AN ABSTRACT OF THE THESIS OF

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Japanese knotweed is an invasive perennial shrub that dominates riparian ecosystems. Effective management techniques are currently limited to repeated annual herbicide applications and there is little science-based information about which control tactics result in the greatest management success. Restoration of invaded sites to a functioning riparian plant community is needed to prevent re-infestation of Japanese knotweed or other invasive weed species. Field and greenhouse experiments were initiated in fall of 2007 to generate this information on Japanese knotweed management. A greenhouse experiment was conducted to evaluate the efficacy of the experimental herbicide aminocyclopyrachlor methyl ester for Japanese knotweed control. Treated plants were unable to produce new shoots from underground rhizomes indicating that aminocyclopyrachlor is an effective control. This level of Japanese knotweed suppression is comparable to imazapyr and surpasses the level of control that glyphosate provided under greenhouse conditions. A field experiment was initiated in the Nehalem River watershed in western Oregon to evaluate the integration of chemical weed management with restoration of the site to a diverse native grass plant community. We documented that native grasses could be established at this site, but the long-term survival of these

grasses was poor. This result indicates that methods for simultaneous chemical control of invasive knotweed and restoration of sites need to be investigated further. The most effective chemical treatment in terms of visual injury and reduced Japanese knotweed biomass and cost of application was glyphosate applied at a rate of 4.21 kg ae/ha for a cost of $160/ha. A secondary experiment evaluating herbicide tank mixtures of glyphosate with imazapyr, imazapyr with aminopyralid, and triclopyr with 2,4-D was conducted at the same site. The most effective chemical treatment in terms of Japanese knotweed control and cost of application for the tank mixture experiment was imazapyr with aminopyralid applied at rates of 1.12 kg ae/ha (imazapyr) and 0.12 kg ae/ha (aminopyralid) for a cost of $346/ha.

Integrating Restoration and Ecologically Based Weed Management Practices for Invasive Knotweed Control

by
Melody Rudenko

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APPROVED:

Major Professor, representing Crop Science

Head of the Department of Crop and Soil Science

Dean of the Graduate School

I understand that my thesis will become part of the permanent collection of Oregon State University libraries. My signature below authorizes release of my thesis to any reader upon request.

Melody Rudenko, Author

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CONTRIBUTION OF AUTHORS

Dr. Andrew Hulting provided guidance and supervision of this research and editoral assistance throughout all phases of this thesis. Dr. Alejandro Perez-Jones provided training and assistance in conducting the greenhouse herbicide screening trial. Glenn Miller from the Oregon Department of Agriculture facilitated the use of the Nehalem River field research site and offered guidance on herbicide products and spraying equipment.

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1. INTRODUCTION

1.1 Taxonomy

Japanese knotweed (*Fallopia japonica*) is a widely distributed invasive weed in the Polygonaceae family. Japanese knotweed is native to Asia and inhabits a wide variety of environments throughout Europe and North America. This plant has been classified as a member of three genera including *Reynoutria*, *Polygonum*, and *Fallopia* in the past (Doll and Doll, 2006). Though all three classifications are still recognized, *Fallopia japonica* is currently the accepted classification (Flora of North America, 2005). Common names include Japanese knotweed, Mexican bamboo, Japanese bamboo, and Japanese fleece-flower (Forman, 2004).

1.2 Biology

Invasive knotweeds are rhizomatous perennials with shrub-like herbaceous growth that dies back yearly in cold climates. This plant functional type is usually not represented in the native vegetation of most invaded areas (Dassonville *et al.,* 2007). The Oregon Department of Agriculture (ODA) classifies Japanese knotweed and its invasive relatives giant knotweed *(Fallopia sachalinense)* and Bohemian knotweed *(Fallopia bohemicum)* as class B invasive weeds (ODA, 2007). A Class B weed is defined as a weed of economic importance which is regionally abundant, but which may have limited distribution in some counties. Invasive knotweed species are commonly found in riparian areas, coastal habitats, roadsides, and unmanaged areas in the Pacific Northwest (PNW) (ODA, 2007). These species can survive in a variety of soil types, but especially thrive in moist, well-drained, nutrient rich soils (Doll and Doll, 2006). The particular soil types that invasive knotweed most frequently invades in Oregon are unknown.

The invasive Japanese knotweed biotype differs greatly from the native "wild-type." Japanese knotweed functions as a primary successional pioneer plant in barren volcanic slopes in its native habitat in eastern Asia. Intensive study of Japanese knotweed has taken place on Mt. Fuji in a post eruption volcanic desert environment. The last volcanic eruption on Mt. Fuji occurred in 1707 and Japanese knotweed has been a dominant plant in the recolonization of the area, often forming monoclonal stands (Zhou et al., 2003). Patches are believed to be established by seed dispersal rather than rhizome extension or relocation, which is commonly thought to be an important form of dispersal for invasive biotypes. These native Japanese knotweed patches eventually die-back in the center and facilitate establishment of later successional species (Adachi *et al.,* 1996). Conversely, the invasive biotype of Japanese knotweed appears to spread by rhizome extension and relocation and inhibits the succession of other species, potentially by the alteration of the soil properties to favor its own self-replacement (Dassonville *et al.,* 2007).

It is widely believed that all Japanese knotweed plants found throughout the United Kingdom, Europe, and the United States (U.S.) are clones of the same octoploid ($2n=88$) female plant that is sometimes referred to as the British clone (Hollingsworth and Bailey, 2000). It is also commomly believed that sexual reproduction resulting in viable seeds only occurred by hybridization with related species (Mandak *et al.,* 2005). Recent studies have documented the opposite to be true in Japanese knotweed populations found throughout the U.S. Male Japanese knotweed plants are commonly present in established populations and can pollinate female plants to produce viable seeds that are able to germinate and mature (Foreman and Kesseli, 2003). Many infestations of Japanese knotweed in the eastern U.S. are genetically diverse with some populations containing both the British clone biotype and sexually derived offspring (Grimsby *et al*., 2007) while other populations consist of sexually derived genetically dissimilar plants (Wymer *et al.,* 2007). The presence of these dissimilar populations likely indicates that the introduction of the British clone from invaded areas in Europe has occurred along with direct introductions of male Japanese knotweed plants from Asia (Grimsby *et al*., 2007). This is possibly due to horticultural trade of this species.

Japanese knotweed frequently hybridizes with related species in its non-native range. The most common hybridization occurs with tetraploid ($2n$=44) giant knotweed, *Fallopia sachalinensis,* to produce Bohemian knotweed, *Fallopia bohemica* (Bailey and Wisskirchen, 2006). Ploidy levels for Bohemian knotweed vary in the Czech Republic from tetraploid ($2n$=44) to octoploid ($2n$=88) (Tiebre *et al.,* 2007). This hybrid species has proven to be more invasive than either of its parent species and exhibited twice the rate of invasion of either parent species in the Czech Republic (Mandak *et al.,* 2004).

Japanese knotweed can be difficult to distinguish from the hybrid Bohemian knotweed and closely related giant knotweed. Due to this difficulty, the distribution of these species in Oregon is reported and mapped together. Invasive knotweed species are found in all Oregon counties west of the Cascades and 50% of the counties east of the Cascades (ODA, 2007). All three species have broad heart-shaped leaves and stout, cane-like stems that are hollow between the nodes. Though it is difficult to visually differentiate between the three species, reports from Europe indicate that the morphological characteristic of hairs underneath the leaves along the mid-vein can be used for positive identification. Japanese knotweed may have very small hairs, Bohemian knotweed short and broad hairs, and giant knotweed long hairs in this location on the underside of the leaf (Child and Wade, 2000). Unfortunately, there is no defining morphological characteristic that can be used to distinguish Japanese knotweed from closely related hybrids in U.S. populations due to the genetic mixing of knotweed species that has made the genus taxonomically ambiguous (Gammon *et al.*, 2007). Therefore genetic analysis to determine both species identification and relatedness of plants is necessary due to the unique genetic diversity of populations of knotweed species in the U.S. There is currently a genetic analysis study underway for the Western U.S. and British Columbia (Personal Communication, Dr. Fritzi Grevstad).

1.3 Impacts

Invasive knotweed species are difficult to control invasive plants that have detrimental effects on riparian ecosystems and natural areas. Infestations are extremely difficult to manage and over time displace native vegetation, impede wildlife activity, and limit land use (Gerber *et al.,* 2008; Maerz *et al.,* 2005; Siemens and Blossey, 2007; Topp *et al.,* 2008). Native plant diversity and abundance diminishes when an ecosystem becomes dominated by invasive knotweed. Correlated with this change is a total reduction in invertebrate abundance, species richness, and total biomass (Gerber *et al.,* 2008). This reduction in arthropod abundance leads to infestations negatively impacting foraging success of native frogs and other insectivorous species (Maerz *et al.,* 2005). Invasive knotweed infestations negatively affect aquatic invertebrates and connected food-webs, which indirectly impacts native fish species due to the significant reduction of nitrogen in leaf litter inputs from invasive knotweed compared to inputs from native tree and shrub species common to PNW riparian areas (Urgenson *et al.,* 2009).

Invasive knotweed suppresses the growth and prevents the survival of native plant species by physically blocking access to sunlight, facilitating development of soil pathogens, and through allelopathic interference (Siemens and Blossey, 2007). In addition, knotweed species may also facilitate their own reestablishment by altering soil conditions through a positive feedback loop. For example, Japanese knotweed growing in Belgium increased available nutrients in topsoil, drawing them from deeper soil layers, resulting in 35-60% higher concentrations of the exchangeable nutrients Cu, K, Mg, Mn, P, and Zn. These Japanese knotweed populations affected a convergence of soil characteristics at all sites sampled in the study population having a homogenizing effect on the soil properties, thus creating a soil environment that favored continued domination of Japanese knotweed over native species (Dassonville et al., 2007).

