Figure 3.4).

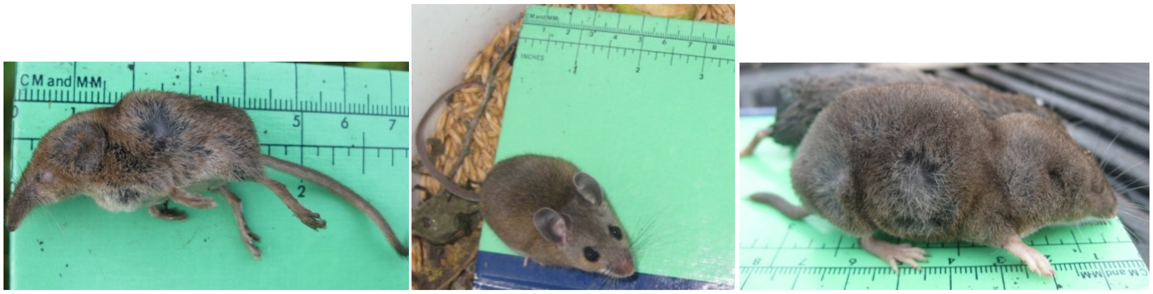

Figure 3.4 Small mammal species from live-trapping in September 2011 at Antigonish and Bible Hill, NS sites. *Sorex* sp. (left), *Peromyscus maniculatus* (centre), and *Blarina brevicauda* (right).

Some variables were plotted to visualize trends and associations with habitats to determine if various animal measures were correlated with the plant community. I thought that the abundance of small mammals could possibly be dependent on plant diversity (Shannon index (H)) (Figure 3.5). Plant diversity does not change in knotweed plots. Mammal track abundance is not strongly related with plant diversity in grass habitats (R^2 = 8.5%), or shrub habitats (R^2 = 3.5%).

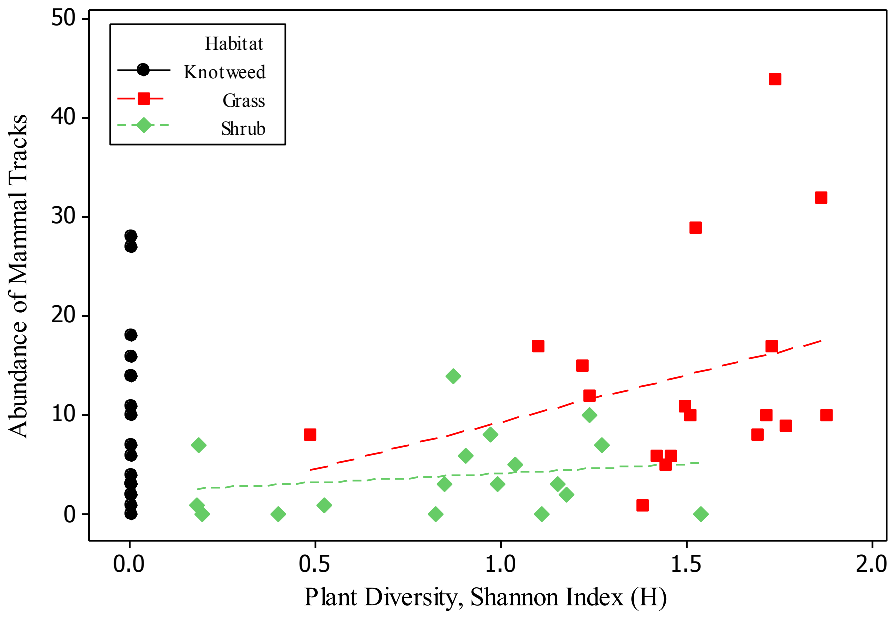

Figure 3.5 Scatterplot showing the relationship between small mammal abundance and plant diversity (H) amongst three habitats at Antigonish and Bible Hill, NS sites together in 2011 and 2012.

Relationships were scrutinized between seemingly correlated variables such as specific mammal tracks and invertebrate abundance. *Sorex* track abundance was not correlated with invertebrate abundance, nor was invertebrate abundance related to plant diversity.

Finally, mammal track abundance was plotted against leaf area index (LAI) and more distinct patterns emerge (Figure 3.6). Within shrub patches, mammal abundance stays relatively low as LAI increases (R^2 = 20.0%). In grass habitats, abundance is a bit higher but there is no relationship with LAI (R^2 = 0.7%). Interestingly, mammal track abundance increases with higher LAI values within knotweed patches (R^2 = 41.6%). Perhaps the denser canopy provides more shelter from predators, creates a more suitable environment for mammal species, or increases prey abundance.

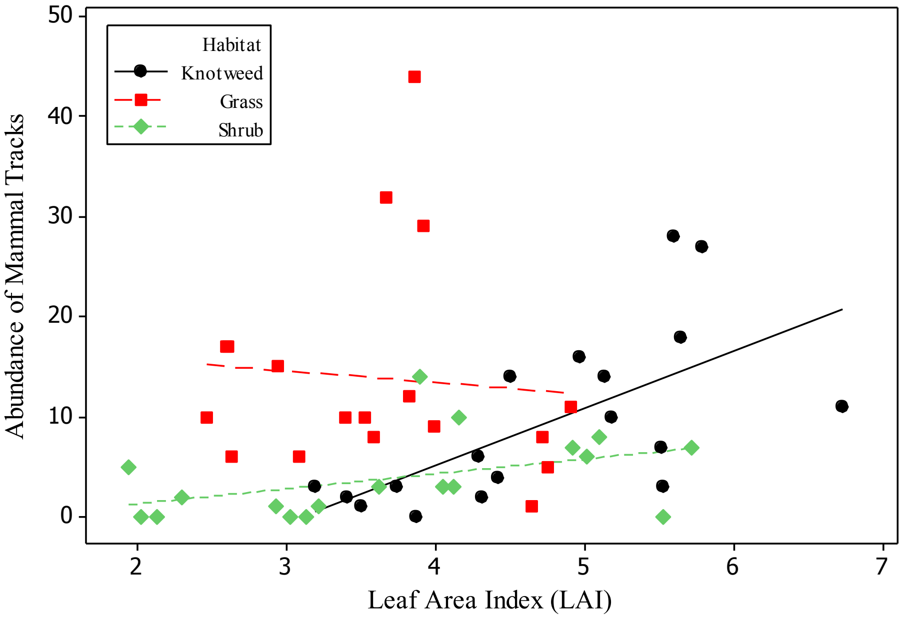

Figure 3.6 Scatterplot showing the relationship between small mammal abundance and leaf area index amongst three habitats at Antigonish and Bible Hill, NS sites together in 2011 and 2012.

By comparing the measured variables within each ecological group it may be possible to notice certain relationships within habitats. Additional data collection of habitat characteristics, environmental conditions within plots, invertebrate diversity, mammal movements, and other aspects of the ecosystem would provide more information about the ecosystem functioning. From this data further correlations between variables can be tested to better understand the impact of Japanese knotweed on other organisms and communities.

This ecological impact assessment at two sites in Nova Scotia over two years both confirmed and contrasted with similar previous studies. The lack of plant diversity in knotweed stands was not unexpected, and is supported by similar studies (Gerber et al. 2008, Aguilera et al. 2010). The survey of plant communities indicated a significant difference between the three habitats in terms of species diversity indices (Simpson (1-D) and Shannon (H)), species richness (S), and amount of cover at different heights. Conversely, this study found no significant difference between habitats in regards to invertebrate diversity, abundance, and richness. In comparison, Gerber et al. (2008) found a reduced abundance and species richness in knotweed plots versus bush and grassland habitats. A study by Topp et al. (2008) also found reduced beetle richness, abundance, and diversity in knotweed versus forb habitats. The data in this study were limited to one sampling period and one collection site and further data collections would strengthen analyses. No other comparable studies regarding invasive plants and small mammals could be found to evaluate results with. The characteristics of each habitat were assessed to determine if certain variables could be linked with preferred conditions for mammals. Data from this study suggest that knotweed patches may provide suitable habitat for some types of mammals. Increased canopy cover within knotweed habitats could create favourable environmental conditions or protection from predators for small mammal species.

3.5 Conclusion

Japanese knotweed patches are practically devoid of any other plant species. However, many animal species are present in these low plant diversity habitats, perhaps due to the high level of ground cover which may alter micro-habitat conditions. This leaf cover may also protect inhabitants from predators, or provide other environmental characteristics. Regardless, the presence of knotweed stands does not have a significant impact on invertebrate abundance, species richness, or diversity. Insectivores such as shrews may find knotweed patches as suitable feeding grounds which is why *Sorex* species are more common in knotweed than shrub habitats. Birds were also observed to be utilizing knotweed patches for nesting, and possibly feeding on insects. On a small scale, knotweed patches may actually contribute to plant and animal diversity by providing additional micro-habitat conditions for riparian inhabitants. Despite these conclusions, there are several other recorded instances where knotweed patches have completely dominated the local ecosystem. This study shows that it is imperative to evaluate many aspects of a plant invasion to assess its impact on the environment.

