1-1-2014

Experimental Assessment of Butomus Umbellatus L. Growth and Expansion Using a Mesocosm Approach

Christian Carter

Experimental assessment of *Butomus umbellatus* L. growth and expansion using a mesocosm approach

By

Christian Carter

A Thesis
Submitted to the Faculty of
Mississippi State University
in Partial Fulfillment of the Requirements
for the Degree of Master of Science
in Biological Sciences
in the Department of Biological Sciences

Mississippi State, Mississippi

August 2014

Experimental assessment of *Butomus umbellatus* L. growth and expansion using a mesocosm approach

By

Christian Carter

Approved:

_____

Gary N. Ervin
(Major Professor/Graduate Coordinator)

_____

Lisa Wallace
(Committee Member)

_____

John D. Madsen
(Committee Member)

_____

R. Gregory Dunaway
Professor and Dean
College of Arts and Sciences

Name: Christian Carter

Date of Degree: August 15, 2014

Institution: Mississippi State University

Major Field: Biological Sciences

Major Professor: Gary N. Ervin

Title of Study: Experimental assessment of *Butomus umbellatus* L. growth and expansion using a mesocosm approach

Pages in Study: 45

Candidate for Degree of Master of Science

Over the last century, flowering rush (*Butomus umbellatus* L.: Butomaceae) has escaped its native Eurasian range and has become a problematic species in North America. As an aquatic invasive species, flowering rush has degraded native wetlands and has interfered with human water usage. Although experimental work has been done regarding the reproductive biology of the species, few empirical studies regarding the ecology of the species have been conducted. The research reported here demonstrates that flowering rush is capable of aggressive clonal growth and propagation, and can perform well along a depth gradient from zero to 132cm. Proper management and control of invasive species relies on sound ecological knowledge of the target species, and this work aims to help gather that information.

ACKNOWLEDGEMENTS

I would like to thank those who assisted in the actualization of this work. Dr. Gary Ervin took me into his lab, provided excellent mentorship, and gave immeasurable support along the way, and I can't thank him enough. I would like to thank Dr. John Madsen for suggesting flowering rush as a study system, and for providing extensive resources, including the use of the mesocosm facility. Gray Turnage provided a great deal of support to the project and contributed great advice in regards to the use of mesocosms. Committee member Dr. Lisa Wallace provided helpful counsel and comments regarding this project. I would like to thank fellow students Tyler Schartel and Cory Shoemaker for being excellent office mates and helping me in any way they could. Finally I would like to acknowledge all of the mesocosm facility workers: Lee Bryant, Cody Blackwell, Olivia Osaji, Parker Davis, Trey Higginbotham, Trey Jackson, Logan White, Julie Gower, and John Perren for their contributions.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER I

INTRODUCTION

1.1 Preface

The world's freshwater systems are vital to human civilization. Water use is essential to the welfare and prosperity of human populations in their current state, and one study values the ecosystem services of freshwater systems as approximately 4.9 trillion U.S. dollars globally per year (Costanza et al. 1997).

A product of globalized trade and transit, alien invasive species threaten to alter local ecosystem function worldwide. In freshwater systems, pest plant species can directly influence human water activities by obstructing transport of irrigation and drainage channels, interfering with navigation, disrupting hydroelectric schemes, hastening sedimentation rates via silt entrapment, disturbing food production (pisciculture, agriculture), and hampering recreational activities, and indirectly by means of increasing evapotranspiration, and creating favorable conditions for disease vectors (e.g. mosquitoes) (Mitchell 1974, Pieterse and Murphy 1990).

As an invasive aquatic macrophyte, flowering rush (*Butomus umbellatus* L.) is capable of creating extensive monotypic stands which have the potential for significant negative ecosystem impacts in its invaded range. Having escaped its large native range, which spans both Europe and Asia, flowering rush has increased its range to include portions of both Europe and North America, where it is considered an invasive pest

species (Anderson et al. 1974, Bailey and Preston 2011, Kliber and Eckert 2005). Flowering rush invasion has been implicated to cause negative ecosystem impacts such as obstruction of water delivery, degradation of recreational waters, and reduction of biodiversity in native wetlands (Boutwell 1990, Countryman 1970, Marko et al. 2012). Its ability to form dense turf, which can float to the surface and accumulate floating material and sediment, may fill in littoral zones around the edges of bodies of water (Madsen et al. 2012).