1.4 Management

In addition to the detrimental characteristics that make knotweed species dominating invasive plants in many ecosystems, these species are also very resilient to current management methods. Currently, chemical control is the only practical management tactic used by most weed management personnel, though even these methods are problematic. Mechanical control of invasive knotweed is often impractical and ineffective do to the difficulty in removing the extensive below ground rhizome biomass that can weigh from 12 to 35 metric tons per hectare (Doll and Doll, 2006).

Cutting and digging of these species usually serves to spread invasive knotweed rather than suppress it because of its ability to regenerate from rhizome fragments as small as 7 g in fresh weight (Doll and Doll, 2006). Dispersal of shoot fragments is also a dispersal mechanism of this species. De Waal (2001) demonstrated that placing 40-mm-long stem fragments into moist soil resulted in the regeneration of new shoots and roots at stem nodes.

Scientists from the ODA, the United States Department of Agriculture (USDA), and from universities across the U.S. are currently investigating biological control possibilities for invasive knotweed. Multiple bio-control agents are being tested for host specificity and are not yet ready to be released in the U.S. (Coombs *et al.*, 2004). The host specific psyllid, *Aphalara itadori*, has shown high fidelity to Japanese knotweed in European tests and has been recommended for release in the United Kingdom (Shaw *et al.*, 2009).

Chemical control of invasive knotweeds is currently the most effective and widely used management practice. However, chemical control of these species is problematic for many reasons including difficultly in timing herbicide applications for maximum control, lack of information on most effective active ingredients for control, tolerance to herbicide active ingredients, the need for repeated annual herbicide applications and because invasive knotweed will establish in sensitive habitats such as riparian areas where the use of some herbicides may be restricted. Herbicides labeled for aquatic use must be used for treatment of invasive knotweed populations adjacent to water. The most common herbicide active ingredient used to date has been foliar applications of glyphosate (Rodeo®, Aquamaster®). More recently an aquatic label for imazapyr has been developed (Habitat®). As this new product became an option for invasive knotweed control, imazapyr has been used at varying rates and has been tank mixed with glyphosate for effective control. The herbicide active ingredient aminopyralid (Milestone®) is not yet widely used, but many managers are interested in its incorporation into chemical management methods. The new herbicide active ingredient aminocyclopyrachlor will soon be labeled for broadleaf weed control in a variety of sites and may also prove to be a valuable addition to the short list of herbicides useful for invasive knotweed control.

Another common method of chemical control of invasive knotweed species is injection of glyphosate into the hollow canes of plants. This method can be effective, but is not without risk. Due to the density of canes in a large patch or population and the amount of herbicide concentrate recommended for injection into each individual cane, the maximum labeled rate per area of glyphosate is easily surpassed without treatment of the entire patch. This leads to incomplete control of invasive knotweed and illegal off-label applications of glyphosate products (Hagen and Dunwiddie, 2008).

Although extensive chemical management of knotweed species is occurring throughout the PNW there is little science-based information about which herbicide application methods and active ingredients will result in the greatest management success (Soll *et al*., 2006). Meta-analysis of multiple control projects from the US and Europe has shown that while treatment of infestations with glyphosate and/or imazapyr will produce a significant decrease in invasive knotweed abundance, these methods will not result in eradication in the short-term (Kabat *et al.,* 2006).

In addition, there is little information available on successful restoration techniques for chemically treated sites. Due to the difficulty in eradicating infestations and necessity to repeat annual treatment, replanting of native species frequently does not occur. However, vegetation surrounding treated sites quickly re-colonizes many areas (Miller, 2005, Ford, 2004). While this can result in desirable natives being restored to the site (Ford, 2004), the possibility for undesirable plant species to re-infest the area is also a concern.

Information regarding the management of invasive knotweeds is improving worldwide, but there is still relatively little known about these species to allow for effective management of infestations in the PNW. Because of the dynamic nature of the mechanisms of spread and establishment management methods need to be combined with restoration methods in order to improve plant community resilience to future invasion. In addition, a greater understanding of the complex reproduction and genetic diversity of invasive knotweed species in the U.S. is needed to improve management and prevent the continuing spread of this species.

A series of experiments were initiated in the fall of 2007 to address our hypotheses. We hypothesized that we could establish a grass community in a invasive knotweed infested area that was undergoing herbicide treatment to reduce weed biomass, and that grass community could be managed as a stepping stone community which could later be restored to mixed tree and shrub species. A multi-year field study that examined replanting native grasses on sites treated with herbicides was conducted in a Japanese knotweed infestation on the coastal Nehalem River near Garabaldi, Oregon. In this study, the efficacy of both restoration methods and herbicide control was quantified. Additionally we hypothesized that the experimental herbicide Aminocyclopyrachlor would have measurable activity on invasive knotweed. A greenhouse experiment was conducted to examine the potential of this new herbicide active ingredient for invasive knotweed control.

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2. GREENHOUSE EFFICACY INVESTIGATIONS USING THE EXPERIMENTAL HERBICIDE DPX-MAT28 (AMINOCYCLOPYRACHLOR METHYL ESTER) TO CONTROL JAPANESE KNOTWEED

2.1 ABSTRACT

Japanese knotweed is an invasive perennial shrub that dominates riparian ecosystems. Currently there are a limited number of active ingredients being used for chemical control of this species. A greenhouse experiment was conducted to evaluate the efficacy of the experimental herbicide aminocyclopyrachlor methyl ester (DPM-MAT28) for Japanese knotweed control. Five rates of aminocyclopyrachlor (0.035 kg ai/ha, 0.07 kg ai/ha, 0.14 kg ai/ha, 0.28 kg ai/ha, 0.56 kg ai/ha) and industry standard treatments of imazapyr (0.84 kg ae/ha) and glyphosate (3.37 kg ae/ha) were applied to Japanese knotweed plants. Plants were approximately 0.5 m tall and had grown for 11 wk prior to herbicide treatments. The highest rate of aminocyclopyrachlor caused the greatest level of control 63 days after treatment (DAT) compared to other treatments. Japanese knotweed dry weight was reduced by aminocyclopyrachlor applied at 0.56 kg ai/ha and treated plants had biomass equivalent to only 48% of the control plants. Biomass of regrowth after clipping the treated plants for all the aminocyclopyrachlor treatments was zero. This lack of Japanese knotweed regrowth indicates that while aminocyclopyrachlor did not initially appear to be more effective at low application rates than current standard herbicide treatments, it may be significantly more effective than glyphosate or imazapyr for controlling regrowth after treatment.

2.2 Introduction

Japanese knotweed (*Fallopia japonica*) is an invasive perennial shrub that can dominate riparian ecosystems. After invading a site, this species negatively impacts the native plant assemblages, invertebrate and vertebrate species, and alters the soil environment to favor continued colonization and persistence of itself rather than other native species (Gerber, 2008, Maerz, 2005, Dassonville, 2007).

In addition to its detrimental characteristics, Japanese knotweed is also very tolerant of current management methods. Currently, chemical control is the only practical management tactic used by most weed management personnel. However, chemical control of Japanese knotweed is problematic for many reasons including difficultly in timing herbicide applications for maximum control, lack of information on most effective active ingredients for control, tolerance to herbicide active ingredients, the need for repeated annual herbicide applications and because Japanese knotweed establishes in sensitive habitats adjacent to natural or protected areas limiting herbicide options.

Mechanical control of Japanese knotweed is often impractical and ineffective due to the difficulty in removing the extensive below ground rhizome biomass that can weigh from 12 to 35 tons per hectare (Doll and Doll, 2006). Cutting and digging usually serves to spread Japanese knotweed rather than suppress it because of its ability to regenerate from rhizome fragments as small as 7 g in fresh weight (Doll and Doll, 2006). Dispersal of shoot fragments is also a dispersal mechanism of this species. De Waal (2001) demonstrated that placing 40-mm-long stem fragments into moist soil resulted in the regeneration of new shoots and roots at stem nodes.

Scientists from the Oregon Department of Agriculture (ODA), the United States Department of Agriculture (USDA), and from universities across the U.S. are currently investigating biological control possibilities for invasive knotweed. Multiple bio-control agents are being tested for host specificity and are not yet ready to be released in the U.S.

(Coombs *et al*., 2004). The host specific psyllid, *Aphalara itadori*, has shown high fidelity to Japanese knotweed in European tests and has been recommended for release in the United Kingdom (Shaw *et al.*, 2009).

Treatment of invasive knotweed populations adjacent to water requires aquatically labeled herbicides to reduce environmental impacts. The most commonly used herbicide active ingredients are glyphosate (Rodeo®, Aquamaster®) and imazapyr (Habitat®) These products have been used alone at varying rates and have been tank mixed and used for invasive knotweed control, though combinations of active ingredients appear to frequently result in levels of control equivalent to using either active ingredient alone (Miller, 2005). The herbicide active ingredient aminopyralid (Milestone®) is not yet widely used, but many managers are interested in its incorporation into chemical management methods. The new herbicide active ingredient aminocyclopyrachlor will soon be labeled for broadleaf weed control in a variety of sites and may also prove to be a valuable addition to the short list of herbicides useful for invasive knotweed control.