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Chapter 4: Japanese Knotweed (*Fallopia japonica*) Management: Efficacy of Aminopyralid and Imazapyr Applications at Various Phenological Stages over Time

4.1 Abstract

The most effective method of managing invasive weeds is by preventing their original establishment, but that is not usually possible. Japanese knotweed is a weed of urban and natural areas that should be managed before it can spread across a landscape. Its extensive rhizome system is a near-limitless source of continual shoot emergence throughout the growing season. Two herbicides were evaluated for their efficacy at controlling knotweed growth over time. Aminopyralid was applied in the early spring on emerging shoots. In an attempt to translocate the herbicide throughout the plant, imazapyr was applied at three phenological stages: (i) maximum plant height, (ii) flowering, and (iii) prior to senescence in the fall. Also, all possible combinations of these two herbicides and four times were utilized for a total of 16 treatments at two locations in Nova Scotia in 2011. Stem height, density, leaf area index, and damage ratings were measured in the short term (2, 4, and 8 weeks after treatment), along with biomass one year after treatment to determine the most effective herbicide treatment. Results suggest that aminopyralid is most effective in the short-term, but imazapyr produces significantly better damage results one year after treatment. The recommended herbicide treatment is imazapyr at a rate of 720 g ae $ha^{-1}$when knotweed is fully grown in mid-June or during flowering in August. Further monitoring of these treatments will support these results over a longer time span.

4.2 Introduction

Invasive plants have a significant impact on the global landscape. The negative effects of these weeds are well-known and widely publicized, and include: loss of biodiversity (Maerz et al. 2005); threats to human health (Groves and Burdon 1986); damage to structures (Nantel et al. 2002); costs of control (Pimentel et al. 2000), and other factors (Child and Wade 2000). Japanese knotweed (*Polygonum cuspidatum* Sieb. & Zucc. syn. *Fallopia japonica* (Houtt.) Ronse Decr.) is of particular concern in Europe and North America due to its rapid growth (Davenport 2006) and persistent stands which outcompete other plants (Aguilera et al. 2010). Knotweed readily establishes at disturbed sites, often associated with high soil moisture such as along roadways, in ditches, and adjacent to riparian zones (Barney et al. 2006).

There are many management options with varying environmental impacts, costs, labour, and overall efficacy. Effective annual control is likely to rely on a combination of methods, timing, rates, and follow-up (Bram 2002). Mechanical or physical control can be quite cost- and labour-intensive, requiring extensive follow-up, and may even promote spread (Child and Wade 2000; Nickerson, personal communication). Biological controls such as insects and disease are currently being evaluated (Shaw et al. 2009; Bourchier, personal communication) although it is unlikely they can be solely relied upon to solve this problem. Herbicide application has been proven to be very effective after several years of follow-up treatment (Bashtanova et al. 2009), but there are still unanswered questions regarding the timing of herbicide application.

The use of herbicides is an efficient means of knotweed control (Bashtanova et al. 2009) although it may have some disadvantages such as cost, killing non-target species, and environmental risks. Published reports indicate that a systemic approach is necessary for effective eradication (Bashtanova et al. 2009). Much of the plant’s energy is stored in the extensive rhizome system (Price et al. 2002), and it is important to use a chemical that will be translocated to this area. The most common and proficient means of herbicide application is by spraying the chemical on the leaves of the plant during key growth stages. The timing of herbicide application is also important due to the movements of nutrients, carbohydrates, and other resources during plant growth (Bashtanova et al. 2009).

Glyphosate (Roundup®, Monsanto Co.) is one of the most commonly used herbicides for knotweed because it has little residue in the soil and can be applied close to water (Child and Wade 2000). However it may not be as effective as imazapyr (Arsenal®, BASF) for knotweed control (Child and Wade 2000; Barney et al. 2006; Bashtanova et al. 2009). Recent studies suggest that imazapyr is more effective than glyphosate because it disrupts phloem transport and has a low rate of decomposition (Bashtanova et al. 2009). Imazapyr is rapidly absorbed through plant tissue and transported to meristematic zones via the xylem and phloem. By inhibiting the acetolactate synthase (ALS) enzyme it disrupts protein synthesis and cell growth, which causes slow plant death (Tu et al. 2004). The application of imazapyr during key growth stages (maximum growth, flowering, and prior to senescence) could direct the ALS-inhibitor towards growth zones and/or rhizome parenchyma. The Group 4 herbicide Aminopyralid (Milestone®, Dow AgroSciences) is also generally recommended to reduce vigorous shoot growth earlier in the season. Aminopyralid is similarly translocated to meristematic tissues, but results in unregulated growth and rapid plant death (The Dow Chemical Company 2012).

These herbicides can be applied using various methods, including backpack sprayer, boom sprayer, stem injection, or wiping. With the appropriate application method, rate, frequency, and timing it may be possible to effectively manage knotweed populations while limiting overexposure of herbicides to non-target species, as well as reduce costs and labour. The overall objective of this study was to determine the most effective herbicide or combination of treatments for knotweed control in terms of reduced height, density, leaf cover, biomass, and an overall knotweed damage rating.

4.3 Materials and Methods

4.3.1 Site Description Herbicide studies were conducted at two sites in Nova Scotia: Antigonish and Bible Hill. The Antigonish site was located along the south side of the

Rights River (45.622°N, 61.972°W), which flows through the town of Antigonish and empties into Antigonish Harbour. The research plots were situated at approximately 1-2 m above sea level. A soil analysis of particle size distribution, as described by Sheldrick and Wang (1993) and Brewster (2001) revealed a composition of 62% sand, 13% clay, and 25% silt. In the spring and fall the area is commonly subjected to flooding, and as a result, parts of the river have been channelized and armoured. Historically, the floodplain supported freshwater wetlands but was subsequently cleared and drained for agriculture. Japanese knotweed is found in several persistent patches along both sides of the river for about 2 km. These stands likely established from the dumping of yard waste and successive movement of knotweed fragments by ice and water. Although it is difficult to estimate patch age, the oldest knotweed specimen from the site preserved in the herbarium of the local St Francis Xavier University was collected in 1986.

The Bible Hill site was located along the Salmon River which separates the towns of Bible Hill and Truro as it flows into the Bay of Fundy (45.371°N, 63.277°W). The herbicide experiment was on the east side of the river and also experiences periodic flooding. Part of the site had been used as pastureland and crops, but has been left fallow for several years. A soil analysis of particle size distribution, as described above revealed a composition of 70% sand, 15% clay, and 15% silt. The age of the well-established knotweed patches are unknown but they were likely introduced to the site from flooding events.

4.3.2 Experimental Design At each site a knotweed patch was located with sufficient size for 16 plots which were each 2 m x 2 m with a 2 m buffer between treatments. The treatments were arranged in a randomized complete block design with three blocks. In

Antigonish, three small patches were used as blocks whereas in Bible Hill there was enough space in one large patch to contain three separate blocks, each with 16 randomized plots. In early May 2011 both sites were cleared of standing knotweed stalks from the previous year. The experiment was set up as a 2 x 8 factorial design with two herbicide levels (aminopyralid presence versus absence) and eight imazapyr application combinations.

4.3.3 Herbicide Treatments Aminopyralid (Milestone) was applied at an early emergent stage of knotweed growth, when the ramets were less than 30 cm tall. This was done at Bible Hill on May 12th 2011 and in Antigonish on May 18th 2011. Aminopyralid was applied at a rate of 120 g ae ha^{-1} in 200 L/ha water (Table 4.1). A handheld CO_2-pressurized boom sprayer was used at about 40-60 p.s.i. with four TeeJet 8002VS nozzles spaced 50 cm apart. The boom was held 50 cm above the knotweed ramets for complete coverage of each plot.

Imazapyr (Arsenal) was applied at all possible combinations of three timings: (1) M- Maximum plant growth, (2) F- Flowering, (3) S- prior to Senescence, (4) M+F, (5) F+S, (6) M+S, (7) M+F+S, and (8) an untreated control. Most knotweed stems had reached maximum growth in July, flowering occurred in August, and senescence typically occurred in October. Knotweed is very frost-susceptible so it was necessary to spray in advance of an autumn killing frost which usually occurs in late October across much of the province (Agriculture and Agri-Food Canada 1997). The herbicide was applied at a rate of 720 g ae ha^{-1} in 1200 L/ha of water with Agral90 acting as a surfactant (50 mL/100 L spray mix). A large spray volume was used to ensure coverage of the thick canopy. A 15 L Solo® backpack sprayer with a single TeeJet 11004VP nozzle was employed to spray all sides and the top of each 4 m^2 knotweed plot.

Table 4.1 Herbicide treatments for Japanese knotweed at two sites in Nova Scotia, 2011.

