THESIS

RIPARIAN AREA INVASIVE PLANT MANAGEMENT ALONG THE NIOBRARA RIVER, TARGETING YELLOW FLAG IRIS (*Iris pseudacorus* L.)

Submitted by

Jordan L. Spaak

Department of Forest and Rangeland Stewardship

In partial fulfillment of the requirements

For the Degree of Master of Science

Colorado State University

Fort Collins, Colorado

Summer 2016

Master's Committee:

Advisor: Paul J. Meiman

Troy W. Ocheltree
K. George Beck

ABSTRACT

RIPARIAN AREA INVASIVE PLANT MANAGEMENT ALONG THE NIOBRARA RIVER, TARGETING YELLOW FLAG IRIS (*IRIS PSEUDACORUS* L.)

Yellow Flag Iris (YFI) (*Iris pseudacorus* L.) is an invasive exotic species that is causing substantial changes to the ecology of the Niobrara River and the adjacent riparian area habitat. Options for invasive plant management along the Niobrara River, like most riparian wetlands, are quite limited. Currently, herbicides offer the best opportunities for successful YFI management in riparian habitats such as those along the Niobrara River but irrigation diversion and livestock grazing are prevalent which impose further limitations on management options. In this study, we analyzed the efficacy of multiple potential YFI management methods, including; chemical (glyphosate), mechanical (cutting), plant competition, and trampling. A combination of field and greenhouse studies were used. Field studies were conducted at Agate Fossils Beds National Monument, Harrison Nebraska, U.S.A. and greenhouse experiments were completed at Colorado State University, Fort Collins, Colorado, U.S.A. The greenhouse studies were conducted to determine how temperature, light, seed scarification, and trampling, affected germination, seedling growth and survival of YFI. The field studies focused on the effectiveness of glyphosate, cutting, planting native plants, and trampling on YFI. Results from field studies indicate that cutting established plants stimulates YFI growth, spring application of glyphosate resulted in a short-term reduction of YFI abundance, and planting native plants did not reduce YFI abundance. Findings indicate that YFI prefer shaded areas and cooler temperatures for emergence and warmer shaded areas for growth. Seed scarification did not affect emergence. Trampling in the field reduced YFI density by 75% and plant height by 58%. Simulated trampling that targeted the plant crown and 1-2-cm above the crown reduced survival, though trampling that targeted the crown was five times more likely to kill the plant than trampling 1-2-cm above the crown.

ACKNOWLEDGEMENTS

Dr. Paul J. Meiman

Dr. Rita Beard

National Park Service Agate Fossil Beds National Monument -James Hill, Superintendent and staff

NPS Northern Great Plains EPMT Liaison-Brennan Hauk and staff

National Park Service Biological Resources Division

PRIDE WMA - Panhandle Research Integration and Discovery Education (Nebraska)

Sioux County Nebraska FFA chapter students

Sioux County Weed District Superintendent/FFA advisor-Nick Sanderson

Colorado State University Department of Forest and Rangeland Stewardship

Dr. K. George Beck

Dr. Troy W. Ocheltree

Kelly Coy

Nic Medley

Charlie Stump

Kenda Cruz

Kyle Biehl

Sam Fowler

PREFACE

Yellow flag iris (*Iris pseudacorus* L.) (YFI) is an invasive exotic species in the United States. This perennial plant reproduces vegetatively and by seed, reaching heights of 25-90 cm tall and can take 3 years to mature (Stone 2009). A large amount of energy is devoted to rhizome development early in the YFI growth cycle so plants can be resilient to fluctuating water levels (Whitehead 1971). The rhizomes of YFI are 1-5 cm in diameter and form dense networks or mats that can easily support a 100-kg person. Flower stalks are round in cross-section, erect and support one to several flowers per inflorescence (Jacobs et al. 2011, Tu 2003). Each flower is 8-10 cm in diameter and composed of 3 downward-pointing sepals (larger) and 3 upward-pointing petals (smaller). Sepals and petals are pale to deep yellow (sometimes orange) and may have purple flecks or veins. On average, 6 seed pods are produced on each plant. Seed production seems to be variable (6-120 seeds per pod) and many seeds remain undeveloped and not viable. Germination is also variable (48-80%) due to a multitude of environmental conditions including air and soil temperature, and amount of time a seed has spent floating in water (Sutherland 1990).

Yellow flag iris is a member of the Iridaceae family and is native to Europe, North Africa, the Mediterranean and temperate Asia (Jacobs et al. 2011). Yellow flag iris grows in a wide variety of wetland habitats on various substrates and as an emergent wetland plant. In the United States YFI is used in phytoremediation and phytoextraction to remove heavy metals and some species of bacteria in water treatment processes (Xu et al. 2015). The most common use of YFI in the U.S. is as an ornamental and it has escaped cultivation and spread to 40 of the 50 states in the U.S (at the time this thesis was written, YFI had not been documented in North and South Dakota, Iowa, Wyoming, New Mexico, Arizona, Oklahoma, Alaska, Colorado and Hawaii; USDA NRCS PLANTS Database 2016). As early as 1771, YFI was documented in Virginia, and this is likely the earliest documentation of it in the U.S. (Stone 2009). Neither livestock nor wildlife prefer to graze YFI as a result of gastrointestinal irritations caused by the glycosides that are found in this plant (Tu 2003). Though the iris is considered poisonous, some herbivores, specifically sheep, have grazed this plant late in the fall and early in the spring when other sources of suitable forage and browse were not available (Stone 2009).

Management strategies to reduce or eliminate YFI are limited. Discussion and research has focused on herbicide application, fire, smothering (with tarps or plastic), excavation of the plant and rhizomes, and mechanical cutting (Simon 2008, Stone 2009). Limited information is available on the effects of fire and the responses of YFI to that type of disturbance. Predictions suggest that fire will likely kill the above ground plant parts but the rhizomes and the seeds present in the soil or water will lead to persistence of, or re-invasion by YFI (Stone 2009). No biological agent has been identified as a viable management option for YFI (Tu 2003). Simon (2008) observed a significant reduction in the number of YFI stems 1 year after a mechanical cutting treatment, though below ground YFI effects where not discussed. The most common approach for management of YFI is through the use of herbicides. The non-selective herbicides glyphosate (e.g. Rodeo™ or Aquamaster™), and imazapyr (e.g. Habitat®) or a combination of both herbicides are commonly identified as successful management methods (Simon 2008). Because this plant prefers wetland habitats, herbicides with an aquatic label are needed. Glyphosate and imazapyr are non-selective, so non-target native plant species growing in treated areas will likely also be killed. In certain ecosystems, residues from imazapyr can be found in the growing season following treatment (residues can be present for up to 120 days post treatment), so extreme caution should be exercised to prevent undesirable impacts in riparian areas (BASF Corporation 2005).

This study took place at Agate Fossil Beds National Monument (AGFO) which is located between Mitchell and Harrison, Nebraska (UTM Zone 13T604131 E, 4697768 N) and has an elevation of 1,372 meters above sea level (MASL). The mean annual precipitation ranges from 36 – 41 cm and the mean annual air temperature ranges from 7 to 9 ℃ (USDA NRCS 2013). Located in the northern mixed grass prairie ecosystem, AGFO occurs on soils that are predominantly sandy loam to loam textures (USDA NRCS Web Soil Survey 2016). The Niobrara River flows through the National Monument and is a perennial, spring-fed, sinuous river system with an average flow of 0.4 $m^3 \cdot s^{-1}$ (1958-1991; the USGS stream gauge was reactivated in February of 2014; USGS National Water Information System 2016). The riparian area vegetation includes monocultures of YFI, dense cattail (*Typha latifolia* L.) stands, narrowleaf willow (*Salix exigua* Nutt.), native graminoids including woolly sedge (*Carex pellita* Muhl. Ex Willd.), Arctic rush (*Juncus arcticus*), western wheatgrass (*Pascopyrum smithii* (Rydb.) Á. Löve), and areas of Kentucky bluegrass (*Poa pratensis* L.) (Prowatzke and Wilson 2015). Plant nomenclature follows the USDA Plants database (USDA NRCS 2016).

The National Park Service (NPS) has been monitoring aquatic vegetation and wildlife species, including fish, since 1979 and noted a sharp decline in fish species richness (Pegg and Pope 2008). In addition to the decline in fish, native riparian plant species are being replaced by YFI, which in turn affects waterfowl, fish, and domestic livestock. In 1989 the Niobrara River flowing through AGFO had eight fish species present [one species was a non-native brown trout (*Salmo trutta*)] and by 2011 only three fish species were recorded [one was a non-native Northern pike (*Esox lucius*)] (Pegg and Pope 2008). These changes to the aquatic wildlife have been accompanied by vegetation changes in the riparian area along the Niobrara River driven by YFI. It was suggested that the YFI invasion might have brought about the observed changes in aquatic wildlife (N. Medley, personal communication, May 2013). Observations of the Niobrara River within the National Monument reveal that the river channel is deeper and narrower when compared to upstream, non-infested river locations. Outside of the YFI infestation, the river has more native vegetation occupying the riparian area, which provides habitat for aquatic wildlife species. The dense rhizomes of YFI and lack of heterogeneity in the riparian area vegetation could be reducing preferred habitat for wildlife species and be a contributing factor to the decline in fish.

The strength and prevalence of the rhizomatous mats produced by YFI have likely increased bank stability and Wohl (2006) stated that the increased bank stability can cause the river channel to narrow and flow velocity to increase. This can be expected to result in increased ability of the river to carry sediment in the narrowed reaches, and subsequent increases in sediment deposition downstream (Wohl 2006) of the YFI infestation. The USGS Nonindigenous Species database (2013) stated that the rhizomatous growth associated with YFI can influence changes in channel geometry by narrowing the channel width by up to 25 cm per year. Alteration of sediment and flow regimes by YFI is likely to result in morphological changes to the channel, that will alter structure and function of the river and impact fish and other aquatic organisms (Jones et al. 1999).

The YFI infestation at AGFO is one of the first documented cases in the State of Nebraska where this plant escaped cultivation to become an aggressive exotic invasive weed. One of the early homesteads in the Niobrara River Valley belonged to James Cook. Cook and his family maintained a swimming hole associated with the Niobrara River. After reviewing National Park Service records and archives, it was determined that the Cook family likely planted YFI seeds which were acquired from Europe around 1900, around the swimming hole. Through time and multiple floods, YFI went from being an ornamental plant surrounding a swimming hole, to an aggressive exotic invader of the Niobrara River. Our observations and those of several NPS employees suggest that YFI is causing drastic changes to the river morphology, vegetation, and wildlife along the river. Presently, approximately 20 km of the river supports riparian habitat dominated by dense stands of YFI, many of which are monocultures. To the west of the national monument an irrigation dam has prevented YFI from spreading westward (upstream), but the easterly flow of the Niobrara is moving seed and rhizomes to new locations downstream. Yellow flag iris is currently on the State of Nebraska "weed watch list" and has a high potential to spread to new areas (USDA APHIS 2013).

Given the negative impacts of YFI on the Niobrara River ecosystem at AGFO, the National Park Service wanted to identify a treatment strategy that would effectively reduce the YFI population. NPS felt that partnering on a research project to evaluate different treatment options was the best way to identify effective management methods. The NPS and a faculty member from the Department of Forest and Rangeland Stewardship at Colorado State University (CSU) began collaborating on treatment tools for management of YFI.

During the initial visit to AGFO in the summer of 2013, we noted that YFI was less abundant on the working cattle ranches that surround the national monument. As a result of this observation, research studies attempted to identify any clues or indicators about how livestock might be affecting YFI abundance.