Although extensive chemical management of invasive knotweed is occurring throughout the Pacific Northwest, there is little science-based information about which herbicide application methods and active ingredients will result in the greatest management success (Soll *et al*., 2007). While glyphosate and imazapyr are considered effective when applied to invasive knotweed in the fall, repeated treatment is often necessary. Sites are often subject to re-infestation with new invasive knotweed clones and other invasive weeds if repeated treatments are not conducted.

The experimental herbicide DPX-MAT28 (aminocyclopyrachlor methyl ester) has been tested on many other agricultural and invasive weeds (Personal Communication, Norman McKinley). The results from these experiments have indicated aminocyclopyrachlor may be effective for the control of many broadleaf weed species. Aminocyclopyrachlor is the only herbicide in the new chemical family pyrimidine carboxylic acids. This product is mobile in both the xylem and phloem of plants, has an auxin-type mode of action, and has the potential to control invasive knotweed by interfering with plant hormones effecting root and shoot development. Additionally, aminocyclopyrachlor has been shown to have a favorable environmental safety profile and low freshwater fish toxicity (trout LC_{50} >122 mg ae/L, bluegill LC_{50} >120 mg ae/L) (DuPont, 2009). Because of these characteristics aminocyclopyrachlor may be a useful herbicide for invasive weed management in sensitive environments such as riparian areas. This herbicide is expected to be available for public use in 2010 and will be labeled as part of four different product blends. Aminocyclopyrachlor will be blended with sulfometuron for bareground applications, with chlorsulfuron for selective broadleaf control, with metsulfuron alone and with metsulfuron plus imazapyr for two brush management products.

Development of management methods that are effective over a shorter period of time and identification of active ingredients that will cause less injury to surrounding desirable vegetation will be of great benefit to those managing invaded ecosystems. A greenhouse evaluation of new herbicides can be a useful first step in finding new chemical treatment options for invasive knotweed. The objective of this greenhouse study was to evaluate the efficacy of aminocyclopyrachlor for invasive knotweed control. Based on other study results we hypothesized that this compound would have measurable activity on invasive knotweed.

2.3 Materials and Methods

Greenhouse experiments were conducted at the Oregon State University Crop and Soil Science departmental greenhouses using clones propagated from plant cuttings. Rhizomes were hand pulled or dug from the surface soil of Japanese knotweed populations in the Luckiamute Watershed (southern Polk county, Oregon) in the fall of 2007. The harvested rhizomes were divided and planted in potting soil in the greenhouse to be used as a source of cutting materials for the propagation of clones to be used in the herbicide efficacy trial.

Clones were used in greenhouse experiments due to the unavailability of Japanese knotweed seeds that could be used for plant propagation. The use of rhizomes to produce the plants used in this trial would have lead to plants of less consistent above and belowground biomass. Though the use of shoot cuttings to produce experimental plants resulted in reduced genetic variation in experimental groups this experimental population appropriately represents a model of the clonal Japanese knotweed populations targeted for herbicide treatment in the field.

Clones were propagated from young Japanese knotweed shoot cuttings. The diameter of cuttings used for propagation was under 1 cm and their length was approximately 16 cm. Each cutting included at least one stem node and one leaf. The basal end of the cutting was dipped in rooting powder (Rootone® containing: 0.20% 1-

Naphthaleneacetamide, 4.04% Thiram [tetramethylthiuramdisulfide] and 95.76% inert ingredients) and planted into moist Sunshine® Professional Growing Mix potting soil. Cuttings were transplanted to 2.8L containers after 6 wk of growth. Plants were approximately 0.5 m tall and had grown for 11 wk prior to herbicide treatments.

Greenhouse temperature was maintained at an average nighttime temperature of 18.4°C and an average daytime temperature of 20.1°C and day length was extended to 14 hours through the use of supplemental lighting. Plants were watered and fertilized as needed, typically watered three times per week and fertilized once per month with Miracle Gro® all-purpose plant food (15-30-15 with micronutrients).

The herbicide evaluation consisted of seven treatment groups and an untreated control. The treatments included five rates of aminocyclopyrachlor (0.25x, 0.5x, 1x, 2x, 4x; 1x rate=0.14 kg ai/ha) plus 0.25% v/v non-ionic surfactant, and standard treatments of imazapyr and glyphosate for comparison to current recommended treatment practices (1x rates were 0.84 kg ae/ha for imazapyr and 3.37 kg ae/ha for glyphosate, plus 1% v/v crop oil concentrate). An experimental unit consisted of three plants and each treatment was replicated three times.

Three separate experiments were conducted that each included control plants and plants treated with the standard rates of imazapyr and glyphosate and combinations of aminocyclopyrachlor rates. Experiment A was initiated on August 8, 2008, and included aminocyclopyrachlor at the 0.5x, 1x, 2x rates. Experiment B was initiated on November 25, 2008, and included aminocyclopyrachlor at the 0.25x, 1x, 4x rates. Experiment C was initiated on June 25, 2009, and included aminocyclopyrachlor at all five treatment rates (0.25x, 0.5x, 1x, 2x, 4x).

Data collection included visual evaluation of the percent control of Japanese knotweed (the percent of injury observed when compared to the control group) evaluated at 7, 21, and 63 days after treatment (DAT). Above ground biomass was clipped 70 DAT dried and weighed. The dry weight of each plant was converted to percent of control dry weight by dividing each measured value by the average control dry weight (percent of control dry weight = dry weight of plant/mean dry weight of control). This clipping and weighing process was repeated after regrowth of the treated plants had emerged 186 DAT.

Individual data points from experiments A, B and C were examined for statistical outliers. Two plants (one from experiment B and one from C) had been identified as having unusually high injury compared to others in treatment groups and the data points connected to those plants were found to have an unusual response value compared to other points in the treatment group based on a high residual value. However, all points had a low Cook's distance indicating that no individual point had significant influence on statistical outcomes (Ramsey and Shafer, 2002). Based on these results no outliers were detected.

A linear regression model was used to determine if experiments A and B could be combined. The model excluded the four treatments that were not common to both experiments, the aminocyclopyrachlor rates 0.25x, 0.5x, 2x, 4x. The model included both the herbicide treatment and experiment group as explanatory variables. To determine if the experiment group had a significant affect on the outcome of the experiment, the interaction effect of treatment by experiment was examined. The high p-value (a=0.05) for the majority of variables indicated the treatments resulted in the same effect regardless of experiment. Based on these results, data from experiments A and B were combined and 95% individual confidence intervals were calculated for the treatment means.

A linear regression model was used to determine if experiments A and C could be combined. The model excluded the two treatments that were not common to both experiments, aminocyclopyrachlor rates 0.25x, 4x. A similar model was used for experiments C and B that excluded the two treatments that were not common to both experiments, aminocyclopyrachlor rates 0.5x and 2x. The models included both the herbicide treatment and experiment group as explanatory variables. To determine if the experiment group had a significant effect on the outcome the interaction effect of treatment by experiment was examined. The very small p-values (a=0.05) for all variables indicate that it was not appropriate to eliminate experiment as an explanatory variable for comparing experiments A and C or experiments B and C (unlike experiments A and B). Based on these results, experiment C was analyzed separately from experiments A and B (which were combined). The reduced regression model was used for experiment C data using only treatment as an explanatory variable, and 95% individual confidence interval were calculated for the treatment means.

2.4 Results and Discussion

One week after treatment plants treated with imazapyr exhibited very little injury, and were equivalent to control plants. The imazapyr treatment exhibited the least injury 21 DAT, but by 63 DAT injury symptoms were equal to aminocyclopyrachlor used at the highest rate in experiments A and B (Table 2.1) and equal to glyphosate in experiment C (Table 2.2). The mean percent of control dry weight 70 DAT was moderately reduced by the imazapyr treatment (Table 2.3 and 2.4). At 186 DAT, the plants treated with imazapyr had no regrowth (after clipping at 70 DAT).

The glyphosate treatments exhibited different levels of injury in experiments A and B compared to experiment C. In experiments A and B, glyphosate treated plants exhibited slightly more injury than those treated with imazapyr 7 DAT, but were still equivalent to the control plants (mean observed injury 5.6%) (Table 2.1). However, in experiment C, glyphosate treated plants exhibited the highest levels of injury 7 DAT (mean observed injury 75.0%) (Table 2.2). Glyphosate treated plants had moderate amounts of injury at 21 DAT in experiments A and B (mean injury 19.2%) and the highest levels of injury in experiment C (mean injury 89.4%). At 63 DAT glyphosate treated plants had high levels of injury in all experiments, with experiment C still showing greater levels of injury than A and B (mean observed injury 84.4% compared to 45.3%). The mean percent of control dry weight 70 DAT was moderately reduced by the glyphosate treatment in experiments A and B (Table 2.3) and greatly reduced in experiment C (Table 2.4). Plants treated with glyphosate had the most regrowth after clipping when measured at 186 DAT, but the percent of control dry weight was still equivalent to zero (Table 2.3).

It is not known why the glyphosate treatments in experiment A and B had much lower observable injury than in experiment C but it could be related to the greenhouse conditions such as humidity and temperature and their impacts on plant physiology such as thickness of the plant cuticles. Though greenhouse conditions are regulated with artificial light, heat, and air conditioning to remain stable throughout the year, the differences may be connected to the time of year when each experiment was initiated. Experiment A began on August 8, 2008, Experiment B began on November 25, 2008, and experiment C began on June 25, 2009. The conditions of the plants and environment of the greenhouse may have been ideal for glyphosate uptake when experiment C treatments were applied leading to higher amounts of herbicide uptake and greater observable injury.