Herbicide	**Rate**	**Site**	**Application Date (2011)**			
			Emergence	**Max Growth**	**Flowering**	**Senescence**
Aminopyralid (Milestone)	120 g ae ha^{-1} in 200 L ha^{-1} water	Antigonish	May 18			
		Bible Hill	May 12			
Imazapyr (Arsenal)	720 g ae ha^{-1} in 1200 L ha^{-1} water + Agral90 (50 mL/ 100 L spray mix)	Antigonish		July 20	Aug 30	Oct 12
		Bible Hill		July 11	Aug 23	Oct 12

4.3.4 Damage Assessment The effect of each herbicide treatment was measured with several variables including damage ratings and height at 2, 4, and 8 weeks after treatment (WAT), density at 2 and 8 WAT, and leaf area index (LAI) at 8 WAT. Herbicide damage was estimated on a 0-10 rating scale of above ground stems with 0 being no damage and 10 being completely dead. Height was measured using a tape measure from the ground to the highest point of the plant, but not including the terminal leaf. By late June the knotweed ramets began to bend over due to their height and weight, so the height was measured to the highest point of the arch. In order to get a good representation of plant height within each 2 x 2 m plot, five ramets were chosen for measurement. One side of the plot was separated into five, 40 cm wide rows and the first ramet was picked from each row. The density of knotweed ramets was determined by counting all stems over 10 cm tall within each 4 m^2 treatment. LAI was measured using the optical instrument LAI-2000 (LI-COR® Lincoln, NE). A 90° cap was placed on the sensor's eye to block 270° of light outside of the desired area. Four readings were taken within each plot at approximately 30 cm above the ground to estimate an average LAI value.

In addition to the short-term damage assessments, data were also collected in the subsequent growing season in June 2012. Damage ratings were taken one year after aminopyralid application, as the knotweed ramets were beginning to emerge in May. Additional measurements were taken when the knotweed plants had reached their maximum growth, including damage ratings, height, density, LAI, and biomass. Biomass in 2012 was measured by cutting down all knotweed ramets over 10 cm tall and weighing them on a portable scale in the field (fresh weight).

4.3.5 Data Analysis ANOVA of all data was completed using PROC MIXED in SAS ver. 9.3 (SAS Institute Inc., Cary, NC) to determine the effects of site, herbicide, and time. Normality and constant variance assumptions were verified. In some cases, transformations were used to achieve normality and back-transformed means are presented. A sequential analysis of data was used to test for interactions ($p \leq 0.05$), so if the highest level of interactions was not significant it was removed and retested. For significant effects, the least squares means were compared with Tukey's adjustment ($p \leq 0.05$). Then the subroutine pdmix800.sas (Saxton 1998) was utilized to provide letter groups. For the short-term data there were up to three dates of assessment so a repeated measures model was used with week as the time factor. The analyses for 2012 data were similar, except it was not a repeated measure.

4.4 Results and Discussion

4.4.1 Short-term Effects of Herbicides Sites were significantly different, so were analyzed separately. Data were tested for normality using a Shapiro-Wilk test ($p \geq 0.10$). This was achieved with all variables except for Rating at the Bible Hill site. Height in Antigonish and LAI in Bible Hill were normalized with square and square-root transformations, respectively, but Rating in Bible Hill could not be normalized with any transformations.

All other variables were normally distributed (Tables 4.2, 4.3).

Only Rating in Antigonish had significant effects with a three-way interaction between pre*post*week. There was a significant interaction between the pre and post herbicide application on damage ratings and this changed with time. All other variables had significant two-way interactions, except for Height in Antigonish and Density in Bible Hill which had one-way interactions. Pre herbicide applications impacted knotweed density and this impact varied with post herbicide applications at both sites. Aminopryalid decreased shoot height, and all herbicide combinations impacted leaf area.

Table 4.2 Significance levels (*p*-values) for all combinations of herbicide treatments (effects) on short-term damage assessments at Antigonish, NS in 2011.

Effect [a]	Damage Rating	Density	Height	Leaf Area Index
week	0.0001	0.0138	0.5716	0.0001
pre	0.0001	0.0001	0.0001	0.0001
week*pre	0.0001	0.2530	0.7045	0.0307
post	0.0001	0.0001	0.2006	0.0001
week*post	0.0001	0.8535	0.1328	0.0001
pre*post	0.0001	0.0161	0.4161	0.0001
week*pre*post	0.0033	0.8966	0.8811	0.3705

[a] Effects were: week (2, 4, and 8 Weeks After Treatment); pre (use of Aminopyralid); and post (use of Imazapyr), as well as combined interaction effects.

Table 4.3 Significance levels (*p*-values) for all combinations of herbicide treatments (effects) on short-term damage assessments at Bible Hill, NS in 2011.

Effect [a]	Damage Rating [b]	Density	Height	Leaf Area Index
week	0.0001	0.0458	0.2342	0.0001
pre	0.0001	0.0001	0.0001	0.0001
week*pre	0.1129	0.7108	0.5250	0.0087
post	0.0001	0.0003	0.6310	0.0001
week*post	0.0001	0.2185	0.7461	0.6542
pre*post	0.0001	0.2954	0.0059	0.0014
week*pre*post	0.8409	0.9592	0.9722	0.3957

[a] Effects were: week (2, 4, and 8 Weeks After Treatment); pre (use of Aminopyralid); and post (use of Imazapyr), as well as combined interaction effects.
[b] Data could not meet normality assumptions.

Damage ratings at the Antigonish site showed the efficacy of various herbicide treatments on Japanese knotweed over an eight-week period (Table 4.4). There are no data at weeks 4 and 8 for any treatments with Imazapyr at the Senescence (S) stage because the plant had succumbed to frost shortly after the final fall application. The control plots showed little to no damage, as should be expected. The plots with the most damage –ratings of 8 and 9- with solely imazapyr treatments were MS and MFS at 2 WAT and MF at 8 WAT. Those with aminopyralid + imazapyr that were most damaged with ratings from 8-10 include MF at 2, 4, and 8 WAT, and MS and MFS at 2 WAT. The plot with only aminopyralid maintained a high level of control of 5 and 6 over eight weeks. In general, the imazapyr treatments had a slow but significant increase in damage ratings over time, as well as with additional treatments. In comparison, the aminopyralid + imazapyr treatments maintained a moderate level of damage ratings over time, and gradually increased with additional treatments.

These results suggest that damage ratings are highest for treatments which have more than one application of imazapyr. In particular, application at Maximum growth (M) along with Flowering (F) and/or Senescence (S) are better than application at just single applications or at Flowering + Senescence (FS). The use of aminopyralid in combination with imazapyr has moderate to high short-term efficacy, but is not significant after eight weeks. The most effective treatment with the least amount of herbicide applications is Imazapyr at Max growth + Senescence (MS). This treatment is not significantly different from the other high ratings two weeks following the final application.

Table 4.4 Japanese knotweed short-term mean damage ratings (0=no damage, 10=dead) following various herbicide treatments at Antigonish in 2011.

Treatment		**Weeks After Treatment** [a]			
Aminopyralid	**Imazapyr**	**2**	**4**	**8**	**Std Err** [b]
No	Untreated	A 1 a	A 0 a	A 0 a	0.33
No	(M) Max growth	A 1 ab	AB 1 a	B 3 b	0.33
No	(F) Flowering	A 1 abc	A 2 ab	B 6 c	0.30
No	M+F	A 3 bc	A 4 bc	B 9 d	0.19
No	(S) Senescence	2 abc			
No	M+S	8 fg			
No	F+S	6 e			
No	M+F+S	9 g			
Yes	Untreated	A 6 e	A 5 cd	A 6 c	0.30
Yes	M	A 5 de	A 6 d	A 6 c	0.38
Yes	F	A 5 de	A 5 cd	A 6 c	0.47
Yes	M+F	A 8 fg	AB 8 e	B 10 d	0.38
Yes	S	3 cd			
Yes	M+S	9 g			
Yes	F+S	6 ef			
Yes	M+F+S	10 g			
Std Err [b]		0.41	0.36	0.45	

[a] Mean values for a given treatment or date with the same letter were not significantly different using Tukey's test at $p \leq 0.05$. Uppercase letters indicate differences within rows (over time); lowercase letters indicate differences within columns (between treatments).

[b] Standard Error used for means comparisons.

Density values at the Antigonish site during the 2011 season varied depending on the type of treatment (Figure 4.1). The lowest densities were found at the treatment with aminopyralid + imazapyr at M, F, and MF, with MF being the lowest at 17 stems per 4 m^2 plot. The control plots had a mean density of 77 stems per 4 m^2 plot. Though not statistically significant for all treatments except MF, there is a trend for plots with aminopyralid to have a lower density than those without. This can be explained because aminopyralid was applied during shoot emergence and the young shoots died following application. These results suggest that an initial aminopyralid treatment can reduce knotweed stem density when followed up with subsequent imazapyr applications.