The goal of this project was to identify effective management strategies for reducing YFI growing in wetlands along the Niobrara River and in similar habitats, with similar management and conservation objectives. Sutherland (1990) pointed out that limited information is available on how YFI responds to light, water, temperature, and nutrients affecting photosynthesis and respiration. We attempted to address some of these unanswered questions and identify effective YFI treatment options. Chapter 1 of this thesis reports on the effects of cutting, herbicide application, and planting native plants on YFI abundance. Chapter 2 describes a greenhouse study that investigated how shade, temperature, and seed scarification affect YFI germination and growth. Chapter 3 covers the effects of trampling on YFI survival and growth.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LITERATURE CITED

BASF Corporation. 2005. Supplemental labeling -Habitat Herbicide ®, 26 Davis Drive, Research Triangle Park, NC, 27709, USA.

Jacobs, J., M. Graves and J. Mangold. 2011, Plant guide for paleyellow iris (*Iris pseudacorus*). USDA-Natural Resources Conservation Service, Montana State Office. Bozeman, Montana 59715.

Jones, E.B.D., G.S. Helfman, J.O. Harper and P.V. Bolstad. 1999. Effects of Riparian Forest Removal on Fish Assemblages in Southern Appalachian Streams. Conservation Biology 13: 1454–1465.

Medley, N. 2013. Personal communication. Fisheries Biologist. USNPS Natural Resources Stewardship and Science Directorate.

Pegg, M. and K. Pope. 2008. Agate Fossil Beds National Monument Fish Inventory 2008 Survey. Final Report.

Prowatzke, M. and S. K. Wilson. 2015. Plant community composition and structure monitoring for Agate Fossil Beds National Monument: 2014 annual report. Natural Resource Data Series NPS/NGPN/NRDS—2015/774. National Park Service, Fort Collins, Colorado.

Simon, B. 2008, Yellow-flag Iris Control and Education. ://www.ecy.wa.gov/programs/wq/plants/weeds/YFI%20Final%20Report%20to%20DOE%206-30-08.pdf.

Stone, K.R. 2009. *Iris pseudacorus*. In: Fire Effects Information System, [Online]. U.S. Department of Agriculture, Forest Service, Rocky Mountain Research Station, Fire Sciences Laboratory (Producer). Available: http://www.fs.fed.us/database/feis/ [2016, March 28].

Sutherland, W.J. 1990. Biological flora of the British Isles: *Iris pseudacorus* L. Journal of Ecology. 78(3): 833-848. [73513].

Tu, M. 2003. Element stewardship abstract: *Iris pseudacorus* L.--yellow flag iris, water iris, [Online]. In: Management library: Control methods--plants. In: Global Invasive Species Team (GIST). Arlington, VA: The Nature Conservancy (Producer). Available: http://www.invasive.org/gist/esadocs/documnts/irispse.pdf [2016, March 22].

United States Department of Agriculture, Animal and Plant Health Inspection Service, Weed Risk Assessment for *Iris pseudacorus* L. (Iridaceae)-Yellow flag iris, [2015, September 24].

United States Department of Agriculture, Natural Resource Conservation Service, Plants Database. Available online at: http://plants.usda.gov. [2016, March 16].

United States Department of Agriculture, Natural Resources Conservation Service. Web Soil Survey. Available at: http://websoilsurvey.sc.egov.usda.gov/App/WebSoilSurvey.aspx. Accessed 10 March 2016.

United States Department of Agriculture, Natural Resources Conservation Service, and United States Department of the Interior, National Park Service. 2013. Soil Survey of Agate Fossil Beds National Monument, Nebraska. (Accessible online at: http://soils.usda.gov/survey/printed_surveys/).

United States Department of the Interior, United States Geological Survey (USGS). National Water Information System. Available at: http://waterdata.usgs.gov/nwis/rt. [2016, March 10].

United States Department of the Interior, United States Geological Survey (USGS). 2010. Non indigenous Aquatic Species, *Iris pseudacorus*, Yellow iris, yellow flag iris. [2016, May 7].

Whitehead, F. H. 1971. Comparative autecology as a guide to plant distribution. In: Duffey, Eric O.; Watt, A. S., eds. The scientific management of animal and plant communities for conservation: Proceedings of the 11th symposium of the British Ecological Society; 1970 July 7-9; Norwich, UK. Oxford: Blackwell Scientific Publications: 167-176. [40245].

Wohl, E. 2006, Human Impacts to Mountain Streams. Geomorphology 79: 217-248.

Xu, B., S. Yu, J.Ding, S. Wu, J.Ma. 2015. Metal-Dependent Root Iron Plaque Effects on Distribution and Translocation of Chromium and Nickel in Yellow Flag (*Iris pseudacorus* L.). Journal of Phytoremediation 17(1-6):175-81.

EXPERIMENT #1: EFFECTIVENESS OF SPRING CUTTING, SPRING GLYPHOSATE APPLICATION AND NATIVE PLANTINGS FOR YELLOW FLAG IRIS MANAGEMENT (*IRIS PSEUDACORUS* L.) ALONG THE NIOBRARA RIVER

SUMMARY

Yellow flag iris (YFI) (*Iris pseudacorus* L.) is a non-native, invasive weed that is causing substantial changes to the ecology of the Niobrara River and adjacent riparian habitat in Sioux County, Nebraska. Native plant species are being replaced by YFI, which in turn affects waterfowl, fish, and domestic livestock. Options for invasive plant management along the Niobrara River, like most riparian wetlands, are quite limited. Previous studies on the effectiveness of the herbicides imazapyr and glyphosate suggest that imazapyr is more effective. However, use restrictions related to irrigation diversion limit the feasibility of using imazapyr. This study was conducted to determine the extent to which cutting iris early in the spring improves the effectiveness of subsequent glyphosate application for reduction of YFI. Since irrigation diversion and land use are prevalent along the Niobrara River we attempted to identify a treatment, or combination of treatments that could increase the efficacy of a spring glyphosate application so treatments would not be applied during irrigating season. We are also interested in determining the extent to which cutting and herbicide treatments affect the survival of *Carex nebrascensis* and *Juncus arcticus* planted after herbicide application and whether these native plants can compete with surviving or recolonizing YFI. Forty-eight, 2- x 2- m study plots were located in an approximate 260-ha portion of riparian habitat along the Niobrara River in western Nebraska. Eight treatment combinations including all combinations of early spring cutting (cut and uncut), spring herbicide application (sprayed with an 8% solution of glyphosate and unsprayed), and planting natives (planted and unplanted) were each replicated six times. Iris density was recorded three times throughout the growing season. The two native plant species were planted to assess how well native plants can compete with YFI and recolonize treated plots. Results indicate that glyphosate did not affect YFI density

after 1 growing season, spring cutting of YFI increased density, and native plant abundance was not affected by treatments. Planted native plants did not reduce YFI abundance. Further work should focus on identifying a group of native plants that could compete and recolonize a site after YFI. Due to a lack of success with the chemical and mechanical treatments that were employed during this project, future research could focus on improving treatment techniques and mechanisms.

1. INTRODUCTION

Yellow flag iris (YFI) (*Iris pseudacorus* L.) is a non-native, invasive weed in North America that is usually found in wetland areas, and is native to Europe, North Africa, and Western Asia. This invasive species is distributed in 40 of 50 states across the U.S. (USDA NRCS PLANTS Database 2016). Primarily used as an ornamental plant, YFI escaped cultivation and has become an invasive species. The United States Geological Survey Nonindigenous Species database (2013) stated that the rhizomatous growth associated with YFI can influence changes in channel geometry by narrowing the channel width by up to 25 cm per year. Due to its uptake and mass release of heavy metals, bacteria, total nitrogen, total phosphorus, ammonium, and other nutrients (Xu et al. 2015, Wu et al. 2011), YFI has also been found to negatively impact aquatic ecosystems. The ability to absorb metals, nutrients, and reduce bacteria loads make YFI a preferred species for use in phytoremediation (Xu et al. 2015, Sutherland 1990).

This study investigates a YFI infestation in the State of Nebraska. Agate Fossil Beds National Monument (AGFO) is a park unit managed by the National Park Service (NPS). The Niobrara River is a perennial, spring-fed river system that runs through AGFO. The headwaters for this river occur approximately 80 km west of the National Monument near Torrington, Wyoming. Since 1979, the NPS has been monitoring the composition and density of aquatic wildlife and noted a decline in the number and species richness of fish (Pegg and Pope 2008). In addition to the decline in fish, native riparian area plant species are being replaced by YFI, which in turn affects waterfowl, fish, and domestic livestock. In

1989 the Niobrara River flowing through AGFO had eight fish species present [one species was a non-native brown trout (*Salmo trutta*)] and by 2011 only three fish species were recorded [one was a non-native Northern pike (*Esox lucius*)] (Pegg and Pope 2008). These changes to the aquatic wildlife have been accompanied by vegetation changes in the riparian area along the Niobrara River driven by YFI. It was suggested that the YFI invasion may have brought about the observed changes in aquatic wildlife (N. Medley, personal communication, May 2013). Observations of the Niobrara River within AGFO reveal that the river channel is deeper and narrower when compared to upstream, non-infested river locations. Outside of the YFI infestation, the river has more native vegetation occupying the riparian area, which provides habitat for aquatic wildlife species. The dense rhizomes of YFI and lack of heterogeneity in the riparian area vegetation could be reducing preferred habitat for wildlife species and be a contributing factor to the decline in fish.

The NPS mission includes management and control of invasive and exotic species (NPS Management Policies 2006). Given the concerns about YFI at AGFO, NPS was interested in identifying suitable management options that could be used. Some individuals at NPS felt that partnering on a research project to evaluate different treatment options was the best way to identify effective management methods. The NPS and a faculty member from the Department of Forest and Rangeland Stewardship at Colorado State University (CSU) began collaborating on treatment tools for management of YFI.

YFI is often found in wetland habitats, so herbicides labeled for aquatic use are required. Simon (2008) reported on herbicide and non-herbicide management efforts for YFI in Washington State. Spring and fall herbicide trials included glyphosate, imazapyr, and combinations of the two products (Simon 2008). Simon (2008) found that fall treatments were slightly more effective than spring treatments, and that imazapyr was notably more effective than glyphosate 12–17 months after treatment. Non-herbicide management tools included cutting or covering with tarps, plastic, and landscape fabric (Simon 2008). Covering treatments were not replicated, but did suggest that covering might reduce iris abundance however, spring cutting reduced iris density by about 60% 1 year after treatment.

The conditions along the Niobrara River at AGFO are representative of conditions where YFI management may need to be applied. The riparian area and the associated wetlands are dominated by YFI and in many areas, this plant has out-competed the native vegetation and created a monoculture. Agriculture is an economic driver surrounding AGFO that is a further complication in treating invasive weeds. Invasive species managers need to consider treatments that do not interfere with agricultural interests, such as irrigation diversions or plant-back intervals that interfere with the growing season and crop production. Because irrigation diversions from the Niobrara River are common and occur well into October of each year, opportunities for summer and fall herbicide applications of products with irrigation restrictions are limited and would require landowners to stop irrigating much earlier than preferred. As a result of these limitations the preferred management approach would include effective reduction of YFI, flexibility in herbicide application time so irrigation restrictions would not be an issue, and the use of herbicides that can be followed immediately or soon after by planting (short or no plant-back interval).

Because of the previously identified limitations, we chose to use glyphosate (Rodeo®) to treat our study plots. This product has an aquatic label and does not have a plant-back interval so we identified it as a preferred treatment option. Simon (2008) indicated that imazapyr was more successful at reducing YFI abundance in the spring, but because imazapyr residues can be long lived (25 d-142 d depending on a variety of conditions; Douglass et al. 2016), it might not be appropriate to apply near agricultural areas, as residues could be present during the growing season. Although glyphosate is usually more effective when used later in the growing season, Simon (2008) reported 70 – 80% control of YFI 12 months following spring application of glyphosate. We identified this as a potentially successful management option that was a good fit to the site and the land management scenario if its effectiveness could be increased. To attempt to increase the effectiveness of a spring glyphosate treatment, we cut YFI before herbicide application to reduce the standing dead plant material and to stimulate above-ground growth. Lastly, in an attempt to determine the ability of native plants to compete with YFI and increase in abundance post-treatment, we planted two native plant species in our treatment plots.