Plants treated with aminocyclopyrachlor were the first to exhibit injury symptoms. Injury symptoms that resulted from treatment with aminocyclopyrachlor were similar to expected symptoms after treatment with synthetic auxin herbicides (Group 4 herbicides). Within the first day after treatment stems began to curl or twist and leaves to cup. Within one week new shoots were emerging from the rhizome and growing in a twisted manner. These symptoms continued to progress and after five weeks the tips of new shoots often swelled into a bulbous bud-like structure. White calluses also formed on shoot tips. Necrotic tissue first appeared three weeks after treatment.

Aminocyclopyrachlor treatments had greater injury than other active ingredients 7 and 21 DAT in experiments A and B (Table 2.1). The level of injury was correlated to the rate of aminocyclopyrachlor applied. The highest rate of aminocyclopyrachlor resulted in the greatest injury of all treatments 63 DAT. In experiments A and B, the mean percent of control dry weight 70 DAT was not affected by aminocyclopyrachlor applied at the 0.5x or 2x rates, was moderately reduced by the 0.25x rate, and greatly reduced by the 4x treatment (Table 2.3). In experiment C the mean percent of control dry weight 70 DAT was not affected by aminocyclopyrachlor applied at the 0.25x, 0.5x, 1x or 2x rates, and it was greatly reduced by the 4x treatment (Table 2.4). At 186 DAT the 2x and 4x aminocyclopyrachlor treatments had no regrowth after clipping and all other treatments had regrowth equivalent to zero (Table 2.3).

The aminocyclopyrachlor appears to cause two physical responses that have contradictory effects on biomass and are correlated with rate. As rates increase the amount of leaf loss increases, causing a reduction in biomass. Additionally as rate increases the amount of new shoots and callus tissue increases causing an increase in biomass. The observation of these contradictory effects may explain the lack of a true rate response in the measured biomass.

The lack of Japanese knotweed regrowth after treatment with aminocyclopyrachlor indicates that while this product did not initially appear to be more effective than current standard herbicide treatments for Japanese knotweed control in terms of visual injury symptoms, it may be significantly more effective for controlling Japanese knotweed regrowth than glyphosate and may have control comparable to that of imazapyr when used at the rates tested here. Other synthetic auxin (Group 4) herbicides have been shown to cause initial injury but over time are ineffective for Japanese knotweed control. It is not known why aminocyclopyrachlor has a greater effect on Japanese knotweed than other auxin-type herbicides but it is possible that it is due to the unique chemical structure of this new herbicide. It is also possible than this chemical has greater translocation into rhizomes and that metabolism of the chemical inside of plant cells is slow, resulting in long term shoot growth suppression.

These findings indicate that aminocyclopyrachlor may have potential for Japanese knotweed control in the field. Based on these data, it appears that the standard rate for Japanese knotweed control would be approximately equivalent to the 4x rate used in this experiment (0.56 kg ai/ha). Rates in the range of 0.28 kg ai/ha to 0.56 kg/ha should be further examined under western Oregon field conditions to determine a standard application rate of this product for Japanese knotweed control.

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Table 2.1 Japanese knotweed average percent injury from herbicide treatment in greenhouse experiments A[2] and B as quantified by visual rating 7, 21, and 63 DAT.

Herbicide	Rate	Ave. % injury 7 DAT	Ave. % injury 21 DAT	Ave. % injury 63 DAT
aminocyclopyrachlor	0.035 kg ai/ha	13.3 ± 1.9 b[1]	23.9 ± 2.5 b	21.7 ± 0.8 c
aminocyclopyrachlor	0.07 kg ai/ha	17.0 ± 4.0 ab	30.6 ± 4.8 ab	45.0 ± 4.2 b
aminocyclopyrachlor	0.14 kg ai/ha	23.6 ± 4.4 ab	42.2 ± 3.7 a	61.4 ± 4.6 b
aminocyclopyrachlor	0.28 kg ai/ha	29.4 ± 5.0 a	47.2 ± 4.9 a	53.9 ± 5.6 b
aminocyclopyrachlor	0.56 kg ai/ha	13.9 ± 1.6 b	54.4 ± 1.3 a	77.8 ± 2.1 a
imazapyr	0.84 kg ae/ha	1.2 ± 0.6 c	11.4 ± 1.9 c	76.4 ± 2.2 a
glyphosate	3.37 kg ae/ha	5.6 ± 1.6 bc	19.2 ± 3.3 bc	45.3 ± 4.3 b
control	-	0.0 c	0.0 d	0.0 d

[1] Means (plus/minus the standard error of the means) followed by the same lower-case letter are not significantly different as indicated by 95% individual confidence intervals.

[2] Experiment A was initiated on August 8, 2008 and experiment B was initiated on November 25, 2008.

Table 2.2 Japanese knotweed average percent injury from herbicide treatment in greenhouse experiment C[2] as quantified by visual rating 7, 21, and 63 DAT.

Herbicide	Rate	Ave. % injury 7 DAT	Ave. % injury 21 DAT	Ave. % injury 63 DAT
aminocyclopyrachlor	0.035 kg ai/ha	22.2 ± 0.9 b[1]	23.9 ± 0.7 c	35.6 ± 1.0 c
aminocyclopyrachlor	0.07 kg ai/ha	22.8 ± 1.2 b	27.2 ± 0.9 c	38.9 ± 1.6 c
aminocyclopyrachlor	0.14 kg ai/ha	23.9 ± 1.1 b	27.2 ± 1.2 c	33.3 ± 1.2 c
aminocyclopyrachlor	0.28 kg ai/ha	25.6 ± 1.0 b	30.0 ± 1.2 bc	52.2 ± 6.5 c
aminocyclopyrachlor	0.56 kg ai/ha	25.0 ± 1.2 b	42.8 ± 4.9 b	96.7 ± 2.2 a
imazapyr	0.84 kg ae/ha	1.1 ± 0.5 c	16.7 ± 9.2 bcd	83.3 ± 2.4 b
glyphosate	3.37 kg ae/ha	75.0 ± 1.7 a	89.4 ± 1.5 a	84.4 ± 1.8 b
control	-	0.0 c	0.0 d	0.0 d

[1] Means (plus/minus the standard error of the means) followed by the same lower-case letter are not significantly different as indicated by 95% individual confidence intervals.

[2] Experiment C was initiated on June 25, 2009

Table 2.3 Japanese knotweed above ground biomass (AGB) 70 DAT and mean dry AGB of regrowth 186 DAT (after clipping) expressed as a percentage of control biomass in greenhouse experiments A[2] and B.

		Mean dry biomass as % of control	
Herbicide	Rate	70 DAT	186 DAT
aminocyclopyrachlor	0.035 kg ai/ha	59.0 ± 3.5 ab[1]	18.8 ± 10.5 b
aminocyclopyrachlor	0.07 kg ai/ha	107.9 ± 11.8 a	1.6 ± 1.6 b
aminocyclopyrachlor	0.14 kg ai/ha	75.5 ± 7.8 ab	9.6 ± 9.6 b
aminocyclopyrachlor	0.28 kg ai/ha	92.9 ± 12.2 a	0.0 b
aminocyclopyrachlor	0.56 kg ai/ha	48.3 ± 6.3 b	0.0 b
imazapyr	0.84 kg ae/ha	68.9 ± 8.9 ab	0.0 b
glyphosate	3.37 kg ae/ha	54.7 ± 7.0 ab	6.1 ± 2.2 b
control	-	100.1 ± 10.3 a	100.0 ± 11.6 a

[1] Means (plus/minus the standard error of the means) followed by the same lower-case letter are not significantly different as indicated by 95% individual confidence intervals.

[2] Experiment A was initiated on August 8, 2008 and experiment B was initiated on November 25, 2008.

Table 2.4 Japanese knotweed mean dry above ground biomass (AGB) 70 DAT expressed as a percentage of control biomass in greenhouse experiment C[2].

Herbicide	Rate	Mean dry biomass as % of control 70 DAT
aminocyclopyrachlor	0.035 kg ai/ha	95.9 ± 5.8 a[1]
aminocyclopyrachlor	0.07 kg ai/ha	108.0 ± 8.0 a
aminocyclopyrachlor	0.14 kg ai/ha	95.7 ± 6.0 a
aminocyclopyrachlor	0.28 kg ai/ha	78.2 ± 9.1 a
aminocyclopyrachlor	0.56 kg ai/ha	71.4 ± 3.1 b
imazapyr	0.84 kg ae/ha	53.8 ± 5.7 b
glyphosate	3.37 kg ae/ha	23.8 ± 3.9 c
control	-	100.0 ± 18.0 a

[1] Means (plus/minus the standard error of the means) followed by the same lower-case letter are not significantly different as indicated by 95% individual confidence intervals.