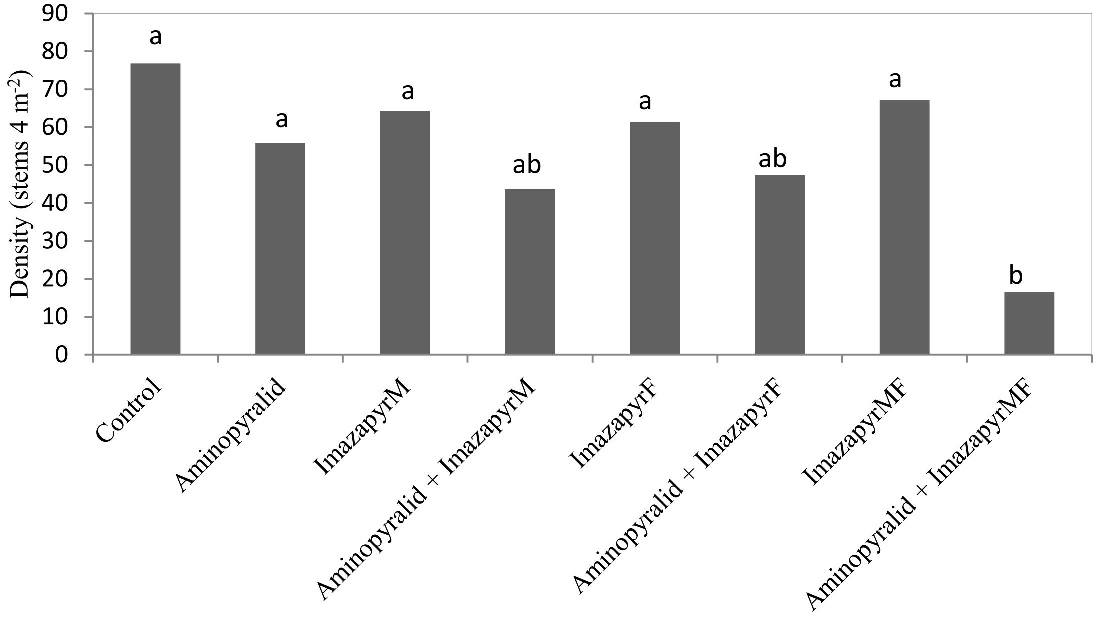

Figure 4.1 Density of Japanese knotweed stems under various herbicide treatments at the Antigonish site in 2011. Treatments indicate timing of imazapyr application: M=Maximum growth; F=Flowering. Treatments with the same letter were not significantly different using Tukey's test at $p \leq 0.05$. Standard error = 6.72.

Height values at the Bible Hill site during the 2011 season varied depending on the type of treatment (Figure 4.2). The control plots had a mean height of 192 cm; whereas the aminopyralid treatment had the lowest height of 102 cm. Imazapyr applications did not impact shoot height because all applications occurred following peak vegetative growth. Aminopyralid applications had a significant reduction in height when compared with the control and imazapyr M. The other imazapyr treatments (F and MF) had a shorter height when combined with aminopyralid treatments versus those without. It is interesting that the treatment with exclusively aminopyralid application has reduced height, but this difference lessens instead of compounding with the addition of imazapyr treatments. The purpose of aminopyralid treatments was to reduce canopy height to facilitate post application coverage. This outcome was achieved, but not as well as desired.

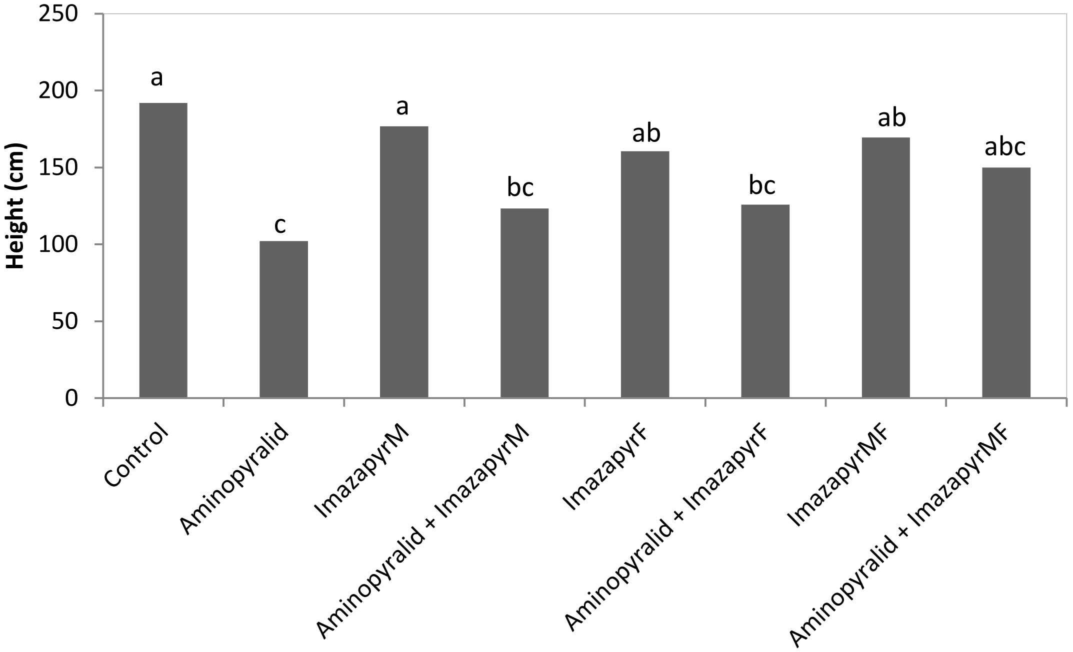

Figure 4.2 Mean height of Japanese knotweed stems under various herbicide treatments at the Bible Hill site in 2011. Treatments indicate timing of imazapyr application: M=Maximum growth; F=Flowering. Treatments with the same letter were not significantly different using Tukey's test at $p \leq 0.05$. Standard error = 9.85

LAI data showed that there was no significant difference in leaf cover 2 WAT between treatments with and without aminopyralid. Knotweed plots with aminopyralid had a rapid visible effect on the leaves with curled and stunted leaf growth, but the quantity of leaves did not have a short-term effect on leaf cover. Eight WAT showed a significant difference in LAI values between the control plot and all other treatments, which were all significantly similar to each other. Over time the imazapyr plots lost a majority of leaves but the stems remained standing.

For short-term effects of these herbicides, several conclusions may be drawn from these results. Effective Japanese knotweed control can be observed with very high damage ratings and very low to nil density, height, and LAI. Over an eight-week period following treatment, results suggest that aminopyralid alone significantly damaged the knotweed plots and decreased density, height, and LAI. Imazapyr alone was significantly different than the control plot for damage ratings and LAI, but otherwise was similar to the control in density and height. The combination of aminopyralid + imazapyr had significantly better values for damage ratings, density, and LAI. Paradoxically knotweed stems were actually taller in the combined herbicide plots than those in just aminopyralid alone (Table 4.5).

Table 4.5 Efficacy of herbicide types on various damage assessments on Japanese knotweed over an 8-week period at two sites in Nova Scotia, 2011.

Treatment	Damage Rating [a]	Density	Height	Leaf Area Index
Control	X	X	X	X
Aminopyralid	*	*	**	**
Imazapyr	*	X	X	**
Aminopyralid + Imazapyr	**	**	*	**

[a] X = not significantly different from control; * = significantly different from control (good); ** = significantly different from * (excellent)

4.4.2 Continuing Effects of Herbicides The effect of the herbicides was measured in the following summer (June 2012) when the knotweed plants reached maximum growth. All herbicide treatments were significantly different from the control plot. The sites were significantly different and analyzed separately.

Normality assumptions were achieved with all variables except for Rating data at the Bible Hill site which could not be normalized. Several other variables required data transformation in order to be considered normally distributed. Density at both sites, Biomass in Antigonish, and LAI in Bible Hill were normalized with square-root transformations, and Biomass in Bible Hill was normalized with a cube-root transformation. All other variables were normally distributed without any transformations.

In Antigonish there were significant interactions between aminopyralid and imazapyr herbicide application on knotweed density, LAI, and biomass. All other variables had significant interactions with imazapyr, resulting in higher damage ratings and decreased density, height, LAI, and biomass (Tables 4.6, 4.7). Aminopyralid was more effective on knotweed control in Antigonish versus Bible Hill, possibly due to knotweed growing conditions or previous herbicide applications.

Table 4.6 Significance levels (*p*-values) for the effects of herbicide applications on damage assessments one year after treatment at Antigonish, NS in 2012.

Effect [a]	Damage Rating	Density	Height	Leaf Area Index	Biomass
pre	0.7845	0.6939	0.3840	0.0243	0.0375
post	0.0001	0.0001	0.0001	0.0001	0.0001
pre*post	0.1618	0.0267	0.1756	0.0585	0.0001

[a] Effects were: pre (use of Aminopyralid); post (use of Imazapyr), and the combined interaction of both.

Table 4.7 Significance levels (*p*-values) for the effects of herbicide applications on damage assessments one year after treatment at Bible Hill, NS in 2012.

Effect [a]	Damage Rating [b]	Density	Height	Leaf Area Index	Biomass
pre	0.7441	0.5074	0.3680	0.4804	0.7446
post	0.0001	0.0001	0.0001	0.0001	0.0001
pre*post	0.8410	0.3611	0.1937	0.6656	0.5805

[a] Effects were: pre (use of Aminopyralid); post (use of Imazapyr), and the combined interaction of both.