The overall goal of this project was to identify a treatment, or a combination of treatments that could be applied in the spring, and provide invasive plant managers with more flexibility to manage YFI. Also, we wanted to identify if any treatments could suppress YFI and allow native plant abundance to increase in areas that were formerly dominated by YFI.

Objective 1: Determine the effects of spring cutting, glyphosate and planting natives on YFI density and the effects of spring cutting and glyphosate on survival of Nebraska sedge (*Carex nebrascensis* Dewey) and Arctic rush (*Juncus arcticus* Willd.).

Hypothesis 1.1: YFI density will be lowest in plots that are cut, sprayed and planted, intermediate in plots that planted and either sprayed or cut and greatest in control plots.

Hypothesis 1.2: Nebraska sedge and Arctic rush survival will be greatest in plots where YFI was cut and sprayed, intermediate in plots where YFI was only sprayed or only cut and least in control plots.

Objective 2: Determine if Nebraska sedge and Arctic rush abundance will increase when YFI abundance is reduced.

Hypothesis 2.1: Nebraska sedge and Arctic rush abundances will both increase with decreasing YFI abundance.

2. METHODS

2.1 Study Site:

This study took place at Agate Fossil Beds National Monument (AGFO), which is located between Mitchell and Harrison, Nebraska (UTM Zone 13T 604131 E, 4697768 N) at an elevation of 1,372 MASL. The mean annual precipitation ranges from 36 to 41cm and the mean annual air temperature ranges from 7 to 9℃ (USDA NRCS 2013). Located in the northern mixed grass prairie ecosystem, AGFO occurs on soils that are predominantly sandy loam to loam textures (USDA NRCS

Web Soil Survey 2016). The Niobrara River flows through AGFO and is a perennial, spring-fed, sinuous river system with an average flow of 0.4 $m^3 \cdot s^{-1}$ (1958-1991; the USGS stream gauge was reactivated in February of 2014) (USGS National Water Information System 2016). The riparian area vegetation includes monocultures of YFI, dense cattail (*Typha latifolia* L.) stands, narrowleaf willow (*Salix exigua* Nutt.), native graminoids including woolly sedge (*Carex pellita* Muhl. Ex Willd.), Arctic rush (*Juncus arcticus*), western wheatgrass (*Pascopyrum smithii* (Rydb.) Á. Löve), and areas of Kentucky bluegrass (*Poa pratensis* L.) (Prowatzke and Wilson 2015). Plant nomenclature follows the USDA Plants database (USDA NRCS PLANTS Database 2016).

This study was conducted in the wetlands along the Niobrara River in AGFO. In April of 2014, 48, 2- x 2- m plots were located in areas where the highest YFI abundances were observed. Plots were located in the riparian area and the associated wetlands on the eastern half of AGFO. Some plots had a mix of cattails and willows, but YFI was the dominant plant species in all plots. Plots were located between 1 and 30 m from the main river channel on both the north and south banks of the river. Plot locations were variable and occurred in standing and flowing water, and slightly drier areas above the river channel. Figure 1 illustrates how plots were marked. Plots were randomly assigned to one of eight treatments for a completely randomized experimental design using a 2 x 2 x 2 factorial arrangement of treatments.

Figure 1. Plot layout- Plots were marked with two t-posts and were laid out by forming a right isosceles triangle with a tape measure. The right isosceles triangle had a hypotenuse 2.82 meters ($\sqrt{\mathbf{2}} * \mathbf{A} =$

$\mathbf{hypotenuse}, \mathbf{A} = \mathbf{triangle\ side\ length}$; mathworld.wolfram.com) long and each side of the triangle was 2-m in length. Visualizing a right isosceles triangle, both t-posts were placed in the location of the 45°angles. The third point occurred at the location of the 90°angle. The fourth point was determined by forming a second right isosceles triangle and the hypotenuse was formed between the two t-posts previously identified. The location of the 90°angle marked the fourth point.

2.2 Treatments:

To address the stated objectives and associated hypotheses in this study, we identified eight treatments that included all possible combinations of cutting (cut and uncut), spraying (sprayed and unsprayed), and planting native graminoids (planted and not planted). The cutting treatment was performed on April 17 and 18, 2014; 1 month before the glyphosate application. A handheld scythe was used to cut YFI plants 5-10 cm above the plant crown. Cutting the new YFI growth was also effective at removing aboveground growth from previous years from each of the plots assigned to cutting. Cutting was used because Simon (2008) observed a 60% reduction in YFI density as a result of a standalone cutting treatment. Our idea was that a cutting treatment combined with glyphosate application would be more effective for YFI reduction than either treatment alone. Our decision to use an 8% concentration of glyphosate for our sprayed treatments was also based on the Simon (2008) findings. On May 14, 2014 an 8% solution of glyphosate (Rodeo®) and Brewer 90-10® surfactant was applied to plots assigned to sprayed treatments. Glyphosate application took place one month prior to YFI reproductive growth stage. The NPS Northern Great Plains Exotic Plant Management Team applied the herbicide treatment on May 15, 2014 using backpack sprayers holding the nozzle approximately 90 cm above the YFI. A 0.60 m buffer was sprayed around each herbicide treated plot to reduce encroachment of YFI into the treated plots from rhizomes. The weather on the day herbicide was applied was sunny, with no wind, and the temperature was10.5°C.

In June of 2014, Nebraska sedge and Arctic rush plugs were planted in plots assigned to the planted treatments. Planted material was purchased from Aquatic and Wetland Nursery, LLC Fort Lupton, Colorado. Each 2- x 2- m plot was subdivided into four, 1- x 1- m quadrants. A 5-cm diameter metal pipe was pounded into the ground to a depth of 18 cm to create a hole for each native plant plug to be planted into. The black dots in Figure 2 identify the location where native plants were planted. Ten Arctic rush plants and 10 Nebraska sedge plants were planted in study plots that were randomly assigned to the planting treatment. Five Nebraska sedge plants were planted in quadrants “A” and “C” (5 plants/ quadrant, 10 total plants/plot), and 5 Arctic rush plants were planted in quadrants “B” and “D” (5 plants/ quadrant, 10 total plants/plot) (Figure 2). Nebraska Sedge and Arctic Rush were used for this study because the NPS Inventory and Monitoring Division identified these two plant species at AGFO. Both species were abundant at AGFO but were not observed in any study plots before planting. Because of their rhizomatous growth form and other growth characteristics (USDA NRCS PLANTS Database 2016), we predicted that these species would be good candidates to compete with YFI.

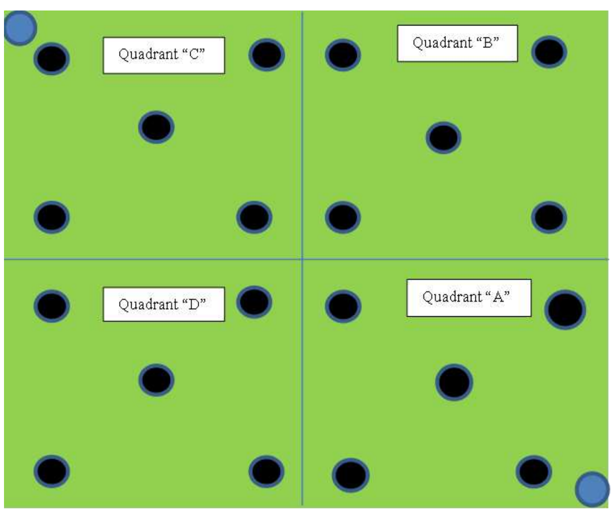

Figure 2. Layout of the 2- x 2- m plots used in this study showing 1- m^2 quadrants, quadrants A-D. Black dots indicate where each plug was planted. Nebraska sedge (*Carex nebrascensis* Dewey) was planted in quadrants “A” and “C” and Arctic rush (*Juncus arcticus* Willd.) was planted in quadrants “B” and “D”. The blue dots signify where the T-posts were placed to mark each plot.

2.3 Data Collection:

Initial YFI and native plant density counts were performed on April 17 and 18, 2014. Subsequent YFI and native plant density counts were taken on May 14, 2014 (before herbicide application) and on August 18 and 19, 2014. For the April and May density counts, a 1- m^2 frame was placed over each quadrant in the 2- x 2- m plot to help keep track of progress while counting. Density of YFI was very high during the August 18 and 19 density counts, so a 0.5 m^2 frame was randomly placed in each of the four quadrants to take a subsample of the 2- x 2- m plot. The observed YFI densities in the subsampled frames were averaged together to obtain an overall plot density. All density counts were expressed as shoots·m^{-2}. The final YFI and native plant density counts were taken in May of 2015, at the conclusion of this study to determine if treatment effects were carried into the next growing season. Individual YFI plants were identified by the distinct point that was formed at the tip of the leaves and the presence of equitant leaves. Cattails featured a rounded tip on the leaf blade and later in the growing season grew taller than YFI. These characteristics made it possible to distinguish between the two species.

2.4 Statistical Analysis:

Analysis of Variance (ANOVA) was used to determine the effects of cutting, planting and herbicide on YFI density, and the effects of cutting and herbicide on native plant survival. Residuals were inspected to confirm that assumptions of the analyses were met. Treatment means were compared only if the ANOVA yielded a significant F-test. Separate analyses were run for the May 2014, August 2014 and May 2015 data sets. Relationships between native plant densities and YFI density were explored using simple linear regression. All analyses were conducted using JMP® version 12.0 (JMP®) and $\alpha = 0.05$.

3. RESULTS

Plots 8, 32, and 44 were clear outliers and were omitted from analysis based on QQ plot analysis in JMP. These plots were obvious visual outliers based on the line of best fit and therefore omitted. Plot 22 was also omitted from May 2015 ANOVA and regression analysis due to an imazapyr treatment that killed the entire plot. The imazapyr treatment was not part of this research study. May 2014 YFI densities were affected by cutting but not by herbicide ($P = 0.0690$), planting ($P = 0.4451$) or any of the interactions ($0.2611 < P < 0.8269$). May 2014 YFI density was 119 ± 7.1 plants·m^{-2} (mean ± SE, n=22) in cut plots and 143 ± 7.1 plants·m^{-2} (n=23) in uncut plots. By August 2014 YFI densities were no longer affected by cutting ($P = 0.9808$), herbicide ($P = 0.0636$), planting natives ($P = 0.9088$) or any of the interactions ($0.2290 < P < 0.7782$). Mean YFI density across all plots was 161 ± 6.6 plants·m^{-2} (n=45). In May of 2015 YFI densities were not affected by cutting ($P = 0.5539$), herbicide ($P = 0.1194$), planting natives ($P = 0.1232$), or any of the interactions ($0.1571 < P < 0.7698$). The mean YFI density across all plots was 149 ± 13.8 plants·m^{-2} (n=44).

Native plants were planted in June 2014 and by August 2014 the Arctic rush density was affected by the May herbicide treatment ($P = 0.0085$), but not by cutting ($P = 0.3921$) or the interaction of cutting and herbicide ($P = 0.1055$). Plots treated with herbicide had 1.4 ± 0.2 plants·m^{-2} (means ± SE) of Arctic rush and the unsprayed plots had 0.5 ± 0.2 plants·m^{-2}. The 2014 Nebraska sedge density was not affected by cutting, herbicide, or the interaction ($0.2708 < P < 0.6458$). The overall 2014 native plant abundance was not affected by cutting, herbicide, or the combination of treatments ($0.1381 < P < 0.9420$). May 2015 Arctic rush density was not affected by any of the treatments or combination of treatments ($0.2437 < P < 0.5546$). May 2015 Nebraska sedge density was also not affected by any of the treatments (main effects or interactions; $0.3373 < P < 0.7660$). Overall, native plant density was also not affected by treatments ($0.3022 < P < 0.9074$). Interestingly, native plant density was low and not affected by any treatments but a small number of plots did support a large increase in native plant abundance (Table 1).