[2] Experiment C was initiated on June 25, 2009

3. MANAGEMENT AND RESTORATION OF JAPANESE KNOTWEED INVADED SITES

3.1 ABSTRACT

Japanese knotweed is an invasive perennial shrub that dominates streamside ecosystems throughout North America and Europe. Effective management techniques are currently limited to repeated annual herbicide applications. Restoration of these sites to a functioning riparian plant community is needed to prevent re-infestation of Japanese knotweed or other weed species. A field experiment was initiated to evaluate the integration of chemical management with restoration to a diverse native grass community. A mixture of the native grass species that included *Elymus glaucus* (blue wild rye), *Hordeum brachyantherum* (meadow barley), *Bromus carinatus* (California brome), *Deschampsia cespitosa* (tufted hairgrass), and *Deschampsia elongata* (slender hairgrass) was seeded May 1, 2008, prior to Japanese knotweed shoot emergence, at two rates (10 kg/ha and 40 kg/ha) in experimental plots. The 4 fold increase in seeding rate resulted in a 2.4 fold increase in grass seedling establishment. While grasses established well initially, long-term survival was poor and all grasses were dead one year after seeding. Concurrent to the grass establishment treatments, foliar herbicide applications were made on October 13, 2008 to the Japanese knotweed, and included glyphosate applied at 4.21 kg ae/ha, imazapyr at 1.12 kg ae/ha, triclopyr at 10.1 kg ae/ha, and 2,4-D at 4.26 kg ae/ha. Percent Japanese knotweed injury from each treatment was evaluated 11, 32, 210, and 378 days after treatment (DAT). The glyphosate treatment resulted in 89.5% mean observed injury one year after treatment and was equivalent to the imazapyr treatment which resulted in 81.3% mean observed injury. Reduction in Japanese knotweed biomass was quantified by measuring new shoot density, height and diameter

at 25 centimeters aboveground 196 DAT. None of the herbicide treatments had an effect on shoot diameter. The glyphosate, triclopyr, and imazapyr treatments had an equal effect on reducing the shoot density resulting in approximately three times lower new shoot density than control plots (85, 72, and 66 shoots/9 m^2 compared to 248 shoots/9 m^2). The mean height of the Japanese knotweed was moderately reduced by triclopyr and imazapyr treatments (24 cm and 13 cm tall) and greatly reduced by glyphosate treatment (10 cm tall) when compared to the control plants (57 cm tall) 196 DAT. Due to the failure of the planted grasses to survive the winter the integration of restoration concepts with a herbicide treatment program was unsuccessful in this field study. However, the success of the initial grass establishment may support the feasibility of the concept and provides important information that can be used to direct the continued testing of this methodology in the future.

3.2 Introduction

Japanese knotweed is a widely distributed invasive weed in the Polygonaceae family. Japanese knotweed is native to Asia and inhabits a wide variety of environments throughout Europe and North America. Japanese knotweed and its invasive relatives giant knotweed *(Fallopia sachalinense)* and Bohemian knotweed *(Fallopia bohemicum)* are difficult to control invasive species that have detrimental effects on riparian ecosystems and other natural areas. Infestations are extremely difficult to manage and over time displace native vegetation, impede wildlife activity, and limit land use (Gerber *et al.,* 2008, Maerz *et al.,* 2005, Siemens and Blossey, 2007, Topp *et al., 2008*).

Japanese knotweed is a rhizomatous perennial with shrub-like herbaceous growth that dies back yearly in cold climates. This plant functional type is not represented in the native vegetation of most invaded areas (Dassonville *et al.,* 2007). The Oregon Department of Agriculture (ODA) classifies Japanese knotweed and its invasive relatives giant knotweed *(Fallopia sachalinense)* and Bohemian knotweed *(Fallopia bohemicum)* as class B invasive weeds (ODA, 2007). A Class B weed is defined as a weed of economic importance which is regionally abundant, but which may have limited distribution in some counties. Invasive knotweed species are commonly found in riparian areas, coastal habitats, roadsides, and unmanaged areas in the Pacific Northwest (PNW) (ODA, 2007). These species can survive in a variety of soil types, but especially thrive in moist, well-drained, nutrient rich soils (Doll and Doll, 2006).

In addition to these detrimental characteristics that make invasive knotweed species dominating invasive plants in many ecosystems, these species are also very resilient to current management methods. Currently, chemical control is the only practical management method used by most weed management personnel, though even these methods are problematic. Mechanical control of invasive knotweed is often impractical and ineffective do to the difficulty in removing the extensive below ground rhizome biomass that can weigh from 12 to 35 tons per hectare (Doll and Doll, 2006). Cutting and digging of these species usually serves to spread invasive knotweed rather than suppress it because of its ability to regenerate from rhizome fragments as small as 7 grams in fresh weight (Doll and Doll, 2006). Dispersal of shoot fragments is also a dispersal mechanism of this species. De Waal (2001) demonstrated that placing 40-mm-long stem fragments into moist soil resulted in the regeneration of new shoots and roots at stem nodes.

Chemical control of invasive knotweeds is currently the most effective and widely used management practice. However, chemical control of these species is problematic for many reasons including difficultly in timing herbicide applications for maximum control, lack of information on most effective active ingredients for control, tolerance to herbicide active ingredients, the need for repeated annual herbicide applications, and because these species establish in sensitive habitats adjacent to natural or protected areas where the use of some herbicides may be restricted.

Aquatically labeled herbicides must be used for treatment of Japanese knotweed populations adjacent to water. The most common herbicide active ingredient used to date has been foliar applications of glyphosate (Rodeo®, Aquamaster®) More recently an aquatic label for an imazapyr has been developed (Habitat®). As this new product became an option for invasive knotweed control, imazapyr has been used at varying rates and has been tank mixed with glyphosate for effective control. The herbicide active ingredient aminopyralid (Milestone®) is not yet widely used but many managers are interested in its incorporation into chemical management methods. The new herbicide active ingredient aminocyclopyrachlor will soon be labeled for broadleaf weed control in a variety of sites and may also prove to be a valuable addition to the short list of herbicides useful for invasive knotweed control.

Another common method of chemical control of knotweed species is injection of glyphosate into the hollow canes of plants. This method can be effective, but is not without risk. Due to the density of canes in a large patch or population and the amount of herbicide concentrate recommended for injection into each individual cane, the maximum labeled rate per area of glyphosate is easily surpassed without treatment of the entire patch. This leads to incomplete control and illegal off-label applications of glyphosate products (Hagen and Dunwiddie, 2008).

Although extensive chemical management of knotweed species is occurring throughout the PNW there is little science-based information about which herbicide application methods and active ingredients will result in the greatest management success (Soll *et al*., 2006). Meta-analysis of multiple control projects from the US and Europe has shown that while treatment of infestations with glyphosate and/or imazapyr will produce significant decrease in invasive knotweed abundance, these methods will not result in eradication in the short-term (Kabat *et al.,* 2006).

In addition there is little information available on successful restoration techniques for chemically-treated, knotweed-invaded sites. Due to the difficulty in eradicating infestations and necessity of repeat annual treatments, replanting desirable plant communities frequently does not occur. Vegetation surrounding treated sites quickly re-colonizes the area (Miller, 2005, Ford, 2004). While allowing natural re-colonization can result in desirable natives being restored to the site (Ford, 2004) it can also provide an opportunity for undesirable plant species to re-invade the treated area.

Information regarding the management of invasive knotweeds is improving worldwide, but there is still relatively little known about these species to allow for effective management of infestations in the PNW. Because of the dynamic nature of invasive knotweed mechanisms of spread and establishment, methods of management need to be combined with restoration methods in order to improve ecosystem resilience to future invasion.

Many invasive knotweed sites were ecologically degraded or disturbed prior to infestation. Restoration to ecologically desirable species may increase these sites resilience to future infestations. In the PNW a diverse mixture of native tree and shrub species is most suitable for riparian sites, but it is difficult to move directly from invasive knotweed to these species due to the altered site conditions and need for repeated herbicide applications to control invasive knotweed. Establishment of a transitional plant community can act as a restoration stepping stone moving the site towards a desirable stable state.

The objective of this field research was to integrate the planting of PNW native grasses with herbicide treatments to reduce invasive knotweed biomass and to establish a native grass plant community in invaded sites. We hypothesized that as this grass community becomes established it can be managed as a stepping stone community that can later be restored to mixed tree and shrub species. This management scenario differs from one that seeks to move directly from an invaded site to a mixed tree and shrub community that is currently proposed by many land managers.

3.3 Materials and Methods

Field studies were initiated in the spring of 2008 and continued through fall of 2009 with the purpose of determining the establishment success of native grass species in a Japanese knotweed infestation subjected to herbicide treatment. This study was designed to investigate two different grass seeding rates and four herbicide treatments.

The experimental design of this study was a complete randomized block with split plots. Each of the four randomized complete blocks contained five 9 m^2 plots. Each 9 m^2 plot was randomly assigned a herbicide treatment and subdivided into three 3 m^2 subplots. Native grasses (Table 3.1) were hand-seeded into 3 m^2 subplots and herbicide treatments were applied to the entire 9 m^2 plot (Figure 3.1). Timing of individual seeding and herbicide applications did not vary in this study.

The PNW native grass species adapted to the local environment and selected for this study were *Elymus glaucus* (blue wild rye), *Hordeum brachyantherum* (meadow barley), *Bromus carinatus* (California brome), *Deschampsia cespitosa* (tufted hairgrass), and *Deschampsia elongata* (slender hairgrass). Table 3.1 includes further details on the amount of each species in the grass mix and on seeding rates. Grass species were chosen based on the local availability, low cost, desirability and competitiveness of species, and species suitability to variable environmental conditions at field site.

Native grasses were hand-seeded and lightly raked into the moist soil of the Japanese knotweed infested plots at two rates (10 kg/ha and 40 kg/ha) on May 1, 2008. On this date Japanese knotweed plants were just beginning to sprout from underground rhizomes. The timing of emergence of new shoots from underground rhizomes varies based on the temperature and precipitation at the site, and cold, wet conditions had delayed Japanese knotweed emergence. Grass establishment was evaluated by estimating percent grass cover 30 days after seeding (DAS) and measuring grass seedling density 45 DAS. Grass survival was evaluated by measuring grass plant density 176 DAS.