[b] Data could not meet normality assumptions.

Damage ratings at both sites showed the efficacy of various herbicide treatments on Japanese knotweed (Table 4.8) the year after application when the plants were at their maximum growth (June 2012). The 2-way interaction between both herbicides was not significant, so only the imazapyr treatments are included. The control plots showed little to no damage, as should be expected. All other plots were significantly controlled, with a damage rating of 9 or 10 at all plots. At the Bible Hill site all treatments were equally effective, while at Antigonish the treatments MF and MFS were significantly more effective than S.

Table 4.8 Japanese knotweed damage ratings (0=no damage, 10=dead) one year after treatment at Antigonish and Bible Hill, NS in 2012.

	Damage Rating[a]	
Treatment	Antigonish	Bible Hill
Untreated	0 a	1 a
(M) Max growth	9 bc	9 b
(F) Flowering	9 bc	9 b
M+F	10 c	9 b
(S) Senescence	9 b	9 b
M+S	10 bc	10 b
F+S	10 bc	10 b
M+F+S	10 c	10 b
Std Err[b]	0.21	0.36

[a] Mean values for a given treatment with the same letter were not significantly different in each site using Tukey's test at $p \leq 0.05$.
[b] Standard Error for means comparisons.

Density values at the Antigonish site during the 2012 season had little variation between treatment types (Figure 4.3). Density was highest in the control plots with 130 knotweed stems in each 4 m^2 plot, as well as in the aminopyralid treatment at 76 stems per 4 m^2. These had significantly higher densities than all other treatments which all contained less than 13 stems per 4 m^2 plot.

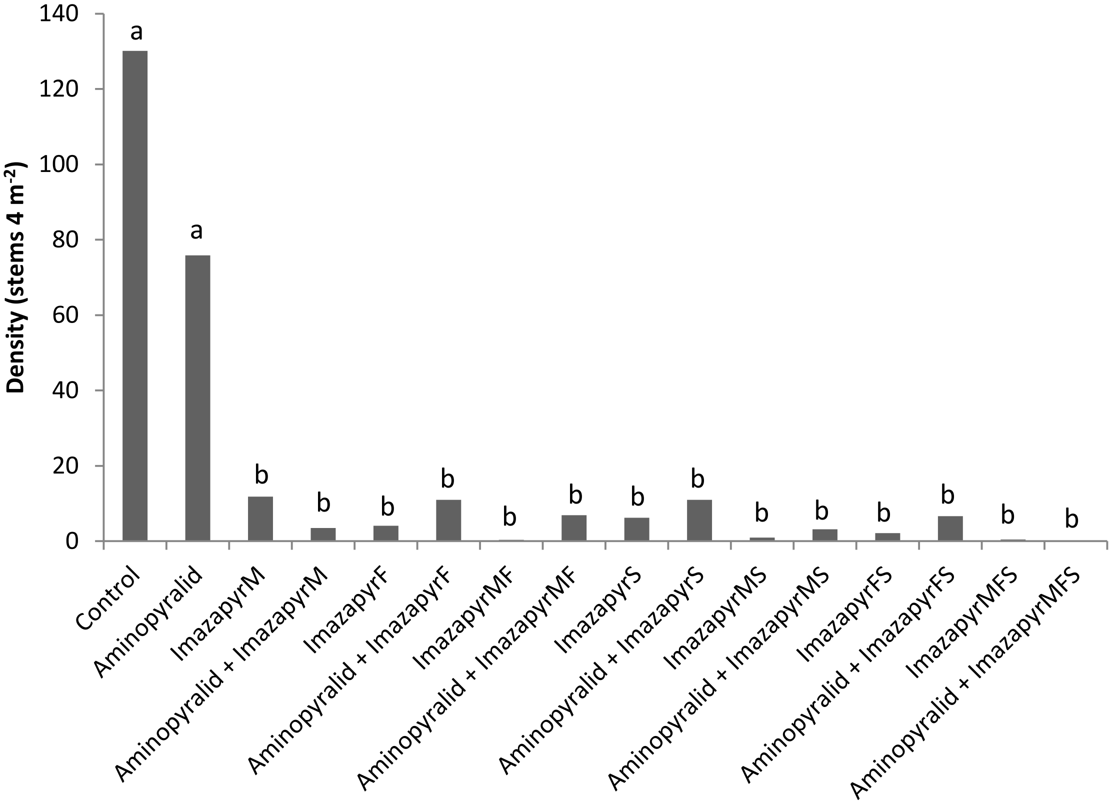

Figure 4.3 Density of Japanese knotweed stems under various herbicide treatments at Antigonish site in June 2012. Treatments indicate the use of aminopyralid and/or imazapyr, and the timing of imazapyr application: M=Maximum growth; F=Flowering; S=Senescence. Treatments with the same letter were not significantly different using Tukey's test at $P \leq 0.05$. Standard error = 0.49

Height values at both sites in June 2012 varied depending on the type of treatment (Figures 4.4, 4.5). The control plots had the tallest mean heights of 189 cm and 159 cm respectively at Antigonish and Bible Hill. These were significantly taller than all other treatments which were all less than 63 cm. At both sites there was a significant height difference between the tallest imazapyr treatment (S) and the shortest imazapyr treatments, MFS, as well as FS in Bible Hill.

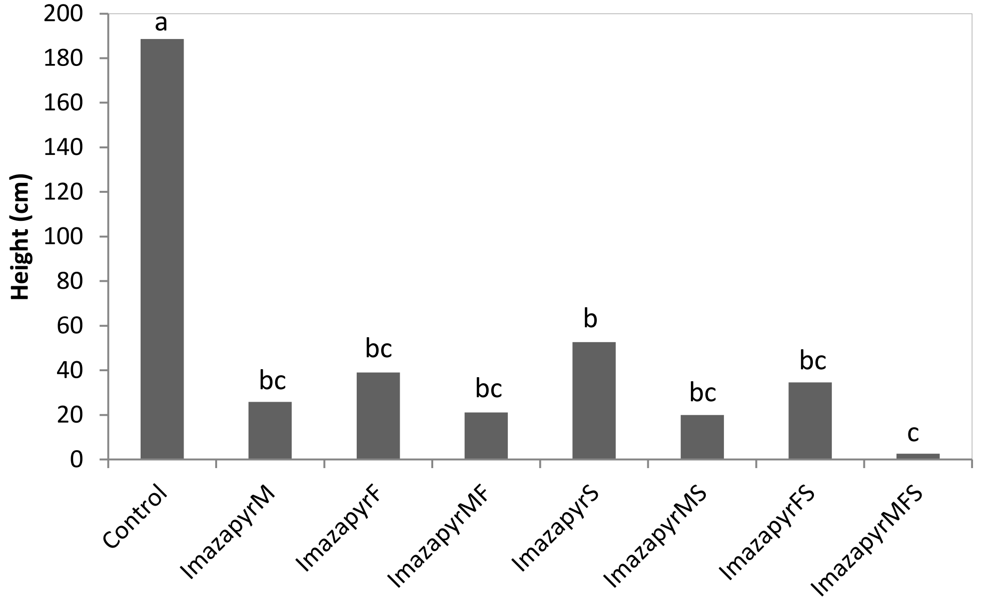

Figure 4.4 Height of Japanese knotweed stems under various herbicide treatments at Antigonish site in June 2012. Treatments indicate the timing of imazapyr application: M=Maximum growth; F=Flowering; S=Senescence. Treatments with the same letter were not significantly different using Tukey's test at $p \leq 0.05$. Standard error = 8.27.

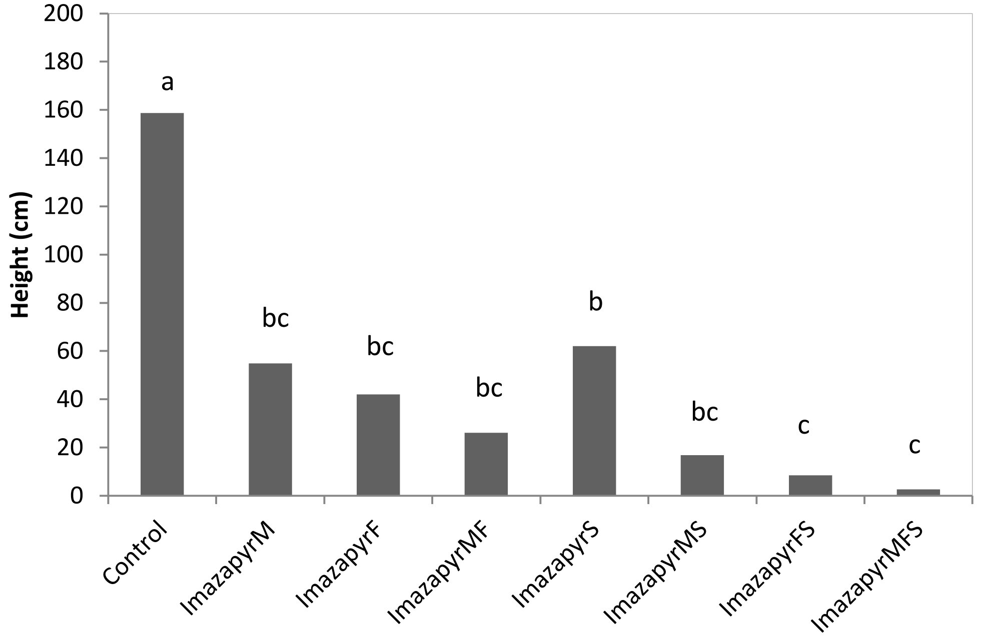

Figure 4.5 Height of Japanese knotweed stems under various herbicide treatments at Bible Hill site in June 2012. Treatments indicate the timing of imazapyr application: M=Maximum growth; F=Flowering; S=Senescence. Treatments with the same letter were not significantly different using Tukey's test at $p \leq 0.05$. Standard error = 10.57.