Regressions that compared the relationship between YFI abundance and the abundance of Nebraska sedge and Arctic rush, across all plots for 2014 revealed that native plant abundance did not vary with YFI abundance ($0.3305 < P < 0.9045$) (Table 2). Regressions revealed a relationship between YFI abundance and Nebraska sedge, Arctic rush, and native plants abundance in May 2015 (Table 2).

Table 1. Plots where a large increase in native plant abundance was observed between August 2014 and May 2015, Agate Fossil Beds National Monument (near Harrison, Nebraska).

Native Plant Density			
Plot #	**Treatment**	**August 2014 Density (plants·m^{-2})**	**May 2015 Density (plants·m^{-2})**
1	Uncut, Herbicide, Planted	2	27
9	Uncut, No Herbicide, Planted	5	32
24	Cut, Herbicide, Not Planted	0	31
30	Uncut, Herbicide, Not Planted	0	21
31	Uncut, Herbicide, Planted	9	26
48	Cut, Herbicide, Planted	2	76

Table 2. Regression analyses from Agate Fossil Beds National Monument of the relationships between YFI density (plants·m^{-2}) and the density of Nebraska sedge (*Carex nebrascensis* Dewey), Arctic rush (*Juncus arcticus* Willd.), and native plants (Nebraska sedge and Arctic rush combined) density from August 2014 to May 2015. Native plants were planted in June 2014.

Analysis	R^2 Value	P-value	Regression Equation
Nebraska sedge vs. YFI August 2014	0.000822	0.9045	Nebraska sedge = 2.03 - 0.007*YFI
Arctic rush vs. YFI August 2014	0.052653	0.3305	Arctic rush = 2.14 - 0.03*YFI
Native plant vs. YFI August 2014	0.013852	0.6212	Native plants = 4.17 - 0.04*YFI

Nebraska sedge vs. YFI May 2015	0.686945	<0.0001	Nebraska sedge = -17.55 + 0.17*YFI
Arctic rush vs. YFI May 2015	0.843723	<0.0001	Arctic Rush = -6.13 + 0.05*YFI
Native plant vs. YFI May 2015	0.76562	<0.0001	Native plants = -23.69 + 0.2*YFI

4. DISCUSSION

Addressing Hypothesis 1.1, our treatments, alone or in combination, did not reduce YFI density by the end of the first growing season. Simon (2008) applied glyphosate (Aquamaster® at 2.5 and 5%) on May 4, 2005 when YFI plants were in the bud stage. We applied glyphosate on May 14, 2014 prior to YFI being in the bud stage or reproductive stage. The early application of glyphosate (before the reproductive growth phase) could have contributed to the ineffectiveness of glyphosate in this study. We also hypothesized that cutting YFI 1 month before the glyphosate application might stimulate leaf growth and increase the herbicide to leaf contact, which could increase the effectiveness of a spring glyphosate application.

Another problem that likely affected the success of this study was that the Niobrara River reached record high water levels and was flowing over its banks during the early part of the 2014 growing season. Much of the leaf material that would normally be exposed above the water level was likely underwater and the herbicide to leaf contact was not maximized. The success of killing YFI with glyphosate could have potentially increased by applying glyphosate during a different growth phase and/or by using a different concentration and/or rate of application. Kyser and DiTomaso (2012) utilized a “drizzle application” method designed by the University of Hawaii that employed a backpack sprayer and handgun. The drizzle method utilized low rates of concentrated herbicide (20% glyphosate applied at 38L/4047m^2) during the fall just before the plants senesced and the research team observed a successful kill of YFI in California.

Due to the uncertainty of flow on the Niobrara River during the spring, it might be more effective to use a fall application of glyphosate when the river levels are at the lowest point and YFI is beyond the reproductive growth stage.

The Simon (2008) results suggested that a spring application of glyphosate appeared to be effective early on, but a personal communication with Dr. Tim Miller; (February 20, 2015) (Simon 2008) revealed that a large number of YFI shoots arose in their spring glyphosate treated plots late in the season. Much like the Simon (2008) findings, our spring glyphosate treatments looked very promising for most of the growing season until August, at which time hundreds of iris shoots measuring 5-15 cm tall emerged in the glyphosate treated plots

Although it was hypothesized that native plant survival would be favored by cutting and herbicide treatments on YFI (hypothesis 1.2), that hypothesis was not supported by these analyses. Native Arctic rush and Nebraska sedge plants were planted in an attempt to identify native plant species that could compete with YFI. It is noteworthy to mention that effective YFI suppression did not occur as a result of our treatments prior to planting Nebraska sedge and Arctic rush. Kettenring and Adams (2011) indicate that long-term repeated control methods are needed to effectively manage invasive plant species. Surprisingly, Arctic rush, Nebraska sedge, and native plant abundance increased during the second growing season and YFI abundance also increased. Much like the findings from Meiman et al. (2009) which found that native vegetation facilitated diffuse knapweed (*Centaurea diffusa* Lam.) emergence, native vegetation could be facilitating YFI growth, or vis-a-versa. Though the findings during the May 2015 density count displayed a relationship between YFI abundance and native plant abundance, only one density count was recorded during the second growing season. As a result of these findings further work should be completed before a recommendation is made about how well Arctic Rush and Nebraska sedge grow in post YFI treatment areas.

Arctic rush density increased for only a short period of time in the glyphosate treated plots. By August the effect of glyphosate on native plant density was not detected. When native plants were planted, the Niobrara River water levels were exceedingly high and multiple plants were planted completely underwater or floated out of the 18 cm deep hole that was dug.

Crawford and Braendle (1996) stated that the inherent variability of all natural systems is also met in relation to levels of anoxia-tolerance in wetland plant communities. At this study site and like many wetland areas, the observed variability in water levels along the Niobrara River and the duration of anoxic conditions was hard to predict. Due to the variability and unpredictability of conditions along the Niobrara River, future YFI management and restoration projects should consider a variety of plants that can adapt to varying natural environments and anoxic conditions. If a large scale treatment for YFI is successful without consideration for post treatment revegetation efforts, the empty niche hypothesis (Masters and Sheley 2001) indicates that another species will fill the void that YFI previously occupied.

5. MANAGEMENT IMPLICATIONS

These data indicated that a spring cutting followed by an application of 8% glyphosate did not reduce YFI abundance. Therefore, we do not recommend the use of these treatments for yellow flag iris management. Further experimentation is warranted looking at the application timing, rate, and use of glyphosate and different herbicides or combination of herbicides. These data indicated that spring cutting might increase YFI abundance. This attempt to identify native plants (Arctic rush and Nebraska sedge) that could compete with YFI or recolonize a site after a successful YFI reduction, only yielded short term success in increasing native plant density. Because Kettenring and Adams (2011) indicate that long-term repeated control methods are needed to effectively manage invasive plant species, it could be suggested that multiple years of YFI suppression is necessary to allow native plants to effectively compete with

YFI. More research is needed to better identify plant species that could compete and recolonize areas with longer term YFI suppression.

LITERATURE CITED

Crawford, R.M.M., and R. Braendle. 1996. Oxygen deprivation stress in a changing environment. Journal of Experimental Botany 47(2):145-159.

Douglass, C. H., S.J. Nissen, P.J. Meiman and A.R. Kniss. 2016. Impacts of Imazapyr and Triclopyr Soil Residues on the Growth of Several Restoration Species. Rangeland Ecology & Management 69(3):199-205.

http://mathworld.wolfram.com/IsoscelesRightTriangle.html. Accessed 30 April 2016

JMP®, Version *12*. SAS Institute Inc., Cary, NC, 1989-2007.

Kettenring, K. M. and C. R. Adams. 2011. Lessons learned from invasive plant control experiments: a systematic review and meta-analysis. Journal of Applied Ecology 48: 970–979.

Kyser, G.B. and J. DiTomaso. 2012. Shoreline Drizzle Applications for Control of Yellow Flag Iris. UC Weed Science-Weed Control, Management, Ecology, and Minutia. November 5, 2012.

Masters, R. and R.L. Sheley. 2001. Principles and Practices for Managing Rangeland Invasive Plants. Journal of Range Management 54(5): 502–517.

Medley, N. 2013. Personal communication. Fisheries Biologist. USNPS Natural Resources Stewardship and Science Directorate.

Meiman, P. J., E.F. Redente and M.W. Paschke. 2009. Diffuse knapweed (*Centaurea diffusa* Lam.) seedling emergence and establishment in a Colorado grassland. Plant Ecology 201(2): 631-638.

Miller, T. 2015. Personal communication. Associate Weed Scientist, Extension Specialist, Crop and Soil Sciences Department. Washington State University-Northwestern Washington Research and Extension Center.

Pegg, M. and K. Pope. 2008. Agate Fossil Beds National Monument Fish Inventory 2008 Survey. Final Report.

Prowatzke, M. and S. K. Wilson. 2015. Plant community composition and structure monitoring for Agate Fossil Beds National Monument: 2014 annual report. Natural Resource Data Series NPS/NGPN/NRDS—2015/774. National Park Service, Fort Collins, Colorado.

Simon, B. 2008, Yellow-flag Iris Control and Education. ://www.ecy.wa.gov/programs/wq/plants/weeds/YFI%20Final%20Report%20to%20DOE%206-30-08.pdf.

Sutherland, W.J. 1990. Biological flora of the British Isles: *Iris pseudacorus* L. Journal of Ecology 78(3): 833-848.

United States Department of Agriculture (USDA), Natural Resource Conservation Service (NRCS), PLANTS Database. Available online at: http://plants.usda.gov. [2016, March 16].

United States Department of Agriculture, Natural Resources Conservation Service, and United States Department of the Interior, National Park Service. 2013. Soil survey of Agate Fossil Beds National Monument, Nebraska. (Accessible online at: http://soils.usda.gov/survey/printed_surveys/)

United States Department of Agriculture, Natural Resources Conservation Service. Web Soil Survey. Available at:6http://websoilsurvey.sc.egov.usda.gov/App/WebSoilSurvey.aspx. [2016, March10].

United States Department of the Interior, United States National Park Service (USNPS). NPS Management Policies 2006. Available at: https://www.nps.gov/policy/MP2006.pdf.

United States Department of the Interior, United States Geological Survey (USGS). 2010. Non indigenous Aquatic Species, *Iris pseudacorus*, Yellow iris, yellow flag iris. [2016, May 7].

United States Department of the Interior, United States Geological Survey (USGS). National Water Information System. Available at: http://waterdata.usgs.gov/nwis/rt. [2016, March 10].

Wu H., J. Zhang, P. Li, J. Zhang, H. Xie and B. Zhang. 2011. Nutrient Removal in Constructed Microcosm Wetlands for Treating polluted River Water in Northern China. Ecological Engineering 37 (4):560-568.

Xu B., S. Yu, J.Ding, S. Wu and J. Ma. 2015. Metal-Dependent Root Iron Plaque Effects on Distribution and Translocation of Chromium and Nickel in Yellow Flag (*Iris pseudacorus* L.). Journal of Phytoremediation 17(1-6):175-81.