Herbicide treatments were applied with a CO_2 pressurized backpack sprayer equipped with a high-pressure spray gun and adjustable nozzle on October 13, 2008 (165 DAS). During the herbicide application the Japanese knotweed plants were sprayed from all four sides of experimental plots resulting in all aboveground plant parts (both the top and underside of leaves and all sides of canes) being sprayed until saturated. Herbicides applied were glyphosate, imazapyr, triclopyr, and 2,4-D at a total spray volume of 2243.2 L/ha (Table 3.3). Glyphosate and imazapyr were chosen because these active ingredients are currently the standard Japanese knotweed control herbicides used by many managers. Triclopyr and 2,4-D were chosen based on the selectivity of the products and potential for causing less injury to the newly established grass plants than glyphosate and imazapyr. The maximum labeled rate for perennial weed control with aquatic formulations of all these products was applied.

Plots were visually evaluated for percent injury to Japanese knotweed during the same growing season as the herbicide treatment prior to normal senescence; 11 days after treatment (DAT) and 32 DAT. Percent injury to Japanese knotweed was evaluated during the next growing season (2009) at seven and 12 months after treatment (210 DAT and 378 DAT). Reduction in Japanese knotweed biomass was evaluated by measuring new shoot density, height, and diameter at 25 centimeters aboveground six months after treatment (196 DAT).

A linear regression model was used for each Japanese knotweed injury and biomass data set that included both the herbicide treatment and experimental block as explanatory variables. Ninety-five percent individual confidence intervals were calculated for the treatment means. Separate linear regression models were used for both of the grass density measurements and for the percent cover data that included both the seeding rate and experimental block as explanatory variables and 95% individual confidence intervals were calculated for the treatment means.

3.4 Results and Discussion

Native Grass Establishment

The two hairgrass species (*Deschampsia cespitosa* and *Deschampsia elongata*) that made up 10% of the grass seed mix (by weight) had percent cover equivalent to the other three species (*Elymus glaucus*, *Hordeum brachyantherum*, *Bromus carinatus*) which made up 90% of the grass seed mix at both seeding rates. This result indicates that lower percentages (5% of each) by weight of the small seed *Deschampsia* species is appropriate when the desirable outcome is equal parts of all species in this mix. The 40 kg/ha seeding rate resulted in significantly higher grass seedling establishment. Percent cover for all grass species was significantly higher at the 40 kg/ha seeding rate (42%) than at the 10 kg/ha (13%) 30 DAS (Table 3.2). Mean grass density 45 DAS was greater at the higher seeding rate (617 plants/m^2 compared to 285 plants/m^2). At 176 DAS mean grass density was measured as two fold greater in plots seeded with 40 kg/ha but statistically equivalent to the mean grass density of subplots seeded at 10 kg/ha (Table 3.2).

Throughout the 2008 growing season grass plants persisted in all experimental plots until the last observation (176 DAS). When native grass density was evaluated at the beginning of the second growing season (2009), one year after seedling, no grass plants were quantified within the experimental plots. When Japanese knotweed leaf litter was brushed away from the soil surface of the plots, dead grass plants were visible.

The 40 kg/ha seeding rate resulted in a dense cover of grasses that is desirable for restoration sites. Though the grass plants did not survive winter conditions at this site, we documented that grasses could be established and survive initial competition with Japanese knotweed. The winter after grass planting the experimental site did not experience flooding that would remove the leaf litter from the soil surface and thus the grass plants were most likely buried in litter and unable to regrow the following spring. It is possible that in a flood year or in a riparian area that normally experiences high water in winter, established grasses might have greater likelihood of survival. In this case, survival would depend on the leaf litter being washed away and a grass rooting depth that allows plants to withstand average amounts of flooding and scouring.

Japanese Knotweed Control

Experimental plots were evaluated for percent injury to Japanese knotweed due to herbicide treatments during the same growing season as treatment, at 11 and 32 DAT (Table 3.4). At 11 DAT most treatment plots exhibited very little injury and additionally showed little signs of seasonal senescence. Most of the mean percent injury ratings were equivalent to zero, the exception being the triclopyr treatment, which caused a mean percent injury of 35% (Table 3.4).

The triclopyr treatment had a higher percent injury than other treatments 32 DAT (mean 77.5% injury). Other treatments were not statistically different than the control. Japanese knotweed plants had begun fall senescence and percent leaf drop was also evaluated for each plot. Most treatments exhibited a mean near 75% leaf drop, except for 2,4-D which showed a lower mean leaf drop of 45% (Table 3.4). The mechanism of action of 2,4-D is not entirely understood thus making it difficult to discern exactly why the leaf senescence was delayed. The similarity of 2,4-D structure to plant growth hormones has lead to many non-herbicidal uses for this compound such as its use for controlling fruit ripening and delaying fruit drop (Senseman, 2007). Such an effect was observed in the delayed leaf senescence of Japanese knotweed plants treated with 2,4-D in this experiment.

Reduction in Japanese knotweed biomass was evaluated by measuring new shoot density, height and diameter at 25 cm aboveground six months after treatment (196 DAT). None of the herbicide treatments had an effect on shoot diameter (Table 3.5). The glyphosate, triclopyr, and imazapyr treatments had an equal effect on reducing shoot density resulting in approximately three times lower new shoot density than control plots (85, 72, and 66 shoots/9 m^2 compared to 248 shoots/9 m^2). The mean height of the new shoots was 2-4 times shorter after the triclopyr and imazapyr treatments (24 cm and 13 cm tall) and was nearly 6 times shorter after glyphosate treatment (10 cm tall) when compared to the control plants (57 cm tall) (Table 3.5).

Percent injury to Japanese knotweed was evaluated during the next growing season seven and 12 months after treatment (210 DAT and 378 DAT). Glyphosate and imazapyr resulted in the greatest injury symptoms 210 DAT (95% and 90% mean observed injury). The triclopyr treatment resulted in much less injury (63.8%), and the 2,4-D resulted in very little injury 210 DAT (20%) (Table 3.4). Final evaluation of percent injury was approximately one year after treatment (378 DAT) when all treated Japanese knotweed plants had grown for an entire season. The 2,4-D treatment resulted in no injury and triclopyr treatment very little injury (22.5% mean observed injury) 378 DAT. Glyphosate resulted in 89.5% mean observed injury 378 DAT, and was equivalent to the imazapyr treatment which showed 81.3% mean observed injury.

Imazapyr and glyphosate resulted in greatest level of injury to the Japanese knotweed of the four herbicides tested. These two products have very different costs for application. The aquatically-labeled glyphosate (Rodeo®, Aquamaster®) treatment has an approximate product cost of $160/ha and imazapyr (Habitat®) has an approximate cost of $302/ha (Ferrel and MacDonald, 2008). Since the amount of injury for one application evaluated a year after treatment was equal with these two products (81-89%) the glyphosate was the most cost effective treatment evaluated in this experiment.

Timing of herbicide treatment and restoration planting needs to be adapted to the unique conditions at any invasive knotweed control site. Fall herbicide treatment was timed as late as possible prior to any predicted killing frost. Given the mild coastal climate at this research site, herbicide application could be delayed until mid-October. For restoration planning the spring planting date should be modified to suit the conditions at the site in a given year. Spring grass seeding was timed so that Japanese knotweed plants were just beginning to emerge. Fall planting may also be feasible at some sites depending on weather and river level. Testing the efficacy of seeding in the fall after herbicide treatment is needed.

Due to the failure of grasses to survive winter conditions at the site the amount of injury to the grasses from the non-selective herbicides (glyphosate and imazapyr) could not be evaluated. Neither of the synthetic auxin herbicides (2,4-D and triclopyr), which were selected for their low potential injury rate to grasses, produced a satisfactory amount of Japanese knotweed injury. In future related experiments, additional untested herbicide active ingredients (such as aminopyralid and aminocyclopyrachlor) should be substituted for the 2,4-D and triclopyr treatments that resulted in poor control of Japanese knotweed. Glyphosate and imazapyr were found to be highly effective in controlling Japanese knotweed one year after treatment causing 81-89% injury with one application. Herbicide treatments of these experimental plots should continue on an annual basis until the Japanese knotweed is eliminated.

There are alternative management scenarios to the one tested in this experiment that may be effective for controlling invasive knotweed. Beginning a restoration project with a fall herbicide treatment, then following with seeding grasses later that fall or the following spring after senescence and before invasive knotweed emergence, may be effective. Site preparation including dry biomass removal may need to be conducted prior to seeding. At most sites the window of opportunity for seeding will depend on rainfall and river depth and these conditions will likely be the determining factor for using a fall or spring seeding date after herbicide treatment. Additionally, the scenario used in this experiment (beginning with spring seeding followed by fall herbicide treatment) may have had more success if dry biomass had been removed from the site following autumn senescence.

Based on the results from this study, it does appear to be plausible, with some modification to our methods, to establish a native grass community at a invasive knotweed restoration site that can be used as a stepping stone community before proceeding to restoration with trees and shrubs. Further experiments designed to test both spring and fall seeding before and after herbicide application should be conducted in order to determine the most effective means of the integration of restoration with herbicide control.

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Figure 3.1 Diagram of research plots at Nehalem River Field Site in 2008.