LAI at the Antigonish site during the 2012 season varied depending on the type of treatment (Figure 4.6). The control and aminopyralid plots had the highest mean LAI values of 5.32 and 3.63 respectively. These were significantly higher values than all other treatments which were all less than 1.63.

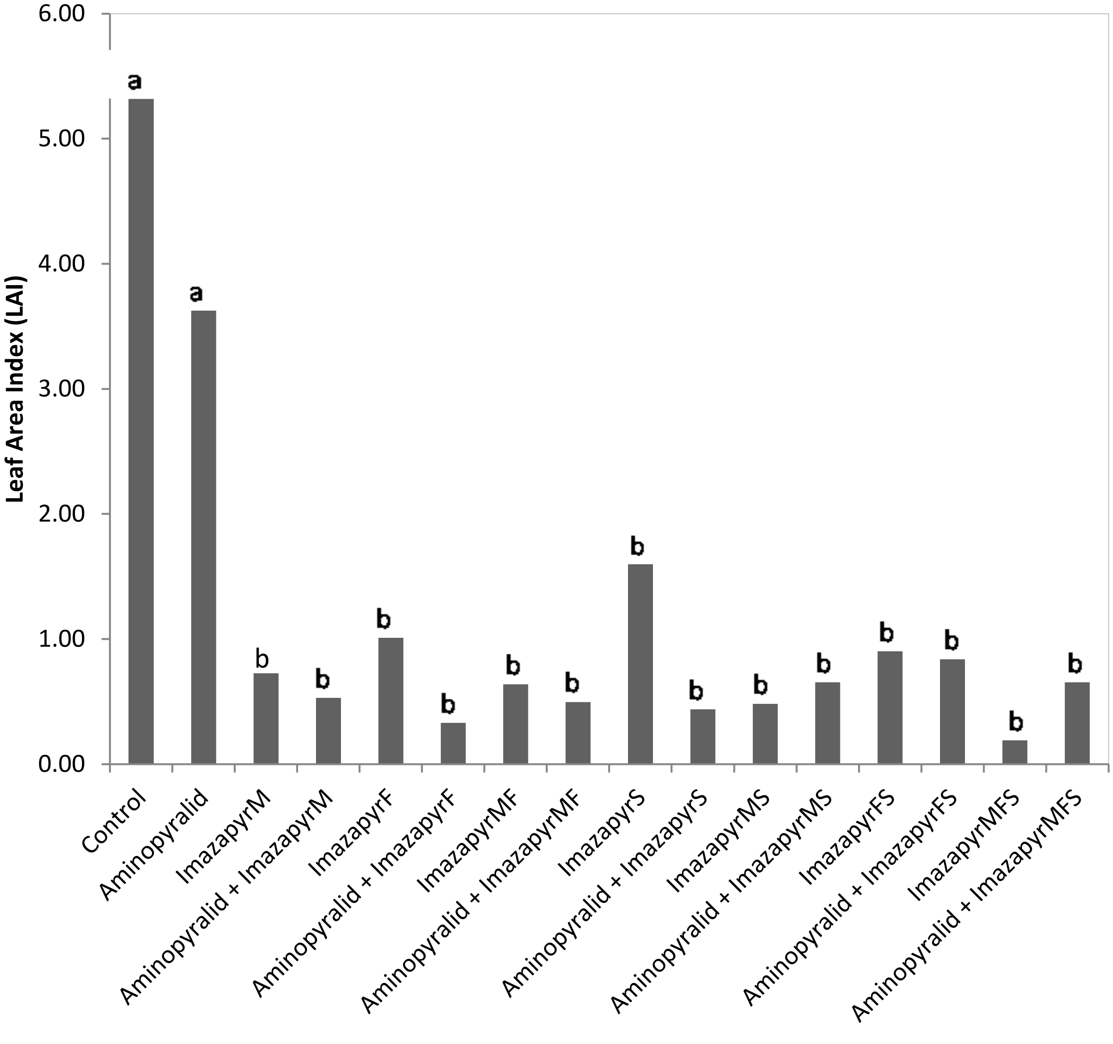

Figure 4.6 Leaf Area Index (LAI) of Japanese knotweed plots with various herbicide treatments at Antigonish site in June 2012. Treatments indicate the use of aminopyralid and/or imazapyr, and the timing of imazapyr application: M=Maximum growth; F=Flowering; S=Senescence. Treatments with the same letter were not significantly different using Tukey's test at $p \leq 0.05$. Standard error = 0.34.

Fresh biomass at the Antigonish site during the 2012 season varied depending on the type of treatment (Figure 4.7). The control plot had the greatest amount of biomass of 34.34 kg, which was significantly greater than the aminopyralid plot of 15.91 kg. These were both significantly greater than all other treatments which were all less than 0.83 kg.

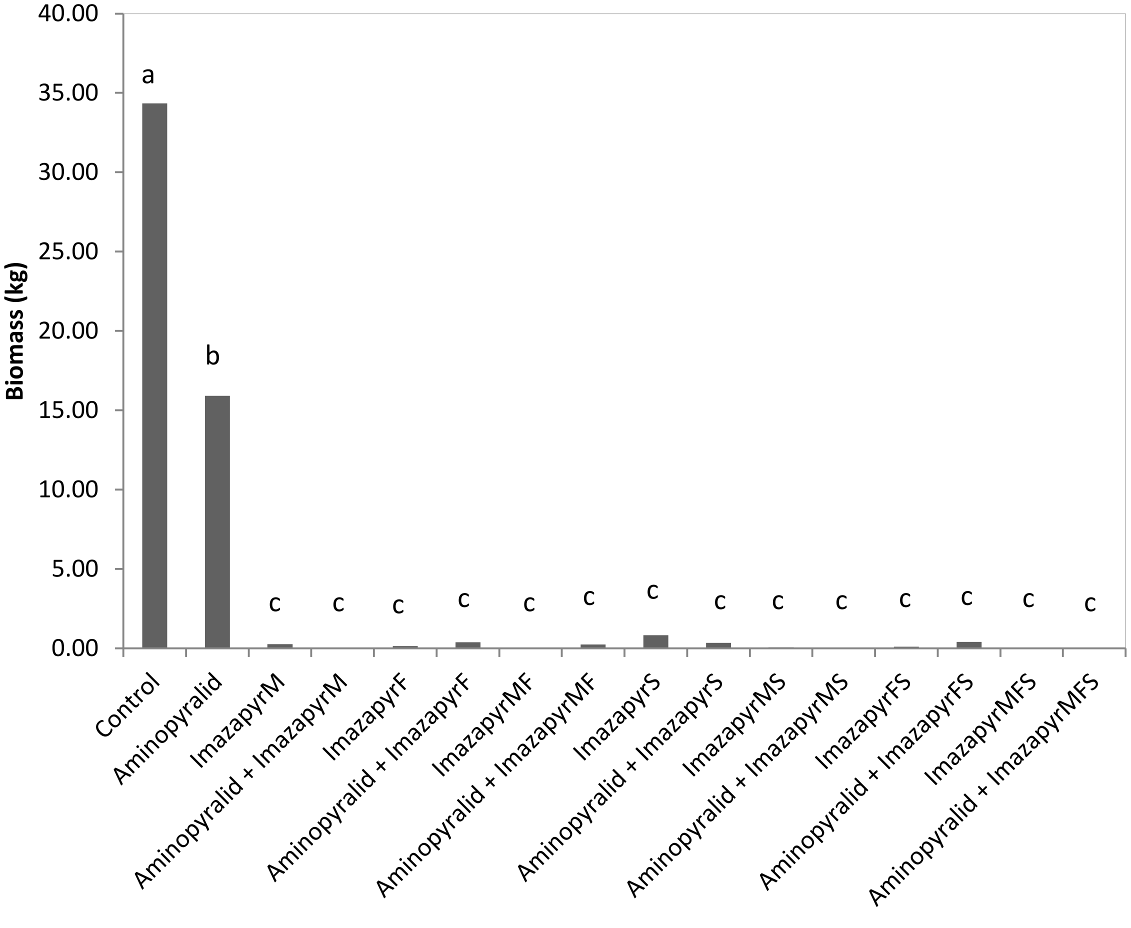

Figure 4.7 Fresh biomass of Japanese knotweed plots with various herbicide treatments at Antigonish site in June 2012. Treatments indicate the use of aminopyralid and/or imazapyr, and the timing of imazapyr application: M=Maximum growth; F=Flowering; S=Senescence. Treatments with the same letter were not significantly different using Tukey’s test at $p \leq 0.05$. Standard error = 0.03.