EXPERIMENT #2: SHADE, TEMPERATURE, AND SEED SCARIFICATION EFFECTS ON YELLOW FLAG IRIS SEEDLING EMERGENCE AND GROWTH

SUMMARY

Yellow Flag Iris (YFI; *Iris pseudacorus* L.) is a non-native, invasive weed that is causing substantial changes to the ecology of the Niobrara River and the adjacent riparian habitat. This study is part of a larger study focused on YFI management at Agate Fossil Beds National Monument (AGFO), Harrison, Nebraska. Early observations indicated that YFI abundance was high at AGFO but lower on working cattle ranches surrounding AGFO. It was speculated that one possible reason for the apparent difference in YFI abundance between the monument and areas grazed by cattle might be that cattle remove enough of the plant canopy, resulting in increased temperature and less shade at the soil surface, which may not be conducive to YFI germination and establishment. In attempt to identify what effect cattle might be having on YFI, a greenhouse study was conducted at Colorado State University to determine how shade, temperature, and seed scarification affect the emergence and growth of containerized YFI seedlings. Two greenhouses with differing temperatures were used for this study. Half of the YFI seedlings in each greenhouse were grown in shaded conditions and the other half were grown in full sun. Half of the seeds were scarified with xylene in an attempt to increase germination rates (Sutherland 1990). Results indicated that seedling emergence in the greenhouse was affected by temperature ($P < 0.0001$). Emergence of YFI was (mean ± SE) 35.5% ± 3.3 in the warm greenhouse versus 57.8% ± 3.3 in the cool greenhouse. Shade increased emergence to 55.0% ± 3.5 versus 38.3% ± 3.53 in full sun ($P = 0.0105$). Seed scarification did not affect emergence ($P = 0.9412$). The interactions among temperature, light, and seed scarification also did not affect emergence ($0.3435 < P < 0.8828$). Plant height was greater in the warm greenhouse (21.9 ± 0.5 cm) than in the cool greenhouse (17.5 ± 0.5 cm; $P = 0.0001$). Plant height was greater in shade (23.1 ± 0.4 cm) versus full sun (16.3 ± 0.4 cm; $P < 0.0001$). Plant height was not affected by seed scarification ($P = 0.9350$) or any of the interactions among

temperature, light, and seed scarification (0.3959 < P < 0.8441). Leaf blade count was not affected by temperature (P = 0.5362), but was lower in full sun (5.05 ± 0.3 leaf blades per plant) than in shade (5.94 ± 0.3 leaf blades per plant; P = 0.0266). The number of leaf blades was not affected by scarification (P = 0.5729) or any of the interactions among temperature, light, and seed scarification (0.3623 < P < 0.9928).

1. INTRODUCTION

Yellow flag iris (YFI) (*Iris pseudacorus* L.) is a non-native, invasive weed in North America that is usually found in wetland areas, and is native to Europe, North Africa, and Western Asia. This invasive species is distributed in almost all fifty states across the U.S. (USDA NRCS PLANTS Database 2016). Primarily used as an ornamental plant, YFI has escaped cultivation and has become invasive. In Nebraska, there is currently a YFI infestation along the Niobrara River in and near Agate Fossil Beds National Monument (AGFO). Agate Fossil Beds National Monument is a park unit managed by the National Park Service (NPS). The Niobrara River is a perennial, spring-fed river system. The headwaters for this river occur approximately 80 km west of AGFO near Torrington, Wyoming. The USGS Nonindigenous Aquatic Species database (2013) states that the rhizomatous growth associated with YFI can influence changes in channel geometry by narrowing the channel width by up to 25 cm per year. Negative impacts of YFI on aquatic ecosystems have been related to its uptake and mass release of heavy metals, bacteria, total nitrogen, total phosphorus, ammonium, and other nutrients (Xu et al. 2015, Wu et al. 2011). Absorption of metals, nutrients, and bacteria make YFI a preferred species for use in phytoremediation (Xu et al. 2015).

Since 1979, the NPS has been monitoring the composition and density of aquatic wildlife and has noted a decline in the number and species richness of fish at AGFO (Pegg and Pope 2008). In addition to the decline in fish, native riparian area plant species are being replaced by YFI, which in turn affects waterfowl, fish, and domestic livestock. In 1989 the Niobrara River flowing through AGFO had eight fish species present [one species was a non-native brown trout (*Salmo trutta*)] and by 2011 only three fish species were recorded [one was a non-native Northern pike (*Esox lucius*)] (Pegg and Pope 2008). These changes to the aquatic wildlife have been accompanied by vegetation changes in the riparian area along the Niobrara River driven by YFI. It was suggested that the YFI invasion might have brought about the observed changes in aquatic wildlife (N. Medley, personal communication, May 2013). Observations of the Niobrara River within the National Monument reveal that the river channel is deeper and narrower when compared to upstream, non-infested river locations. Outside of the YFI infestation, the river has more native vegetation occupying the riparian area, which provides habitat for wildlife species. The dense rhizomes of YFI and lack of heterogeneity in the riparian area vegetation could be reducing preferred habitat for wildlife species and be a contributing factor to the decline in fish.

Vegetation shifts from cattails to YFI appear to be far less drastic in areas where domestic cattle are, and have been grazing. In areas grazed by cattle, plant communities dominated by native plants are more common and YFI is less abundant. On the national monument where there has been no livestock grazing for 50+ years, YFI abundance is high. We speculated that one possible reason for the obvious difference in YFI abundance between the monument and areas grazed by cattle might be that cattle remove enough of the plant canopy, resulting in increased temperature and less shade at the soil surface, which may not be conducive to YFI germination, establishment and growth.

Given the negative impacts of YFI on the Niobrara River ecosystem at AGFO, the National Park Service wanted to identify management strategies to effectively reduce the YFI population. Individuals at NPS felt that partnering on a research project to evaluate different treatment options was the best way to identify effective management methods. The NPS and a faculty member from the Department of Forest and Rangeland Stewardship at Colorado State University (CSU) began collaborating on treatment tools for management of YFI.

Local managers and our research team observed shifts in plant species composition in the riparian areas along the Niobrara River at AGFO. Many areas once dominated by cattails (*Typha latifolia* L.) and various other native riparian wetland plants now support monocultures of YFI. This study is an attempt to identify the ideal conditions for YFI emergence and growth. If managers know what the preferred YFI growing conditions are, and YFI appears in an area, they can work to manage for conditions that favor native species over YFI.

This thesis chapter will address two objectives that seek determine the effects of shade, light, and seed scarification on YFI emergence and growth.

Objective 1: Determine the effects of temperature and shade on YFI seedling emergence and growth.

Hypothesis 1: YFI seedling emergence, leaf number, and plant height will be greatest in the cool/shaded conditions, intermediate in the cool/full sun and warm/shaded conditions and least in the warm/full sun conditions.

Objective 2: Determine whether or not seed scarification consistently increases YFI seedling emergence across shade and temperature treatments.

Hypothesis 2: YFI seed germination rates will be consistently greater in seeds that were scarified with xylene compared to seeds that were not treated with xylene across shade and temperature treatments.

2. METHODS

The objectives of this study were addressed by performing a greenhouse experiment on containerized plants grown from field-collected seed. Eight treatments, that included all possible combinations of temperature (cool greenhouse/warm greenhouse), light (full sun/ 50% shade), and scarification (xylene treatment / no xylene treatment) were used during this study. The random number generator feature in Microsoft Excel was used to randomly assign a treatment to each experimental unit. Each treatment was replicated 6 times. This study occurred at the Colorado State University Greenhouse from February, 2015 to June, 2015 in Fort Collins, Colorado.

2.1 Study Site:

Wild grown YFI seeds were collected from AGFO at an elevation of approximately 1,370 meters above sea level (MASL). The mean annual precipitation ranges from 36 to 41 cm and the mean annual air temperature ranges from 7 to 9 °C (USDA NRCS 2013). Located in the northern mixed grass prairie ecosystem, AGFO occurs on soils that are predominantly sandy loam to loam textures (USDA NRCS Web Soil Survey 2016). The Niobrara River flows through the National Monument and is a perennial, spring-fed, sinuous river system with an average flow of 0.4 $m^3 \cdot s^{-1}$ (1958-1991; the USGS stream gauge was reactivated in February of 2014; USGS National Water Information System 2016). The riparian area vegetation includes monocultures of YFI, dense cattail stands, narrowleaf willow (*Salix exigua* Nutt.), native graminoids including woolly sedge (*Carex pellita* Muhl. Ex Willd.), Arctic rush (*Juncus arcticus*), western wheatgrass (*Pascopyrum smithii* (Rydb.) Á. Löve), and areas of Kentucky bluegrass (*Poa pratensis* L.) (Prowatzke and Wilson 2015). Plant nomenclature follows the USDA Plants database (USDA NRCS PLANTS Database 2016).

Approximately 6,000 YFI seeds were collected in October of 2014 from AGFO, which is outside of Harrison, Nebraska (UTM Zone 13T 604131 E 4697768 N). Mature, hard seeds were collected from AGFO during October 2014.

2.2 Treatments:

After seed collection, seeds were placed in cold storage at 2.8 °C for 5 months to cold stratify seeds and mimic winter conditions before planting. Gedebo and Froud-Williams (1998) stated that the effects of chilling and seed scarification ensured YFI seed germination in the spring at a wide range of temperatures, though our stratification temperature was colder than the temperature used by Gedebo and Froud-Williams (1998). Sutherland (1990) reported that scarifying seeds in xylene would increase germination rates by 80%. In February of 2015, cold stratified seeds randomly selected for the scarification treatment were submerged in xylene for 6 minutes under a ventilation hood, then washed in a deionized water bath for 5 minutes, allowed to dry and were planted 1 hour later at the CSU greenhouse.

Nine hundred and sixty cylindrical pots that measured 6.4 cm in diameter by 35.6 cm deep were completely filled with potting soil (Pro Mix BX®). Racks of 20 pots (pots = subsamples) each were used as experimental units, and 6 racks (replicates) were randomly assigned to each treatment. Two YFI seeds were planted within 1 cm of the soil surface in each pot. If a second seed in a pot germinated, the second shoot was harvested after recording emergence to reduce competition within each pot. The racks were put into clear plastic bins and the bottom of the bin was filled with 14 cm of water. Plants were uniformly fertilized as needed throughout the experiment. Two greenhouses were used for this study. The cool greenhouse temperature was maintained at 22 ± 8°C and the warm greenhouse temperature was maintained at 25 ± 10°C. Supplemental light was supplied by high pressure 400 watt sodium lamps to maintain a 14-h photoperiod.

Forty-eight racks were used during this study. Twenty-four racks were randomly assigned to the cool greenhouse and 24 racks were randomly assigned to the warm greenhouse. Of the 24 racks that were placed in the cool greenhouse, 12 were randomly assigned to be exposed to 50% shade and 12 were randomly assigned to be exposed to full sunlight. The same separation of racks occurred in the warm greenhouse. To provide the shade treatment a 2-cm diameter metal conduit frame was constructed 91-cm above the racks, and a black shade cloth that allowed 50% of light to pass through was placed over the frame and anchored to the conduit with plastic ties. The frame was constructed 91-cm above the racks so the shade cloth would not interfere with plant growth.

Figure 3. The pots and rack system that was used for the greenhouse shade and temperature study. Twenty cylindrical cones in a rack shown below constituted one replication and each rack was placed in a clear plastic bin. Each plastic bin was filled with 14 cm of water.

2.3 Data Collection:

Measurements were taken every 3 days for the duration of the study. Measurements included emergence, plant height (cm), and leaf blade count. The water level was recorded and replenished, as needed. Emergence was recorded as “yes” or “no” depending on whether or not a YFI seedling was visible. Plant height of the tallest leaf tip (cm) in each pot was recorded. Leaf blade count was taken by counting each leaf blade that was present, in each pot.

2.4 Statistical Analysis:

Two separate greenhouses (warm and cool) were used to impose the temperature treatments, and in each greenhouse, all racks of shaded plants were grouped together and all racks of full-sun plants were grouped together, resulting in a split-split-plot design. As a result, whole plot treatment was temperature and the sub-plot treatment was light. There were no restrictions on randomization of the xylene treatments except for those imposed by temperature and light treatments.

Analysis of Variance (ANOVA) was used to determine the effects of temperature, light, seed scarification (xylene) and all interactions on YFI seedling emergence, leaf blade count, and height. Residuals were inspected to confirm that assumptions of the analyses were met. Treatment means were compared only if the ANOVA yielded a significant F-test. All analyses were conducted using JMP® version 12.0 (JMP®) and $\alpha = 0.05$.