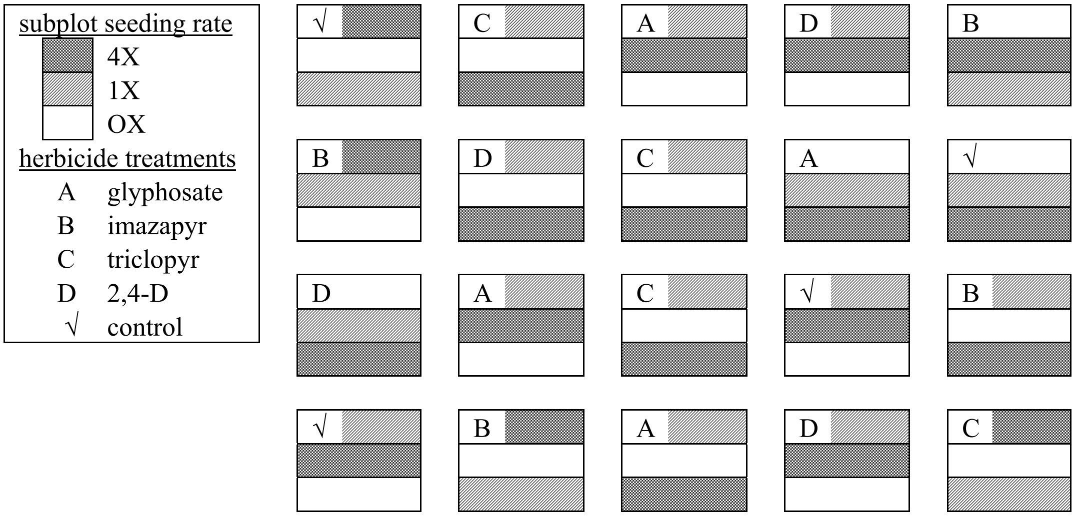

Table 3.1 Pacific Northwest native grass seed mixes seeded into herbicide treatment plots.

Grass species		Percent of mix by seed weight	Weight of species for:	
Common name	Scientific name		10 kg/ha seeding rate	40 kg/ha seeding rate
Blue Wild Rye	*Elymus glaucus*	30%	3 kg/ha	12 kg/ha
Meadow Barley	*Hordeum brachyantherum*	30%	3 kg/ha	12 kg/ha
California Brome	*Bromus carinatus*	30%	3 kg/ha	12 kg/ha
Tufted Hairgrass	*Deschampsia cespitosa*	5%	0.5 kg/ha	2 kg/ha
Slender Hairgrass	*Deschampsia elongata*	5%	0.5 kg/ha	2 kg/ha

Table 3.2 Pacific Northwest native grass estimated percent cover 30 DAS and grass density 45 and 176 DAS.

Seeding rate	Mean percent cover grasses 30 DAS	Mean grass seedlings per 1 m^2 45 DAS	Mean grass plants per 1 m^2 176 DAS
10 kg/ha	13.2 ± 0.9 b[1]	285 ± 50 b	11 ± 3 a
40 kg/ha	42.3 ± 3.5 a	617 ± 86 a	28 ± 19 a

[1] Means (plus/minus the standard error of the means) followed by the same lower-case letter are not significantly different as indicated by 95% individual confidence intervals.

Table 3.3 Herbicide treatments applied to grass seeding plots on October 13, 2008.

Herbicide common name	Rate kg ae/ha	Surfactant	Est. cost per hectare
Glyphosate	4.21	0.5% MSO	$160
Imazapyr	1.12	0.5% MSO	$302
Triclopyr	10.1	0.5% NIS	$520
2,4-D	4.26	0.5% NIS	$35

Table 3.4 Japanese knotweed average percent injury and leaf drop from herbicide treatments applied to grass seeding plots on October 13, 2008, as quantified by visual rating 11, 32, 210, and 378 DAT.

Herbicide treatment	Mean % injury				Mean % leaf drop 32 DAT
	11DAT	32 DAT	210 DAT	378 DAT	
Check	0.0 b[1]	0.0 d	0.0 d	0.0 c	75.0 ± 5.0 ab
2,4-D	1.5 ± 0.6 b	33.8 ± 5.5 b	20.0 ± 4.1 c	0.0 c	45.0 ± 5.4 b
Glyphosate	0.8 ± 0.8 b	15.0 ± 8.4 bcd	95.0 ± 0 a	89.5 ± 0.5 a	78.8 ± 6.3 ab
Imazapyr	1.3 ± 0.8 b	10.0 ± 2.0 c	90.0 ± 2.0 a	81.3 ± 4.3 a	75.0 ± 2.4 a
Triclopyr	35.0 ± 2.0 a	77.5 ± 3.2 a	63.8 ± 2.4 b	22.5 ± 4.3 b	76.3 ± 6.3 ab

[1] Means (plus/minus the standard error of the means) followed by the same lower-case letter are not significantly different as indicated by 95% individual confidence intervals.

Table 3.5 Japanese knotweed new shoot biomass 196 DAT.

Herbicide treatment	Density in 9 m^2 plot	Mean height (cm)	Mean diameter at 25 cm
Check	248 ± 21 a	57 ± 4 a	2.0 ± 0.4 a
2,4-D	230 ± 18 a	42 ± 2 a	1.1 ± 0.0 a
Glyphosate	85 ± 11 b	10 ± 1 c	0.6 ± 0.1 a
Imazapyr	66 ± 9 b	13 ± 3 bc	1.2 ± 0.2 a
Triclopyr	72 ± 11 b	24 ± 2 b	2.3 ± 0.2 a

[1] Means (plus/minus the standard error of the means) followed by the same lower-case letter are not significantly different as indicated by 95% individual confidence intervals.

4. SUMMARY AND CONCLUSIONS

4.1 INTRODUCTION

Invasive knotweed species are difficult to control invasive plants that have detrimental effects on riparian ecosystems and natural areas. Infestations are extremely difficult to manage and over time displace native vegetation, impede wildlife activity, and limit land use. Native plant diversity and abundance diminishes when an ecosystem becomes dominated by invasive knotweed. Research that provides land managers with information regarding the biology and management of invasive knotweed species is crucial for developing effective methods for control of these species. Quantifying the effectiveness and cost of control methods aids in planning and prioritizing management projects. Additionally, determining how to integrate multiple approaches for control and restoration of sites will likely result in lasting positive results.

The experiments described in the preceding chapters of this thesis and following appendix are the first step in the investigation of the efficacy and integration of current control practices of invasive knotweed with restoration practices in the PNW. The best methodology for invasive knotweed control may still lie in the numerous untested combinations of control and restoration practices. Furthering our understanding of invasive knotweed biology and continuing to test new management combinations may lead researchers and land managers to a consistently successful methodology for removing invasive knotweed and restoring invaded areas to desired pre-invasion conditions.

4.2 SUMMARY OF FINDINGS

Experimentation with the new herbicide aminocyclopyrachlor to control Japanese knotweed under greenhouse conditions indicates that this product may have potential for Japanese knotweed control in the field. The lack of Japanese knotweed regrowth after treatment with aminocyclopyrachlor suggests that while this product did not initially appear to be more effective than current standard herbicide treatments for Japanese knotweed control in terms of visual injury symptoms, it may be as effective or more effective for controlling Japanese knotweed regrowth than glyphosate and imazapyr. Based on these experimental results it would appear that the standard rate for Japanese knotweed control would be approximately equivalent to the 4x rate used in greenhouse experiments (0.56 kg ai/ha). Rates in the range of 0.28kg ai/ha to 0.56 kg/ha should be further examined under western Oregon field conditions to determine a standard rate for Japanese knotweed control.

Results from field experiments comparing glyphosate, imazapyr, triclopyr, and 2,4-D for Japanese knotweed control indicate that one year after treatment the control method with the lowest cost and greatest amount of Japanese knotweed control is glyphosate applied at 4.21 kg ae/ha. While imazapyr applied at 1.12 kg ae/ha resulted in similar control the cost of this product is significantly greater than most glyphosate containing products. However, the difference in control between the two products may be greater after multiple years of application and should be further studied.

A mixture of the native grasses that included *Elymus glaucus, Hordeum brachyantherum, Bromus carinatus, Deschampsia cespitosa,* and *Deschampsia elongata* was successfully established in experimental plots with a spring planting date at two seeding rates (10kg/ha and 40kg/ha). It is possible that removing Japanese knotweed biomass after senescence in the fall, when the leaves and canes could no longer regenerate new plants, could have increased the success of grass winter survival in this study. This method could be attempted if spring seeding of grasses is the most appropriate timing for a given control project. Seeding the spring following herbicide application has potential as well when herbicide application has removed some invasive knotweed biomass and new weeds have not established in the bareground left by treatment.

4.3 FUTURE RESEARCH

Continuing the annual herbicide applications to experimental plots in order to determine the timeline and total cost for eradication of invasive knotweed is needed. Further experiments testing both spring and fall seeding of grass and forbs before and after herbicide application should be conducted in order to determine the most effective timing of the integration of restoration with herbicide control. The amount of injury to grasses from the non-selective herbicides (glyphosate and imazapyr) also needs be evaluated. In future related experiments additional less well evaluated herbicide active ingredients (such as aminopyralid and aminocyclopyrachlor) should be substituted into field experiments for the 2,4-D and triclopyr which we found to provide unsatisfactory control of Japanese knotweed.