The first recorded account of flowering rush in North America occurred in 1905 near Montreal along the St. Lawrence River (Core 1941, Countryman 1970). This species has since spread throughout much of the Great Lakes region, with isolated pockets throughout waterways in southern Canada and across the northern U.S. (Kliber and Eckert 2005). Escape from water gardens appears to be the most important process leading to both its introduction and continued spread; flowering rush was advertised and sold by William Tricker, Inc. ("America's oldest water garden specialist"), as early as 1897 (Les and Mehrhoff 1999). Despite flowering rush's designation as an invasive species, a recent survey of Ontario garden centers found one third of those businesses to be carrying flowering rush (Funnell et al. 2009), and a group in Minnesota found little difficulty having the plant delivered in state despite its prohibited status (Maki and Galatowitsch 2004).

1.2 Species Description

Flowering rush is a perennial rhizomatous monocot in the order Alismatales, belonging to the family Butomaceae. *Butomus* is the sole genus in the family, and most taxonomists consider *Butomus umbellatus* the only species, while some consider a second

species in *Butomus juncus* (Anderson et al. 1974). This genus is of particular interest to morphologists and systematists, as some consider flowering rush one of the most primitive monocots, and a possible link to the Magnoliidae, specifically the Nymphaeales (Singh and Sattler 1974). A recent sequencing of its mitochondrial genome recognized *Butomus* as a suitable reference point for early branching in the monocots (Cuenca et al. 2013).

The plant consists of leaves that are triangular in cross section, with a single showy inflorescence atop a cylindrical flowering stalk (Lieu 1979). The inflorescence is umbellate with radially symmetrical flowers consisting of three sepals, three petals, nine stamens, and six carpels (Core 1941). Generally the gynoecium is pink in color, but a white colored phenotype has been found recently in China (Huang and Tang 2008). The rhizome grows monopodially with lateral branches and is typically 1-1.5 cm in diameter and brittle (Lieu 1979). Fragments of broken rhizome can form new ramets, as can axillary buds borne in leaf axils of the rhizome; these buds have very narrow connections and break off easily (Lieu 1979, Madsen et al. 2012). Plants growing in the field have been found to produce approximately one bud for every two grams of rhizome (Marko et al. 2012), and cultivated plants were found to produce an average of 196 buds per plant over six growing seasons (Hroudová 1989). The plant can exist in a wide variety of substrates (Roberts 1972), can grow terrestrially along shores up to depths where the plant is completely submerged, and may exhibit different growth forms in each condition (Countryman 1970, Sarbu et al. 2009). Field surveys have found flowering rush at depths out to 4.88 m (Madsen et al. 2013), but at depths of around 1.5 m, emergent leaf height drops to near zero (Madsen et al. 2012). When completely submerged, the leaves are thin

and ribbon-like (Boutwell 1990), and plants do not flower (Hroudová et al. 1996). However, when emerged, the leaves become rigid, the outer cell walls and cuticle thicken, and stomata increase in number by 15% (Sarbu et al. 2009).

Flowering rush has two cytotypes; a diploid and a triploid, both of which are considered invasive outside the native range (Bailey and Preston 2011, Kliber and Eckert 2005). The diploid can self-fertilize and almost always produces an inflorescence, while tripoids cannot self-fertilize and very rarely flower (Kliber and Eckert 2005, Krahulcová and Jarolímová 1993). Diploid plants do form seeds, but this is thought to rarely serve as a method of propagation (Hroudová and Zákravský 1993a, Lui et al. 2005); thus, both cytotypes mainly reproduce vegetatively. The triploid form, on which this work focuses (Lui et al. 2005, Marko et al. 2012), has been found to have a more branching rhizome (which leads to increased clonal reproductive activity), produce more above and below ground biomass, and to be more resistant to eutrophication than the diploid cytotype (Hroudová and Zákravský 1993a, Hroudová and Zákravský 1993b). Both cytotypes can be acquired commercially (Bailey and Preston 2011, Kliber and Eckert 2005), but North American horticultural stock is believed to be mainly triploid (Lui et al. 2005). Populations are generally believed to consist of only one cytotype, and co-occurrence has not been recorded (Hroudová and Zákravský 1993a, Kliber and Eckert 2005).