3. RESULTS

Results indicated that YFI seedling emergence in the greenhouse was affected by temperature ($P < 0.0001$) and light ($P = 0.0105$), but not by seed scarification ($P = 0.9412$) or any of the interactions ($0.3435 < P < 0.8828$). Emergence of YFI (mean ± SE) was lower (35.5% ± 3.3) in the warm greenhouse versus the cool greenhouse (57.8% ± 3.3; $P < 0.0001$). Shaded conditions increased emergence to 55.0% ± 3.5 versus 38.3% ± 3.53 in full sun ($P = 0.0105$). Plant height was affected by temperature ($P = 0.0001$) and light ($P < 0.0001$), but not by seed scarification ($P = 0.9350$) or any of the interactions ($0.3959 < P < 0.9350$). Plants were taller (21.9 ± 0.5 cm) in the warm greenhouse than in the cool greenhouse (17.5 ± 0.5 cm; $P = 0.0001$). Shaded conditions increased plant height to 23.1 ± 0.4 cm versus 16.3 ± 0.4 cm in the full sun conditions ($P < 0.0001$). Leaf blade count was not effected by temperature ($P = 0.5362$) or any of the interactions ($0.3623 < P < 0.9928$). Light affected leaf blade count ($P = 0.0266$), resulting in 5.05 ± 0.3 leaf blades per plant in full sun compared to 5.94 ± 0.3 leaf blades per plant in the shade.

4. DISCUSSION

Addressing hypothesis 1, YFI seedling emergence was clearly favored by cool, shady growing conditions. In full sun conditions YFI emergence was the lowest. Therefore, it is possible that conditions which maximize the potential for shade will favor yellow flag iris seedling emergence. Plant height was maximized in warm and shady environments. It is not surprising that warm temperatures favored yellow flag iris growth, but it is interesting that the effects of shade persisted to affect plant height at the end of the study. Germination of YFI did not increase in the seeds that were scarified with xylene, contrary to hypothesis 2. This was a surprise given the success that Sutherland (1990) observed when seeds were scarified with xylene. Germination rates were 30-60% across all treatment types. One potential explanation for the reduced germination in scarified seeds was seed storage. Grime et al. (1981) found that germination of freshly collected seed was reduced from 48% to 8% after 3 months of storage at 5 °C. Because our seeds were stored at 2.8 °C for 5 months, this cold stratification could have reduced the viability of the seed before it was scarified with xylene. Leaf blade count was only affected by full sun conditions (P = 0.0266), and not by any of the other treatments. However, the difference between shade and full sun was only one leaf per plant. Leaf blade count may not be a good indicator of treatment effects.

This study indicated that YFI prefer to germinate in cool shaded environments and growth is maximized in warm shaded environments. This is an important finding as the riparian area along the Niobrara River is dominated by cattails. Due to cool springtime air temperatures at AGFO, and shade that is provided by cattails, a cool shaded environment is present in many parts of the national monument, which is conducive to YFI seedling germination. Later in the growing season, the summer and early fall air temperatures are warm and cattails and other mature vegetation provide a shaded environment. The combination of warm air temperatures and shade from YFI and other mature vegetation provide an environment that is conducive to YFI growth. As previously mentioned, we noted a shift from cattails to YFI in certain areas and this study provides an indicator that cattails, shrubs and ungrazed herbaceous vegetation are creating a preferred microclimate for YFI. Through time, these areas will provide the ideal growing conditions for YFI seedling establishment and growth. Once YFI invades an area and becomes the dominant species, they appear to provide a beneficial microclimate which will perpetuate the growth of this plant.

The findings of this study also speak to our initial field observations in the spring of 2013 at AGFO. At that time, we observed that YFI density was lower on the cattle ranches compared to the national monument. This study likely provides a partial explanation of why YFI density is lower on the ranches. The variation in YFI density on the ranches can likely be attributed to the vegetation height being lower due to grazing. Because the canopy has been removed by grazing, sunlight is likely reaching the soil surface altering the growing conditions in a manner that is less favorable to YFI compared to the conditions in AGFO which has not been grazed by livestock for decades.

5. MANAGEMENT IMPLICATIONS

Though light and temperature likely only explain a portion of the YFI issue, land managers should think about ways to manage the plant canopy to reduce shaded microclimates for YFI emergence and growth. More light reaching the soil surface combined with ambient temperatures at AGFO may create an environment that is less favorable to YFI.

Differences in temperature and light could be part of the reason that yellow flag iris abundance is lower in riparian areas grazed by domestic livestock both upstream and downstream of AGFO. Areas grazed by domestic livestock would likely have a shorter vegetation height which provides more light and less shade to the soil surface.

LITERATURE CITED

Grime, J. P., G. Mason, A.V. Curtis, J. Rodman, and S.R. Band. 1981. A comparative study of germination characteristics in a local flora. The Journal of Ecology 1017-1059.

Gedebo, A., R. J.Froud-Williams, A. Monteiro, T. Vasconcelos, and L. Catarino. 1998. Seed biology of Impatiens glandulifera, Typha latifolia and Iris pseudacorus. In Management and ecology of aquatic plants. Proceedings of the 10th EWRS International Symposium on Aquatic Weeds, Lisbon, Portugal, 21-25 September 1998. (pp. 55-58). European Weed Research Society.

JMP®, Version 12. SAS Institute Inc., Cary, NC, 1989-2007.

Medley, N. 2013. Personal communication. Fisheries Biologist. USNPS Natural Resources Stewardship and Science Directorate.

Pegg, M. and K. Pope. 2008. Agate Fossil Beds National Monument Fish Inventory 2008 Survey. Final Report.

Prowatzke, M. and S. K. Wilson. 2015. Plant community composition and structure monitoring for Agate Fossil Beds National Monument: 2014 annual report. Natural Resource Data Series NPS/NGPN/NRDS—2015/774. National Park Service, Fort Collins, Colorado.

Sutherland, W.J. 1990. Biological flora of the British Isles: *Iris pseudacorus* L. Journal of Ecology 78(3): 833-848.

United States Department of Agriculture (USDA), Natural Resource Conservation Service (NRCS), PLANTS Database. Available online at: http://plants.usda.gov. [2016, March 16].

United States Department of Agriculture, Natural Resources Conservation Service, and United States Department of the Interior, National Park Service. 2013. Soil survey of Agate Fossil Beds National Monument, Nebraska. (Accessible online at: http://soils.usda.gov/survey/printed_surveys/).

United States Department of Agriculture, Natural Resources Conservation Service. Web Soil Survey. Available at:6http://websoilsurvey.sc.egov.usda.gov/App/WebSoilSurvey.aspx. [2016, March 10].

United States Department of the Interior, United States Geological Survey (USGS), N. (2013). United States Geological Survey-Nonindigenous Aquatic Species database (USGS NAS). [2016, May 10].

United States Department of the Interior, United States Geological Survey (USGS). National Water Information System. Available at: http://waterdata.usgs.gov/nwis/rt.[2016, May 10].

Wu H., J. Zhang, P. Li, J. Zhang, H. Xie, B. Zhang. 2011. Nutrient Removal in Constructed Microcosm Wetlands for Treating polluted River Water in Northern China. Ecological Engineering 37(4):560-568.

Xu B., S. Yu, J. Ding, S. Wu, J. Ma. 2015. Metal-Dependent Root Iron Plaque Effects on Distribution and Translocation of Chromium and Nickel in Yellow Flag (*Iris pseudacorus* L.). Journal of Phytoremediation 17(1-6):175-81.

EXPERIMENT #3: SIMULATED TRAMPLING REDUCES YELLOW FLAG IRIS SURVIVAL AND GROWTH

SUMMARY

Yellow flag iris (YFI) (*Iris pseudacorus* L.) is a non-native, noxious weed that is causing substantial changes to the ecology of wetland and riparian areas. Due to the limited options for invasive weed management in wetlands and based on observations from earlier work, we explored a non-chemical approach for YFI management. In prior studies on YFI, we noted the development of footpaths around our field study plots. As a result, we decided to conduct both field and greenhouse studies to explore the effects of trampling on YFI and determine if trampling might be an effective tool to reduce YFI abundance. When greenhouse grown YFI plants reached 15-30 cm in height, two groups were "trampled" using a piece of 2 cm diameter metal pipe. Treatments included trampling at the plant crown, trampling 1-2 cm above the plant crown, and a non-trampled control. Three months after treatment, we quantified plant survival and growth (height and number of leaves). We also conducted a field study to determine the effects of trampling on shoot density and height of established YFI in the riparian area adjacent to the Niobrara River, at Agate Fossil Beds National Monument (AGFO) in western Nebraska. Because permitting requirements prevented bringing cattle into the national monument in a reasonable timeframe, we implemented a citizen science project using humans to trample YFI. The Harrison High School (Nebraska), Future Farmers of America Chapter, volunteered to help us trample YFI for this study. Sixteen, 2- x 2- m study plots were randomly located in YFI infestations along the Niobrara River. Half of each plot was trampled in June 2015 and the other half was an untreated control. Iris density and height were recorded 3 months after treatment. Trampling decreased iris density by 75% ($P = 0.0002$) and plant height by 58% ($P = 0.0001$). Trampling containerized, greenhouse grown seedlings at the crown was five times more likely to kill YFI than trampling 1-2 cm above the crown ($P = 0.0001$). Greenhouse study results indicate trampling reduced the number of leaf blades ($P < 0.0001$) and plant height (cm) (P <

0.0001). Though the findings of this project are promising, trampling has only occurred in the greenhouse setting and under the human foot. In certain situations YFI trampling with humans could be an effective treatment option, but in other locations and at larger scales, research utilizing trampling with large ungulates could be a management consideration.

1. INTRODUCTION

Yellow flag iris (YFI) (*Iris pseudacorus* L.) is a non-native, invasive weed in North America that is usually found in wetland areas, and is native to Europe, North Africa, and Western Asia. This invasive species is found in 40 out of 50 states across the U.S. (USDA NRCS PLANTS Database 2016). Primarily used as an ornamental plant, YFI has escaped cultivation to become a problematic invasive species. In Nebraska, there is currently a YFI infestation along the Niobrara River in and near Agate Fossil Beds National Monument (AGFO). Agate Fossil Beds National Monument is a park unit managed by the National Park Service (NPS). The Niobrara River is a perennial, spring-fed river system. The headwaters for this river occur approximately 80 km west of AGFO near Torrington, Wyoming. United States Geological Survey Nonindigenous Species database (2013) stated that the rhizomatous growth associated with YFI can influence changes in channel geometry by narrowing the channel width by up to 25 cm per year. Negative impacts of YFI on aquatic ecosystems related to its uptake and mass release of heavy metals, bacteria, total nitrogen, total phosphorus, ammonium, and other nutrients have been reported (Xu et al. 2015, Wu et al. 2011). The ability to absorb metals, nutrients, and reduce bacteria loads make YFI a preferred species for use in phytoremediation (Xu et al. 2015, Sutherland 1990).

Since 1979 the NPS has been monitoring the composition and density of aquatic wildlife and has noted a decline in the number and species richness of fish (Pegg and Pope 2008). In addition to the decline in fish, native riparian plant communities are being replaced by YFI, which in turn affects waterfowl, fish, and domestic livestock. In 1989 the Niobrara River flowing through AGFO had eight fish species present [one species was a non-native brown trout (*Salmo trutta*)] and by 2011 only three fish species were recorded [one was a non-native Northern pike (*Esox lucius*)] (Pegg and Pope 2008). These changes to the aquatic wildlife have been accompanied by vegetation changes in the riparian area along the Niobrara River driven by YFI. It was suggested that the YFI invasion might have brought about the observed changes in aquatic wildlife (N. Medley, personal communication, May 2013). Observations during this research and from National Park Service staff reveal that the Niobrara River channel is deeper and narrower within the national monument when compared to upstream, non-infested river locations. Outside of the YFI infestation, the river has more native vegetation occupying the riparian area, which provides habitat for wildlife. The dense rhizomes of YFI and lack of heterogeneity in the riparian area vegetation could be reducing preferred habitat for wildlife and be a contributing factor to the decline in fish.