Characterization of the genetic structure of weed populations used in experimental research is important for understanding the history and mechanisms of invasion and spread. Based on the studies conducted in Europe, it was assumed until recently that populations of Japanese knotweed in the U.S. also reproduced asexually (Hollingsworth *et al.*, 1998). Instead, both asexual and sexual reproduction can occur in Japanese knotweed populations in the U.S. (Forman and Kesseli, 2003, Wymer *et al.*, 2007, Grimsby *et al*., 2007). The greater amount of genetic diversity in U.S. populations of invasive knotweed species compared to European populations has been attributed to multiple introductions of the species from Europe and Asia and to hybridization and introgression of the closely related species (Gammon *et al.*, 2007).

This genetic diversity due to genetic mixing of invasive knotweed species has made the genus taxonomically ambiguous, and there is no clear morphological trait that can be used to distinguish Japanese knotweed from closely related hybrids in U.S. populations (Gammon *et al.*, 2007). Genetic analysis to determine both species identification and relatedness of plants in experimental trials is necessary due to this difficultly in taxonomic characterization and the unique genetic diversity observed in the eastern U.S.

Objectives for future genetic research should include confirmation of species identification, characterization of the genetic diversity of the experimental invasive knotweed populations used in this research, and characterization of the genetic diversity of a invasive knotweed infestation spreading through a watershed. Classifying populations by genotype and identifying how individuals within a population are related will be important information to include when publishing findings of both field and greenhouse studies. Genetic characterization of a population will indicate the mode of reproduction and invasion, as well as the speed of invasion. This type of information may make experimental results more transferable to similar populations around the world.

Research that increases our understanding of invasive knotweed biology is also crucially important for determining when these plants are most susceptible to control methods. Nearly all research connected to the invasive knotweeds can be utilized both to increase understanding of these species and identify vulnerabilities that will lead to better management practices.

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APPENDIX

A. COMPARISON OF HERBICIDE TANK MIXTURES FOR JAPANESE KNOTWEED CONTROL

A.1 Abstract

In addition to the studies presented in the previous chapters, a field experiment was completed to determine the efficacy of three herbicide tank mixtures for invasive knotweed control. A brief summary of these results is presented here. A triclopyr/2,4-D treatment resulted in no injury symptoms in treated Japanese knotweed 378 DAT. A imazapyr/glyphosate treatment resulted in 77.5% mean observed injury 378 DAT, and was equivalent to the imazapyr/aminopyralid treatment which resulted in 57.5% mean observed injury. The imazapyr/aminopyralid treatment had the lowest product cost per hectare ($346).

A.2 Materials and Methods

This study was designed to determine the efficacy of three herbicide tank mixture treatments on established Japanese knotweed populations by evaluating three combinations of commonly used herbicides. Herbicide combinations applied to the experimental plots included glyphosate with imazapyr, imazapyr with aminopyralid, and triclopyr with 2,4-D. The imazapyr mixtures were evaluated because anecdotal evidence suggested synergistic effects when used on invasive knotweed. The triclopyr and 2,4-D mixture was evaluated because of the lack of efficacy data on this mixture when used on invasive knotweed and the known high efficacy of this treatment on other invasive broadleaf perennial plants. The total spray volume for all mixtures was 2243.2 L/ha and the application rates were imazapyr at 1.12 kg ae/ha, glyphosate at 4.21 kg ae/ha, aminopyralid at 0.12 kg ae/ha, triclopyr at 10.1 kg ae/ha, and 2,4-D at 4.26 kg ae/ha (Table A.1).

The experimental design was a complete randomized block experiment with 9 m^2 experimental plots. Each 9 m^2 plot was randomly assigned a herbicide treatment. Herbicide treatments were applied with a CO_2 pressurized backpack sprayer outfitted with a high-pressure spray gun and adjustable nozzle on October 13, 2008. Plots were visually evaluated for percent injury to Japanese knotweed during the same growing season as the herbicide treatment; 11 days after treatment (DAT) and 32 DAT. Percent injury to Japanese knotweed was evaluated during the next growing season seven and twelve months after treatment (210 DAT and 378 DAT). Percent leaf drop was visually evaluated 32 DAT. Reduction in Japanese knotweed biomass was evaluated by measuring new shoot density, height and diameter at 25 centimeters aboveground six months after treatment (196 DAT).

A linear regression model was used to evaluate each type of Japanese knotweed injury data that included both the herbicide treatment and experimental block as explanatory variables and 95% individual confidence intervals were calculated for the treatment means.

A.3 Results and Discussion

At 11 DAT most treated plots showed very little herbicide injury and additionally showed little signs of seasonal senescence. Most of the mean percent injury ratings were equivalent to zero 11 DAT, the exception being the triclopyr/2,4-D treatment which resulted in 11% injury. The triclopyr/2,4-D treatment was equivalent to the imazapyr/aminopyralid treatment, and these treatments resulted in the highest mean percent injury 32 DAT (60% and 63.75%, respectively). The imazapyr/glyphosate treatment resulted in a lower mean percent injury (25%). Percent leaf drop varied in general but all treatments had higher percent leaf drop than the control with triclopyr/2,4-D and imazapyr/glyphosate treatments having the greatest means (90% and 85%).

Percent injury to Japanese knotweed was evaluated during the next growing season seven and 12 months after treatment (210 DAT and 378 DAT). The imazapyr/aminopyralid treatment resulted in the greatest injury symptoms 210 DAT (Table A.2). The imazapyr/glyphosate treatment resulted in slightly less injury than the imazapyr/aminopyralid treatment, and the triclopyr/2,4-D treatment resulted in the least amount of injury 210 DAT (Table A.2). The final evaluation of percent injury was 378 DAT when all treated Japanese knotweed plants had regrown for an entire season. The triclopyr/2,4-D treatment resulted in no injury symptoms 378 DAT. The imazapyr/glyphosate treatment caused 77.5% mean observed injury 378 DAT, and was equivalent to the imazapyr/aminopyralid treatment which showed 57.5% mean observed injury.

Reduction in Japanese knotweed biomass was evaluated by measuring new shoot density, height and diameter at 25 cm aboveground six months after treatment (196 DAT). None of the herbicide treatments had an effect on mean shoot height (Table A.3). All three treatments had an equal effect on reducing the shoot density (mean density of treated plots was 3-9 shoots in 0.75 m^2 compared to 24 shoots in 0.75 m^2 for the control). Mean shoot diameter at 25 cm aboveground was slightly reduced by the imazapyr/aminopyralid mixture (0.3 cm) and the triclopyr/2,4-D mixture (0.8 cm), and shoot diameter was significantly reduced by imazapyr/glyphosate (0.1 cm) when compared to diameter of control plants (1.0 cm) (Table A.3).

The three tank mixtures utilized in this study had very different product costs per hectare and cost significantly more than single herbicide applications. The glyphosate/imazapyr mixture approximate product cost of $462/ha, imazapyr/aminopyralid mixture $346/ha and the triclopyr/2,4-D mixture $555/ha. The imazapyr/aminopyralid mixture had the greatest amount of mean observed injury (57.5%) 378 DAT for the lowest product cost per hectare ($346). When utilizing a herbicide tank mixture for invasive knotweed control our data suggest that imazapyr/aminopyralid would be the best choice of the three tank mixtures included in this experiment.

Table A.1 Herbicide treatments applied to tank mixture plots on October 13, 2008.

Tank mix	Herbicide common name	Rate kg ae/ha	Surfactant	Est. cost per hectare
Mix 1	Glyphosate	4.21	0.5% MSO	$462
	Imazapyr	1.12		
Mix 2	Imazapyr	1.12	0.5% MSO	$346
	Aminopyralid	0.12		
Mix 3	Triclopyr	10.1	0.5% MSO	$555
	2,4-D	4.26		

Table A.2 Japanese knotweed injury and leaf drop from herbicide treatments applied to tank mixture plots on October 13, 2008.

Herbicide treatment	Mean % injury				Mean % leaf drop
	11DAT	32 DAT	210 DAT	378 DAT	32 DAT
Check	0.0 b	0.0 b	0.0 c	0.0 b	46.8 ± 3.6 b
Imazapyr/ aminopyralid	0.3 ± 0.3 ab	63.8 ± 3.8 a	90.8 ± 2.7 a	57.5 ± 13.6 a	61.3 ± 2.7 ab
Imazapyr/ glyphosate	0.0 b	25.0 ± 9.1 ab	90.0 ± 5.1 ab	77.5 ± 4.3 a	85.0 ± 3.5 a
Triclopyr/ 2,4-D	10.8 ± 3.3 a	60.0 ± 4.6 a	61.3 ± 3.1 b	0.0 b	90.0 ± 2.0 a

[1] Means (plus/minus the standard error of the means) followed by the same lower-case letter are not significantly different as indicated by 95% individual confidence intervals.

Table A.3 Japanese knotweed new shoot biomass 196 DAT.

Herbicide treatment	Density in 0.75 m^2	Mean height (cm)	Mean diameter at 25cm
Check	24.3 ± 2.6 a	27.1 ± 2.6 a	1.0 ± 0.1 a
Imazapyr/ aminopyralid	3.0 ± 1.7 b	19.1 ± 4.0 a	0.3 ± 0.3 ab
Imazapyr/ glyphosate	6.0 ± 1.8 b	14.0 ± 2.2 a	0.1 ± 0.1 b
Triclopyr/ 2,4-D	9.5 ± 1.3 b	14.2 ± 1.8 a	0.8 ± 0.3 ab

[1] Means (plus/minus the standard error of the means) followed by the same lower-case letter are not significantly different as indicated by 95% individual confidence intervals.