1.3 Objectives

This work is intended to provide basic life history information concerning triploid flowering rush, which has become a problematic species in its invaded ranges. Controlling pest populations and preventing spread requires ecological information regarding the target species. Madsen and colleagues have performed studies examining

biomass allocation, plant height, and rhizome bud productions at field sites, finding biomass, plant height, and density to increase out to around 1.22 m water depths, then begin to decline in deeper waters (Madsen et al. 2012). Rhizome bud production was found to be negatively correlated with depth from zero to 3 m. While these studies use field observations, subject to the inherent variability of natural systems, the present study provides information on viability of vegetative propagules, modes of colonization and spread, and optimal depth of flowering rush in systematic controlled experiments to test individual factors that drive the species' biology in the field.

This work examines the effect of A) propagule size on production and survivorship, B) initial propagule density on production, and C) water depth on production of flowering rush, adding to the body of knowledge of this species to aid management in controlling this species.

CHAPTER II

GROWTH RESPONSE AND SURVIVORSHIP OF FLOWERING RUSH VEGETATIVE PROPAGULES

2.1 Introduction

Triploid flowering rush reproduces solely by vegetative means via rhizome fragments and vegetative buds (Hroudová and Zákravský 1993b). Vegetative buds form on both the rhizome and in inflorescences (Hroudová and Zákravský 1993b, personal observation); however rhizome buds constitute the bulk of vegetative buds produced by triploid flowering rush, as flowering is uncommon in triploids and not all flowers produce pseudoviviparous buds (Hroudová and Zákravský 1993b, personal observation). Fragmentation of the rhizome can also lead to new clonal ramets. This can occur by mechanical disturbance of the rhizome, or autofragmentation in older plants (Hroudová 1989). Triploid flowering rush rhizomes exhibit many branchings, and have been found to produce, on average, a vegetative bud for every two grams of rhizome mass in the field (Marko et al. 2012). While the mechanisms of flowering rush vegetative reproduction are well understood, little is known of how the size of a vegetative propagule affects growth and survivorship of the resulting clone. I hypothesized that survival and biomass production both would be positively influenced by the size of initial vegetative propagules. I tested this hypothesis by examining growth and survival of belowground propagules up to 9cm in length and across a 12-week study period.

2.2 Methods

All experiments described in the following chapters were performed at the mesocosm facility at R.R. Foil Plant Science Research Center, Mississippi State University. All plant materials used in these experiments came from stock tanks housed at the mesocosm facility; these materials were collected from Detroit Lakes, Minnesota and are of the triploid cytotype and are clones of a single genet (Marko et al. 2012). Soil substrates used in these experiments were amended with Osmocote® 18-16-12 corresponding to 2 g fertilizer L^{-1} soil. All mesocosms were housed under 30% shade cloth to reduce heat and direct sunlight in order to closer emulate conditions near the collected range of these plants. Insecticide (cyfluthrin 0.75% liquid) was applied as necessary to prevent biomass loss to herbivory, primarily from Lepidoptera larvae.

This study was conducted in 378.5L (100 gallon) Rubbermaid commercial stock tanks (53"L x 31"W x 25"H) in the mesocosm facility at the R.R. Foil Plant Science Research Center, Mississippi State University. The experiment ran for 12 weeks, beginning May 29, 2013. Propagules of four sizes (a single rhizome bud or a three, six, or nine cm rhizome fragment, with associated axillary buds) were planted into individual pots filled with topsoil and capped with pea gravel to prevent soil loss. These pots were placed eight per stock tank with two of each fragment size randomly assigned to one of twelve tanks. Each tank was randomly assigned to one of four time durations (three, six, nine, or 12 weeks) and served as a block for analysis, resulting in 96 pots total, with six replicates of each treatment combination (fragment size × time).