Longer-term damage to knotweed aboveground matter is necessary for effective management of the plant (Child and Wade 2000; Barney et al. 2006). Results from data collected in June 2012 (one year after herbicide applications) suggest that imazapyr was more effective than aminopyralid. There was no significant difference between the control plot and aminopyralid alone in terms of density and LAI. Aminopyralid yielded a much higher amount of fresh biomass than all imazapyr treatments, suggesting that the plant was able to survive the following season when treated with that herbicide. As previously mentioned it is necessary to use a systemic herbicide that inhibits growth and/or energy storage in the rhizome parenchyma (Bashtanova et al. 2009). Further monitoring of these treatments would be useful in determining herbicide effects beyond one year. Another herbicide experiment could study various spray volumes of imazapyr in an effort to further reduce herbicide use. Also, trials with other herbicides such as glyphosate at these phenological times would expand management options.

In terms of which imazapyr application is best, results suggest that only one application is necessary. All imazapyr treatments showed significant damage results one year after treatment. Damage ratings were higher in MF and MFS treatments in comparison to solely S treatments at the Antigonish site. Similarly, S treatments were taller than MFS at both sites, as well as FS at the Bible Hill site. Although all imazapyr treatments were quite similar, Senescence (S) was the least effective. These results conflict with the hypothesis that this timing would be an optimal time for the translocation of herbicide to the rhizome (Price et al. 2002). It is recommended that imazapyr alone is applied to knotweed stands when the plant has reached Maximum growth (M) in mid June or at Flowering (F) in August.

Aminopyralid treatments yielded excellent results for knotweed management at the end of the growing year in which the plots were sprayed. However, subsequent assessment the following year indicated that the damage was not long-lasting. In an effort to reduce time and costs associated with knotweed management, a minimal number of herbicide applications are desired. This experiment indicated that only one imazapyr application after the plant has reached full height is required for effective growth suppression. Further monitoring and more refined herbicide trials will demonstrate the effectiveness of this recommendation over a longer period and at various locations.

4.5 References

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Chapter 5: Conclusion

As this paper has discussed, there are many aspects to consider when challenged with an introduced species. It is important to gather information pertaining to the plants historical invasiveness, climatic thresholds, distribution, biological attributes, and beneficial and detrimental impacts, among other characteristics. A non-native species that is perceived to become invasive must be dealt with accordingly with a rapid response to avoid establishment. Management options differ according to species, environment, and human factors. Specific knowledge of invasive weeds is paramount when considering management plans.

This research project has answered several questions regarding Japanese knotweed in Nova Scotia in terms of its biology, ecological impacts, and management options. With this knowledge it is possible to make informed decisions regarding how humans choose to deal with knotweed and other similar organisms. Recommendations can be proposed in accordance with tested hypotheses and statistical analyses of data. Finally, additional research projects on similar topics can be better defined after thoughtful consideration of what problems need to be solved.

Several biological characteristics about Japanese knotweed are now more apparent because of this project, in conjunction with the results of other studies. Data collected from Antigonish and Bible Hill knotweed populations indicated that the plant typically reached an average height of about 210 cm in mid to late June, and the canopy actually decreased in height to about 190 cm due to the weight of the mature leaves and branches. The shoots emerged in early spring (late April) and were recorded to grow on average over 170 cm in height over 30 days. The dense monospecific knotweed stands had an average of about 17 stems per m^2, and an aboveground fresh biomass of approximately 8.0 kg per m^2.

The thick vegetative structure may actually create a favourable environment for invertebrate species, birds, and small mammals. Although plant diversity and species richness values were next to nothing, the amount of leaf cover exceeds that of native shrub and grass habitats in riparian ecosystems. There was no significant difference of invertebrate diversity, species richness, or abundance between habitats, suggesting that a knotweed patch may provide habitat and/or food for invertebrates although limited data may have been an issue. There were more small mammal tracks recorded in knotweed stands than shrub, but less than grass habitats. In particular, insectivorous shrew (*Sorex* species) tracks were more commonly recorded in grass and knotweed habitats versus shrub patches. Several bird species were also observed nesting in and visiting knotweed stands. Data suggest that as canopy cover increases within knotweed patches, the abundance of small mammal tracks also increase at a greater rate than in other habitats. Considering the lack of plant diversity, these results completely oppose the hypothesized lack of other organism abundance and diversity. Perhaps knotweed patches are not as harmful to the ecosystem as previously suggested. Conversely an entire landscape dominated by knotweed plants would be a large cause for concern, and likely necessitate a management plan.

Herbicides are the most practical method for large-scale knotweed management. Backpack sprayers are inexpensive, portable, and allow for more directed application. Two herbicides were evaluated in various combinations of dates of application. Aminopyralid yielded promising results within days of application, but the knotweed plants were able to survive beyond the initial treatment and were not significantly different from the control in the following growing season in terms of density and leaf cover. While imazapyr-treated plots were slower to show measurable herbicide damage, all imazapyr treatments were significantly damaged one year after treatment. The best herbicide treatment was an application of imazapyr at a rate of 720 g ae ha^{-1} when knotweed was at its maximum height (mid June), or when it was flowering in August. Additional research on herbicide use can refine this method with an appropriate spray volume, tank mixes, and other application methods such as stem injection.

This project also mapped a baseline knotweed population over three years in Antigonish and two years in Bible Hill. Supplementary mapping will reveal trends of patch spread over time, as a predictor of population expansion. A preliminary map of knotweed locations in the province indicates how widespread the plant is, and no climates seem too harsh for its growth in Nova Scotia. More detailed data collection of these plant and animal variables can show how communities may change over time with a plant invasion. A minor seed viability test revealed that this population did not have viable seeds, which would have dramatic management implications. A psyllid is being considered as a biocontrol agent with Agriculture and Agri-Food Canada in Lethbridge, AB and these sites would be excellent potential release points. Alternative management options such as covering with tarps, mowing, and wire grid could also be further studied. Another important application of this research project is linking it with other invasive species to create a broader-scaled plan for invaded sites. Himalayan balsam (*Impatiens glandulifera*) is a potentially serious invasive plant in Antigonish along with others that could be monitored and further evaluated. Finally, this research should be used for educational purposes to highlight the significance of invasive weeds on natural ecosystems among other valuable messages.

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Appendix A – Plant species richness from three riparian habitats in Antigonish and Bible Hill, NS in 2011 and 2012.

		2011						2012					
		Antigonish			Bible Hill			Antigonish			Bible Hill		
Species name	**Common name**	Knotweed	Shrub	Grass	Knotweed	Shrub	Grass	Knotweed	Shrub	Grass	Knotweed	Shrub	Grass
Acer spp.	maple		2[a]			0			2			0	
Achillea millefolium	yarrow*			4									
Alnus spp.	alder		25										
Alopecurus spp.	foxtail*			240			230			46			320
Artemisia spp.	wormwood*												0
Aster novae-angliae	New England aster						2						
Avena fatua	wild oats*												82
Barbarea vulgaris	yellow rocket*									0			
Centaurea spp.	knapweed*			76						2			
Convolvulus arvensis	field bindweed*			8	0		14			8		0	8
Cornus spp.	dogwood	0	6						9				
Crataegus spp.	hawthorn**		0			5			0			6	
Cyperus esculentus	yellow nutsedge*		1						1				
Echinocystis lobata	wild cucumber	0	0	2			2	0	5	6			2
Epilobium spp.	willow herb**			0									
Equisetum spp.	horsetail		2	4					1	56			
Galeopsis tetrahit	hemp nettle*		5	2		0	14		2	110		3	38
Galium spp.	bedstraw**			80			16			46			10
Glechoma hederacea	ground ivy*			2		1	70						
Helianthus tuberosus	Jerusalem artichoke						108						148
Heracleum maximum	cow parsnip		1			7	74		0	0		31	112
Hesperis matronalis	dame's rocket*	0	95	74		0	62	0	93	66		26	18
Impatiens capensis	jewelweed		68	52	0	132		0	131	172	0	187	2
Lactuca biennis	blue lettuce						0						
Lotus corniculatus	birdsfoot trefoil*												0
Lysimachia ciliata	fringed loosestrife*			0						0			
Lysimachia nummularia	moneywort*		158			93	24		250	0			40
Lythrum salicaria	purple loosestrife*												0
Pastinaca sativa	wild parsnip*			14				0		10			
Phalaris arundinacea	reed canary grass**			268			218			108			524
Phlox spp.	phlox*					0	0						
Plantago spp.	plantain*			32						0			
Polygonum cuspidatum	Japanese knotweed*	69			46			85			89		
Prunus spp.	cherry		19			3			49			6	
Pteridium aquilinum	bracken fern		1										
Rosa spp.	rose**		1	10			4		0	8			0
Rubus spp.	blackberry			54					0	78			
Rumex crispus	curled dock*						0						0
Salix spp.	willow		0										
Sanguinaria canadensis	bloodroot		8						15				