Given the negative impacts of YFI on the Niobrara River ecosystem at AGFO, the National Park Service wanted to identify a management strategy to effectively reduce the YFI population. Individuals with NPS felt that partnering on a research project to evaluate different treatment options was the best way to identify effective management methods. The NPS and a faculty member from the Department of Forest and Rangeland Stewardship at Colorado State University (CSU) began collaborating on treatment tools for management of YFI.

During the initial visit to AGFO in the summer of 2013, it was noted that YFI was less abundant on the working cattle ranches that surround the National Monument. As a result of this observation, research studies attempted to identify clues or indicators of the effects livestock might be having on YFI abundance. The most important observation was made in the summer of 2014 during Experiment #1 (Chapter 1 of this thesis). From April to June of 2014, two to three people walked around the study plots approximately six times while establishing the plots and taking density measurements. By June, a distinct foot path was obvious around the plots where people had previously walked. We observed a large reduction in YFI abundance from such a small amount of disturbance. As a result of this observation, seed was harvested from AGFO in October of 2014 to be used for a greenhouse study on the effects of simulated trampling on YFI. While the greenhouse study was underway, we began exploring the possibility of a field study with livestock at AGFO to attempt to quantify the effects of trampling on YFI abundance. Using livestock to trample established YFI was found to be infeasible, so we identified an alternative approach to study the effects of trampling. This thesis chapter will report on two simulated trampling studies; one greenhouse study and one field study simulating livestock trampling.

Objective 1: Determine the effects of simulated trampling on YFI seedling survival and growth.

Hypothesis 1.1: Survival of YFI seedlings will be greatest for untrampled plants, intermediate for plants trampled high (above the crown) and lowest for plants trampled low (at the crown).

Hypothesis 1.2: YFI plant height and leaf number will be greatest for untrampled plants, intermediate for plants trampled high (above the crown) and lowest for plants trampled low (at the crown)

Objective 2: Determine the effects of trampling on density and height of established YFI plants in the field.

Hypothesis 2.1: Trampling established YFI in the field will reduce both YFI density and plant height.

2. METHODS

2.1 Study Area:

To address objective 1, a greenhouse experiment took place at the Colorado State University Greenhouse from June 26, 2015 to September 1, 2015, in Fort Collins, Colorado. Seedlings from this study were grown from YFI seed collected at AGFO. The field trampling study occurred at AGFO. A site description of AGFO is included below in section 2.2 “Treatments”.

2.2 Treatments:

Greenhouse trampling experiment

Seedlings used in this study were grown from wild YFI seed collected at AGFO. Agate Fossil Beds National Monument has an average elevation of 1,372 meters above sea level. The mean annual precipitation ranges from 36 to 41 cm and the mean annual air temperature ranges from 7 to 9 °C (USDA NRCS 2013). Located in the northern mixed grass prairie ecosystem, AGFO occurs on soils that are predominantly sandy loam to loam textures (USDA NRCS Web Soil Survey, 2016). The Niobrara River flows through the National Monument and is a perennial, spring-fed, sinuous river system with an average flow of 0.4 $m^3 \cdot s^{-1}$ (1958-1991; the USGS stream gauge was reactivated in February of 2014) (USGS National Water Information System 2016). The riparian area vegetation includes monocultures of YFI, dense cattail (*Typha latifolia* L.) stands, narrowleaf willow (*Salix exigua* Nutt.), native graminoids including woolly sedge (*Carex pellita* Muhl. Ex Willd.), Arctic rush (*Juncus arcticus*), western wheatgrass (*Pascopyrum smithii* (Rydb.) Á. Löve), and areas of Kentucky bluegrass (*Poa pratensis* L.) (Prowatzke and Wilson 2015). Plant nomenclature follows the USDA Plants database (USDA NRCS PLANTS Database 2016).

Approximately 6,000 YFI seeds were collected in October of 2014 from AGFO, which is outside of Harrison, Nebraska (UTM Zone 13T 604131 E 4697768 N). Mature, hard seeds were collected from four different sites at AGFO during October 2014.

After seed collection, seeds were placed in cold storage at 2.8 °C for 5 months to cold stratify the seeds and mimic winter conditions before planting. Gedebo and Froud-Williams (1998) stated that the effects of chilling and seed scarification ensured YFI seed germination in the spring at a wide range of temperatures, though our stratification temperature was colder than the temperature used by Gedebo and Froud-Williams (1998). In February of 2015, 60 cylindrical pots which measured 6.4 cm in diameter by 35.6 cm deep were completely filled with potting soil (Pro Mix BX®). Two YFI seeds were planted within 1 cm of the soil surface in each pot. If a second seed in a pot germinated, the second shoot was harvested to reduce competition within each pot. Each individual pot was placed into a rack that could hold 20 pots. The rack was put into a clear plastic bin and the bottom of the bin was filled with 14 cm of water. The plants were allowed to grow until they reached a height of 15 to 30 cm. Plants were fertilized as needed throughout the experiment. For the duration of the study, the greenhouse temperature was maintained at 22 ± 8°C. Supplemental light was supplied by high pressure 400 watt sodium laps to maintain a 14-h photoperiod.

The greenhouse trampling study examined the effects of trampling on YFI seedlings, which was designed to simulate the impacts of trampling at an early stage of YFI invasion. A completely randomized experimental design was utilized with three treatments; trampled high, trampled low and an un-trampled control. Twenty plants were randomly assigned to each of the three treatments. For plants assigned to the trampled low treatment, the lower edge of a 15-cm long and 2-cm in diameter metal pipe was placed as close to the crown as possible and then pushed into the potting soil approximately 5-cm. This most often resulted in breaking the shoot off at the crown. For plants assigned to the trampled high treatment, the pipe was placed on the leaf blade approximately 1- 2 cm above the crown and pushed into the potting soil approximately 5-cm. This treatment most often resulted in breaking the blade off approximately 2-cm above the crown. Simulated trampling was not applied to the control (un-trampled) plants. Sixty cylindrical pots, each containing one YFI plant were used during this study. The trampled high and low treatments were selected to provide information expected to be valuable for informing future decisions about methods that might be useful for YFI reduction efforts (e.g., mechanical treatments with rollers of varying design or use of animals with different body sizes).

Trampling Established YFI Plants- Field Study

This study was conducted in the wetlands along the Niobrara River at AGFO (the conditions at AGFO are described above in section 2.2 “Treatments”). Plots were located in the riparian area and the associated wetlands on the eastern half of the national monument. Some plots had a mix of cattails and willows, but YFI was the dominant plant in all study plots. Plots were located between 1 and 30 m from the main river channel on both the north and south banks of the river. Plot locations were variable and occurred in standing and flowing water, and in slightly drier areas just above bank full. We established sixteen plots in the wetland area of the Niobrara, measuring 2- x 2- m. Plots were placed in accessible areas where the highest YFI abundances were observed. Half (1- x 2- m) of each 2- x 2- m plot was randomly selected for trampling, and the other half was left as an un-trampled control, resulting in a split plot design. Iris plants were between 0.3- 0.9 m tall and the average density was 149.0 plants·m^{-2} when the trampling occurred on June 4, 2015.

The field trampling study was designed to focus on trampling established plants in a long standing infestation with a well-developed rhizome network. Permitting requirements prevented us from bringing livestock to AGFO soon enough to be included in this study, so we decided to implement a citizen science project to help with study treatments. The Future Farmers of America Chapter from Sioux County High School (NE), staff from the National Park Service, and the Sioux County Weed District assisted with plot trampling. On June 4, 2015 half (1 by 2 m) of each of the sixteen, 2- x 2- m plots was trampled. Each 1- x 2- m area (half of each plot) was trampled for 5 minutes, by two to three people weighing between 50 - 100 kg. YFI plants were in both the growth and bud stage when trampling occurred. The goal was to break every YFI shoot in the treatment plot as close as possible to the rhizomatous mat; ideally the plants would break at, or near the crown. The greenhouse simulated trampling study indicated that the trampled low treatment group, in which YFI shoots were damaged at or near the crown was the most detrimental to the iris. Based on these greenhouse findings, the field trampling study focused on breaking the iris off as close as possible to the crown.

2.3 Data Collection:

Greenhouse trampling experiment

Plant heights and leaf blade counts were measured and recorded on a bi-weekly basis for the duration of the study. When the study concluded on September 1, 2015 final plant height (cm), leaf blade count, and the survival data were recorded. Plant height was recorded by measuring from the soil surface to the tip of the tallest leaf.

Trampling Established YFI Plants- Field Study

Initial YFI density data were collected on June 3, 2015, 1 day before trampling to characterize the plots. Density counts were conducted using 2, 1-m^2 frames to outline the 1- x 2- m area being sampled. Each YFI shoot was counted to determine YFI shoot density. Yellow flag iris was distinguished from cattail by the distinct point that forms at the tip of YFI leaves and/or the presence of equitant leaves. The cattails featured a rounded tip on the leaf blade and later in the growing season grew taller than YFI. Individual YFI plants were identified by tracing leaves back to the plant base to ensure that we counted shoots and not leaves. These characteristics made it possible to distinguish the different plants. The heights of YFI plants were measured by placing a tape measure on top of the rhizomatous mat and stretching it to the top of a leaf that represented the average YFI height in each of the half plots. Density and height data for YFI were collected in September of 2015.

Figure 4. The pots and rack system used for the greenhouse trampling study. Twenty cylindrical cones constituted one rack and each rack was placed in a clear plastic bin. Each plastic bin was filled with 14 cm of water.

2.4 Statistical Analysis:

Greenhouse trampling experiment

Logistic regression was used to determine the effects of trampling on plant survival. Odds ratios from the logistic regression were used to characterize the effects of trampling on YFI survival. Analysis of Variance (ANOVA) was used to determine the effects of trampling on plant height (cm) and the number of leaf blades. Residuals were inspected to ensure that assumptions of the analyses were met. Following the ANOVA, a Tukey HSD (Honest Significant Difference) adjustment was performed to compare means where F-tests indicated significant treatment effects. All analyses were conducted using JMP® version 12.0 (JMP®) with $\alpha = 0.05$.

Trampling Established YFI Plants- Field Study

An ANOVA was conducted to determine the effects of trampling on YFI density and plant height. Given the design of this study, the ANOVA essentially resulted in a paired t-test. Residuals were inspected to ensure that assumptions of the analysis were met. The analysis was conducted using JMP® version 12.0 (JMP®) with $\alpha = 0.05$.

3. RESULTS

Greenhouse trampling study

The logistic regression indicated that trampling influenced seedling survival ($P < 0.0001$). According to the odds ratios from the logistic regression, simulated trampling at the plant crown (the trampled low treatment) was 36 million times more likely to kill the YFI seedlings than the un-trampled (control) ($P < 0.0001$). Plants that were trampled high were 7 million times more likely to die than the un-trampled plants ($P < 0.0144$). Finally, YFI plants that were trampled low were over five times more likely to die than those in the trampled high treatment ($P = 0.0203$). The odds ratios for YFI survival were greatly increased due to the fact that 100% of the control plants survived, and should be interpreted with that fact in mind.

Trampling affected the number of leaf blades ($P < 0.0001$) and plant height (cm) ($P < 0.0001$) (Table 3). Both measures of plant size were greatest in the un-trampled control group, intermediate when YFI plats were trampled high and least when YFI plants were trampled low (Table 3).

Trampling Established YFI Plants- Field Study

One of the 16 plots was a clear outlier and was omitted from the statistical analysis. When assessing a QQ plot and line of best fit in JMP, plot 48 was a clear outlier. Plot 48 was considered an outlier due to elevated shoot density (1761 YFI shoots), the apparent lack of anaerobic conditions compared to other plots, and having more soil present. The other 15 plots were located in areas that experience saturated, anaerobic conditions for most of the growing season. Plant height and density of YFI were affected by trampling. Plant density was decreased by 75% in the trampled plots and the mean was 45 ± 10 plants·m^{-2} versus 178 ± 10 plants·m^{-2} in the untrampled plots ($P = 0.0002$; mean ± SE, n=15). Plant height was decreased by 58% in the trampled portions of the plots (0.7 ± 0.04 m) versus non-trampled portions of plots (1.2 ± 0.04 m; $P < 0.0001$).