At each time point, relevant plants were harvested, separated into root, shoot, rhizome, and rhizome buds, and then dried at 100° C until no change in mass was

observed in a 24 hour period (Figure 2.2, 2.3). Only propagules producing shoots were considered for analysis. Survivorship was measured as:

$$Survival = \frac{N_{initial} - N_{no\ growth}}{N_{initial}} \quad (2.1)$$

Where $N_{initial}$ is the number of individuals initially planted per treatment combination, and $N_{no\ growth}$ is the number of individuals exhibiting no significant growth. Significant growth was determined as growth of any shoot material by the propagule (Table 2.1).

2.3 Results

Total biomass produced varied significantly with both time and propagule size, while survivorship of propagules did not vary with initial size. Final biomass generally was greater when plants started from larger propagules, and was greater after longer periods of growth (Figure 2.1).

A two-way ANOVA was performed to examine whether there was a significant difference in total biomass between initial fragment sizes and duration treatments. Total biomass among plants of each initial fragment size ($P < 0.001$), duration of growth ($P < 0.001$), and each size treatment's final biomass at each duration (size×duration interaction term; $P < 0.001$) all varied significantly. A Jonckheere-Terpstra test was performed to determine if initial fragment size had a significant effect on survivorship, and no such effect was found (initial size $P = 0.765$).

2.4 Discussion

While initial size did have an influence over final biomass, size of the propagule did not affect its survivorship. For triploid flowering rush, sexual reproduction and

genetic variability appear to contribute little to successful invasion (Bailey et al. 2009). Due to this, a single flowering rush rhizome bud or fragment of any size appears capable of causing an infestation in a given body of water. The difference in biomass produced during the timespans of this study would likely have a negligible effect on ultimate invasion success assuming the fragment is able to establish and grow successfully. Most invasions experience a time lag from introduction until negative impacts are recognized (Lockwood et al. 2009; Simberloff 2011), and the initial biomass during the first growing season could unlikely play a large role in influencing the length of this stage if it encompasses decades, such as the length of time flowering rush inhabited Detroit Lakes before control was commissioned (Madsen et al 2012).

A study of six aquatic plants examining six vegetative fragment types of each species found high variability in propagule survivorship among propagule type and species but identified two survival tactics (Barrat-Segretain et al. 1998). Fragments either established in sediment and began forming a new ramet or began producing propagules without establishing any vegetative growth (Barrat-Segretain et al. 1998). Tubers of *Potamogeton pectinatus*, planted at a shallow depth produced more ramets per clone and exhibited growth earlier than smaller tubers, and this trend was not seen when planted at greater depths (Spencer 1987). In this study, only two types of propagules were observed (single buds or rhizome fragments) and planting depth was constant, but these aspects could deserve further study.

Triploid flowering rush is fairly unique in its route to invasion, because it has mechanisms to reduce limitations set by one of the most important barriers to the invasion process. Most models of invasion consider propagule pressure to be a critically

important barrier to invasion. Generally, an invader must not only have enough individuals to facilitate establishment in new environments and to ensure successful mating, but may also need a certain amount of genetic diversity to proliferate in the new range following establishment (Blackburn et al. 2011). Although lacking any sexual reproduction, the present work suggests triploid flowering rush can theoretically invade a suitable range with a single propagule of almost any size. If the initial patch goes unnoticed, new propagules dispersing from this patch may make complete eradication an unlikely outcome in reasonable time spans.

Triploid flowering rush also exhibits apomixis (Hroudová and Zákravský 1993b, personal observation), a trait only an estimated 1% of angiosperms are thought to possess (Whitton et al. 2008). This attribute has arisen independently across a variety of taxa, but polyploidy seems often to be associated with the manifestation of apomixis (Whitton et al. 2008). With triploid flowering rush, vegetative bulbils can form within an inflorescence, granting it another pathway to propagation (Eckert et al. 2000).

Another item of note observed during these studies relevant to flowering rush management is the ability of rhizome fragments to remain dormant, but viable. Hroudová (1989) observed a triploid flowering rush rhizome which did not produce shoots one growing season, but did grow the following year. During these studies, I noticed a clear difference between some recovered failed rhizomes when compared to others, especially in this particular experiment. Some rhizomes would appear remarkably similar to their initial appearance prior to planting, either forming a small amount of root or no visible growth, while other recovered failed fragments had deteriorated to a husk surrounding gelatinous material which was undoubtedly incapable of producing

vegetation. While these fragments were not tested for viability, I would speculate that some of these fragments were in fact in some passive state, and could potentially produce vegetation at a later time. The occurrence of this could be a point of concern for control efforts and deserves further investigation.