		2011						**2012**					
		Antigonish			**Bible Hill**			**Antigonish**			**Bible Hill**		
Species name	**Common name**	Knotweed	Shrub	Grass	Knotweed	Shrub	Grass	Knotweed	Shrub	Grass	Knotweed	Shrub	Grass
Saponaria officinalis	soapwort*												28
Satureja vulgaris	wild basil**			0			0						
Sedum purpureum	live forever*											3	
Silene vulgaris	bladder campion*			0						0			
Solanum dulcamara	bittersweet nightshade*					0							
Solidago spp	goldenrod	0	0	248		11	26			256			54
Stellaria graminea	grass-leaved stitchwort*			0					4	18			
Tanacetum vulgare	tansy*						28						0
Taraxacum officinale	dandelion*						8						14
Trifolium spp.	clover*			2						0			0
Vicia spp.	vetch*			28			10			28			4
Zizia aurea	golden alexanders											49	
*=introduced (28)	**N = 6338 plants**	**69**	**392**	**1200**	**46**	**252**	**910**	**85**	**562**	**1018**	**89**	**311**	**1404**
**=possibly introduced (6)	**S = 52 species**	**5**	**18**	**24**	**3**	**12**	**21**	**5**	**16**	**23**	**2**	**10**	**23**

[a] Values indicate total number of plant species per 1 m^2 quadrat within each habitat. Species that were identified within the larger 12.5 m^2 plot are indicated with a 0.

Appendix B – Invertebrate species richness from pitfall traps in three riparian habitats in Bible Hill, NS in July 2012.

Taxa	Total	Knotweed	Shrub	Grass
Annelida				
Oligochaeta				
Haplotaxida				
worm	18	12	2	4
Chelicerata				
Arachnida				
Aranea				
1 spider (med, grey)	19	3	4	12
2 spider (small)	14	2	1	11
Opiliones				
1 harvestmen (brown)	99	34	29	36
2 harvestmen (hourglass)	24	6	10	8
Crustacea				
Malacostraca				
Isopoda				
woodlouse	22	3	11	8
Arthropoda				
Diplopoda				
millipede	33	10	18	5
Chilopoda				
centipede	18	5	2	11
Insecta				
Dermaptera				
earwig	1		1	

Taxa	Total	Knotweed	Shrub	Grass
Arthropoda				
Insecta				
Diptera				
1 orange	59	16	19	24
2 med black	16	4	8	4
3 small	6	1	4	1
4 big	1	1		
5 small black	7	1	5	1
6 mosquito-like	10	3	2	5
7 tiny	5	3		2
8 yellow	1		1	
9 iridescent abdomen	5	3	1	1
10 spot	2	2		
11 sucker face	13		2	11
12 belly	2			2
13 big, iridescent	1			1
14 smaller D2	2			2
15 black, large abdomen	1			1
16 wing	1			1
17 tongue	1			1
18 tail	2		1	1
19 fat	2		1	1
20 midge	2	2		
Syrphidae				
21 Volucella bombylans	1			1
Tipulidae				
22 crane fly	4	2	2	

Taxa	Total	Knotweed	Shrub	Grass
Coleoptera				
Carabidae				
1 Pterostichus melanaricus	109	23	44	42
2 Harpalus rufipes	38	19	7	12
3 small, shiny	153	22	11	120
4 med beetle	9	2		7
5 Chlaenius tricolor	1		1	
6 beetle	3		2	1
7 beetle	4	2	2	
8 Carabus nemoralis	4	2	2	
9 mini C1	7	7		
10 Agonum sp.	4	3		1
*11 Pterostichus lama**	3	2		1
12 square carapace, irid	1			1
13 oval cara, irid	16			16
14 rectangle cara, irid	4			4
15 purple beetle	1	1		
16 tiny beetle	2	2		
17 clear legs, irid	1	1		
18 big thigh	5			5
19 Carabus meander	1		1	
Coleoptera				
Curculionidae				
1 weevil	6	2	4	
2 med weevil	4	1	1	2
3 small weevil	1		1	
4 brown weevil	4		4	
5 med weevil	1		1	
Elateridae				
click beetle	1		1	

Taxa	Total	Knotweed	Shrub	Grass
Hemiptera				
Cicadoidea				
1 hopper	42	10	12	20
2 small gray hopper	16		1	15
3 black hopper	12	3	1	8
Miridae				
Poecilocapsus lineatus	2			2
Nabidae				
damselbug nymph	1			1
Hymenoptera				
Symphyta				
sawfly	1		1	
Formicidae				
1 black ant	193	56	24	113
2 orange ant	32	10		22
3 big ant	1			1
4 winged ant	1	1		
Apocrita				
1 big wasp	1			1
2 small wasp	1			1
Orthoptera				
Rhaphinidophoridae				
Ceothophilus maculates	5		5	
?				
unknown larva	6	3	2	1
Mollusca				
Gastropoda				
Basommatophora				
slug	77	17	42	18

Taxa	Total	Knotweed	Shrub	Grass
Coccinelidae				
ladybird larvae	1		1	
Scarabacidae				
Phyllophaga fusca (June bug)	1	1		
Staphylinidae				
1 rove beetle (brown)	14	2	4	8
2 rove beetle (black)	19	9	4	6
3 rove beetle (long)	3	1	2	
Lepidoptera				
larva	3		1	2
Neuroptera				
larva	10	9	1	
Gastropoda				
Stylommatophora				
snail	17	5	3	9
Chordata				
Amphibia				
Anura				
Bufo americanus	26	7	5	14
Mammalia				
Soricomorpha				
Sorex sp.				
shrew	5		1	4
Total (N)	**1265**	**336**	**316**	**613**
Species number (S)	**81**	**47**	**51**	**55**

Appendix C – Number of bird sightings in three riparian habitats from tree stands in Antigonish and Bible Hill, NS over four and six respective one-hour periods in June 2012.

	Antigonish			**Bible Hill**		
Species	**Knotweed**	**Grassland**	**Shrub**	**Knotweed**	**Grassland**	**Shrub**
Alder Flycatcher	-	-	-	-	-	2
Black-capped Chickadee	-	-	-	-	-	2
European Starling**	-	-	-	2	2	-
Cedar Waxwing	21*	16	-	-	-	-
Yellow Warbler	11	16	12	6	30	21
Yellow-rumped Warbler	3	2	11	1	1	4
American Redstart	-	-	4	-	-	-
Common Yellowthroat	-	-	-	-	-	2
White-throated Sparrow	1	5*	2	-	-	-
Song Sparrow	1	11	4	10	24	14
Dark-eyed Junco	-	-	-	6*	-	-
Red-winged Blackbird	1	1	2	-	-	-
American Goldfinch	2	-	2	-	-	-
Total	**40**	**41**	**37**	**28**	**58**	**50**

* Nest observed in indicated habitat
** Introduced species

Other bird species observed at this site, but not associated with any habitat:

Antigonish	Bible Hill
Double-crested Cormorant	American Black Duck
American Black Duck	Common Merganser
Bald Eagle	Bald Eagle
Osprey	Ring-necked Pheasant**
Ring-billed Gull	Killdeer
Mourning Dove	Mourning Dove
Rock Dove**	Chimney Swift (rare)
American Crow	American Crow
Black-capped Chickadee	Tree Swallow
European Starling**	Cedar Waxwing
Common Grackle	Common Grackle
Purple Finch	Purple Finch

** Introduced species

Appendix D – Abundance of small mammal tracks from tracking tubes in three riparian habitats at Antigonish and Bible Hill, NS over one week in 2011 and 2012.

			2011						**2012**					
			Antigonish			**Bible Hill**			**Antigonish**			**Bible Hill**		
Species name	Common name	**Total**	Knotweed	Shrub	Grass	Knotweed	Shrub	Grass	Knotweed	Shrub	Grass	Knotweed	Shrub	Grass
Sorex spp.	Shrew	238	8	3	36	4	0	15	30	13	56	36	14	23
Blarina brevicauda pallida	Short-tailed shrew	73	12	0	5	5	1	7	22	4	14	0	0	3
Peromyscus maniculatus abietorum	Deer mouse	99	0	4	1	2	0	1	41	20	11	2	7	10
Microtus/ Clethrionomys/ Synaptomys spp.	Vole	68	1	0	20	0	3	1	3	0	40	0	0	0
Zapus hudsonicus acadicus	Meadow jumping mouse	5	0	0	0	1	1	1	0	0	0	0	0	2
Unknown	Unknown	6	2	0	3	0	0	1	0	0	0	0	0	0
	Total	**489**	**23**	**7**	**65**	**12**	**5**	**26**	**96**	**37**	**121**	**38**	**21**	**38**