Table 3. Mean number of leaves per plant and mean plant height for yellow flag iris (*Iris pseudacorus* L.) (mean ± SE, n=20) grown in a greenhouse and subjected to simulated trampling at the plant crown (low), 2-3 cm above plant crown (high) and un-trampled plants. Analysis of variance indicated significant effects of trampling on leaf number ($P < 0.0001$) and plant height ($P < 0.0001$). Means in a column with the same uppercase letter are not different (Tukey HSD, α=0.05).

Treatment	Mean # of Leaves per Plant	Mean Plant Height (cm)
Un-trampled	6.4 ± 0.5 A	52.5 ± 2.6 A
Trampled High	4.5 ± 0.5 B	31.7 ± 2.6 B
Trampled Low	2.3 ± 0.5 C	14.6 ± 2.6 C

4. DISCUSSION

In the greenhouse trampling study the control plants experienced the greatest survival. These findings support objective 1, hypothesis 1.1. The plant height and leaf blade number had the greatest reduction in the trampled low treatment group. The trampled high group had a significant reduction in leaf height and number, and the un-trampled control experienced the greatest growth (plant height and leaf number). These findings support hypothesis 1.2.

Simulated trampling of YFI seedlings reduced survival, leaf blade count and trampling at or below the plant crown is five times more likely to kill the plant, which supports hypothesis 1.2. The plants that experienced trampling close to the crown resulted in the greatest reduction in plant height and number of leaf blades, followed by the plants that were trampled higher above the crown. Liebman et al. (2004) stated that seedlings are often the most vulnerable stage and may be damaged either by grazing or trampling. This could be due to the lack of above ground leaf material that could protect the plant crown. Land managers attempting to reduce YFI abundance may consider the young growth phase a vulnerable time for the YFI and capitalize on the plant vulnerabilities.

Further, our results on YFI are similar to the findings of Anderson (1991), who stated that defoliation of blue bunch wheatgrass [*Pseudoroegneria spicata* (Pursh) Á. Löve] to short stubble heights during the boot stage could essentially eliminate plants within a few years. By simulating trampling just above, or at the crown, and then pushing the residual plant material 5-cm into the soil, we showed that damage at, near, or below the critical growth point can effectively kill YFI seedlings in one growing season.

Finally, research completed on western rangelands by Noy-Meir (1993) found that removing the crown negatively affected plant growth. The low trampling treatment likely had the greatest effect on YFI survival due to the metal pipe being placed as close as possible to the crown to remove all of the plant material above that point. With the high trampling treatment the metal pipe was placed approximately 1-2-cm above the plant crown. This treatment group had a reduction in survival but not as large as the low trampling treatment group.

The field trampling study supported objective 2, hypothesis 2.1. A one-time, short-duration trampling caused a reduction in YFI density and plant height at AGFO. Trampling can likely explain why iris abundance is lower on the working ranches that surround the national monument. Trampling, in combination with the findings from Experiment 2 about the effects of shade and temperature on YFI seedling emergence and growth (Chapter 2 of this thesis) can potentially aid land managers with efforts to decrease YFI abundance and prevent future re-invasion. Experiment 2 identified how YFI seeds preferred to germinate in cool shaded conditions, and grew better in warm shaded conditions. The growing conditions at AGFO likely provide preferred growing conditions similar to what we observed in the greenhouse. Cattails have dominated the riparian area in the past and likely created a cool shaded microclimate in the early spring which Experiment 2 (of this thesis) identified as the optimal conditions for YFI seedling emergence. Later in the growing season the cattails still provide shade and the air temperature can be very warm in the summer months at AGFO, creating the ideal YFI growing conditions. Other vegetation that provides shaded conditions (shrubs and tall, ungrazed herbaceous plants) likely also provide favorable growing conditions for YFI).

Together, findings from our field and greenhouse studies on trampling, shade, temperature, and seed scarification (Chapter 2 of this thesis) suggest that in areas grazed by livestock, the combination of disturbances could have a substantial effect on YFI abundance. Trampling can cause direct damage to YFI and removal of the plant canopy via grazing will allow sunlight to reach the soil surface. The variation in soil surface temperature and available light might be enough to alter the growing conditions for YFI and favor native plant species.

Flooding may be another factor in combination with trampling that may be shifting plant communities outside AGFO from co-dominance between forbs and graminoids to a persistent dominance of graminoids (Striker et al. 2011). Two floods were observed during the study period; however, were not analyzed to identify effects they may have had on the plant community. Nonetheless, the cattle ranches surrounding the national monument experience both disturbance types, whereas AGFO only experienced the effects of flooding and maintained high densities of YFI in the control plots. This observation suggests that a lack of trampling appears to be favoring YFI dominance at AGFO.

The combination greenhouse study and field trampling study yielded promising results for YFI management. The observed successful reduction of YFI density and plant height with simulated trampling could be a promising management tool for dealing with a mature stand of YFI. Gaskin et al. (2016) made the point that YFI managers need to limit seed dispersal and development of mature seeds in the field.

Gaskin et al. (2016) also stated that managers should prevent YFI plants from going to seed because seed dispersal and viability is a prolific dispersal mechanism compared to vegetative spread via rhizomes.

It was observed during the study that a small number of juvenile YFI plants would emerge after trampling; however, the plants would remain slender for the duration of the growing season and never flower or go to seed. This is similar to what Gryseels (1989) observed when YFI above ground vegetation was removed via mowing. Gryseels (1989) noted a slight vegetative spread after mowing but again the plants never flowered or went to seed. Both this study and the Gryseels (1989) study only lasted for a short duration so it is challenging to project what YFI will do after trampling in subsequent growing seasons. Another point that needs to be addressed is the time and timing of trampling. Research plots were trampled in June and only for a short duration. Looking at the effects of a spring trampling event or multiple trampling events per year should be evaluated. Clary (1995) reported that spring, or spring and fall trampling reduced graminoid height by an average of 19.8% (39.4 vs. 48.8 cm) compared to only trampling in the fall. Clary (1995) also observed an interaction between trampling and season of disturbance in a riparian environment. Spring trampling events had the greatest effect on graminoid height, though forb height was not affected by this treatment combination (Clary 1995). Additional work could inform the best time to trample YFI, but anytime throughout the growing season that prevents YFI from going to seed is likely a good time to trample.

When looking at the effects of herbivores on forbs, Damhoureyeh and Hartnett (1997) observed that cattle and bison did not directly consume forb species in their study; rather, the reduced forb growth was attributed to physical trampling of the forbs. Because YFI is a forb and contains glycosides that make iris toxic for herbivores to consume (Stone 2009), it may not be reasonable to expect large herbivores to graze YFI much. Rather, managers should consider the direct impacts of trampling which is consistent with the findings of Damhoureyeh and Hartnett (1997) who reported that forbs can be reduced by physical trampling.

5. MANAGEMENT IMPLICATIONS

This trampling study sheds light on the potentially significant impacts of cattle trampling YFI plants and keeping this invasive plant in-check while allowing the native vegetation to flourish. Other factors may also be affecting YFI densities, such as flooding or herbivory; however, comparisons between sites suggest that trampling alone can reduce YFI enough to encourage native plant growth. It is important to reiterate that only one growing season worth of information is being reported and there is uncertainty as to what effects trampling will have on YFI abundance and height in subsequent growing seasons. Long-term research could help to further ascertain the effectiveness of trampling as a management method for YFI.

LITERATURE CITED

Anderson, L. D. 1991. Bluebunch wheatgrass defoliation, effects and recovery – A Review. BLM Technical Bulletin 91- 2, Bureau of Land Management, Idaho State Office.

Clary, W. P. 1995. Vegetation and soil responses to grazing simulation on riparian meadows. Journal of Range Management 48(1):18-25.

Damhoureyeh, S. and D. Hartnett. 1997. Effects of bison and cattle on growth, reproduction, and abundances of five tallgrass prairie forbs. American Journal of Botany 84:1719-1728.

Gaskin, J. F., M.L. Pokorny, and J.M. Mangold. 2016. An unusual case of seed dispersal in an invasive aquatic; yellow flag iris (*Iris pseudacorus*). Biological Invasions 1:1-9.

Gedebo, A., R. J.Froud-Williams, A. Monteiro, T. Vasconcelos, and L. Catarino. 1998. Seed biology of Impatiens glandulifera, Typha latifolia and Iris pseudacorus. In Management and ecology of aquatic plants. Proceedings of the 10th EWRS International Symposium on Aquatic Weeds, Lisbon, Portugal, 21-25 September 1998. (pp. 55-58). European Weed Research Society.

Gryseels, M. 1989. Nature Management Experiments in a Derelict Reedmarsh II: Effects of Summer Mowing. Biological Conservation 48:85-99.

JMP®, Version 12. SAS Institute Inc., Cary, NC, 1989-2007.

Liebman M, C.L. Mohler, and C.P. Staver, 2004. Ecological Management of Agricultural Weeds. Cambridge University Press. ISBN 0-511-01764-2 eBook.

Medley, N. 2013. Personal communication. Fisheries Biologist. USNPS Natural Resources Stewardship and Science Directorate.

Noy-Meir, I. 1993. Compensating Growth of Grazed Plants and Its Relevance to the Use of Rangelands. Ecological Applications 3(1):32–34.

Pegg, M.and K. Pope. 2008. Agate Fossil Beds National Monument Fish Inventory 2008 Survey. Final Report.

Prowatzke, M. and S. K. Wilson. 2015. Plant community composition and structure monitoring for Agate Fossil Beds National Monument: 2014 annual report. Natural Resource Data Series NPS/NGPN/NRDS—2015/774. National Park Service, Fort Collins, Colorado.

Stone, Katharine R. 2009. Iris pseudacorus. In: Fire Effects Information System, [Online]. U.S. Department of Agriculture, Forest Service, Rocky Mountain Research Station, Fire Sciences Laboratory (Producer). Available: http://www.fs.fed.us/database/feis/ [2016, April 23].

Striker, G.G., F.P.O. Mollard, A.A. Grimoldi, R.J.C. León and P. Insausti. 2011. Trampling enhances the dominance of graminoids over forbs in flooded grassland mesocosms. Applied Vegetation Science 14: 95–106.

United States Department of Agriculture, Natural Resource Conservation Service (NRCS), PLANTS Database. Available online at: http://plants.usda.gov. [2016, March 16].

United States Department of Agriculture, Natural Resources Conservation Service (NRCS), and United States Department of the Interior (USDOI), United States National Park Service (USNPS). 2013. Soil survey of Agate Fossil Beds National Monument, Nebraska. (Accessible online at: http://soils.usda.gov/survey/printed_surveys/).

United States Department of Agriculture, Natural Resource Conservation Service (NRCS), Web Soil Survey. Available online at: http://websoilsurvey.sc.egov.usda.gov/.[2016, March 16].

United States Department of the Interior, United States Geological Survey (USGS), N. (2013). United States Geological Survey-Nonindigenous Aquatic Species database (USGS NAS). [2016, March 10].

United States Department of the Interior, United States Geological Survey (USGS). National Water Information System. Available at: http://waterdata.usgs.gov/nwis/rt. [2016, March 16].

Wu H., J. Zhang, P. Li, J. Zhang, H. Xie and B. Zhang. 2011. Nutrient Removal in Constructed Microcosm Wetlands for Treating polluted River Water in Northern China. Ecological Engineering 37:560-568.

Xu B., S. Yu, J. Ding, S. Wu, J. Ma. 2015. Metal-Dependent Root Iron Plaque Effects on Distribution and Translocation of Chromium and Nickel in Yellow Flag (*Iris pseudacorus* L.). Journal of Phytoremediation 17:175-81.