Here, triploid flowering rush propagules showed no differential survivorship (mean = 58%), with the propagules from larger fragments generally producing more biomas over the examined time periods. The implications of this study may be disheartening to invasive species management, because if the propagule growth seen here is capable of overwintering, a single bud of flowering rush may be capable of an invasion event.

Table 2.1 Survivorship by treatment

Size	3 weeks	6 weeks	9 weeks	12 weeks
bud only	0.33	0.17	0.67	0.67
3 cm	0.50	0.83	0.67	0.50
6 cm	0.83	0.50	0.67	0.50
9 cm	0.67	0.67	0.67	0.50

Survivorship of flowering rush propagules grown in mesocosms at R. R. Foil Plant Research Center, Mississippi State University.

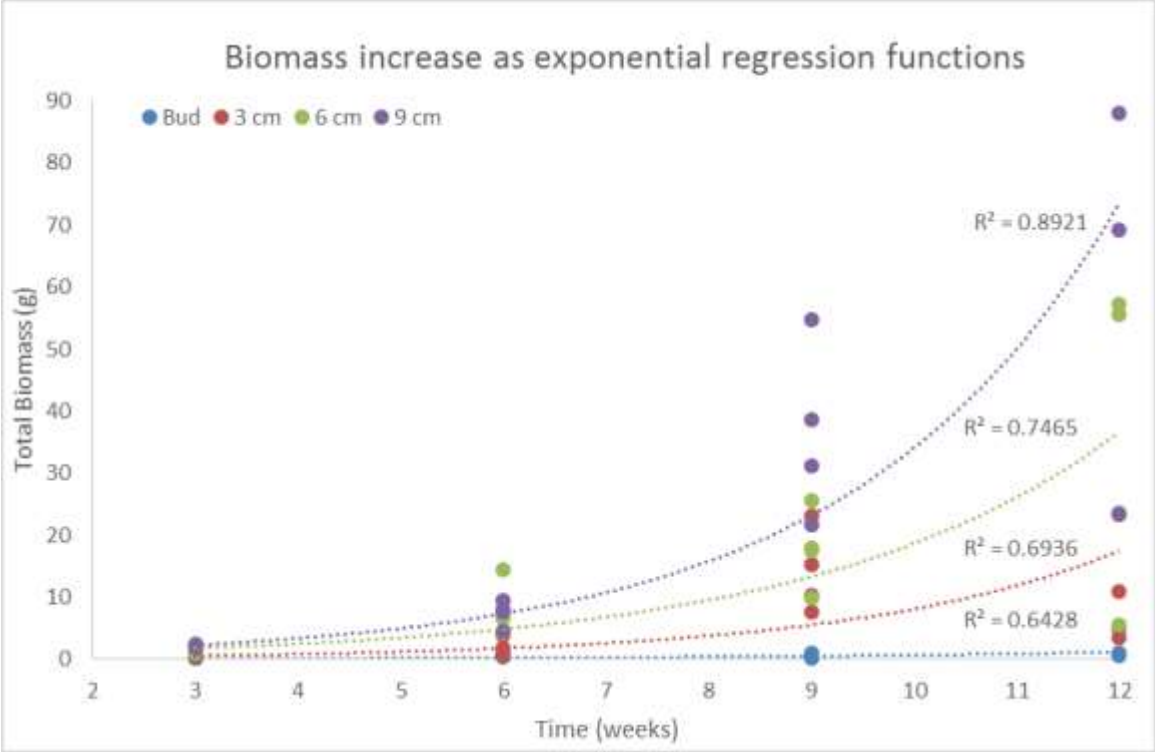

Figure 2.1 Exponential Regression of Biomass Production

Exponential regression of biomass produced by flowering rush propagules grown in mesocosms at R. R. Foil Plant Research Center, Mississippi State University

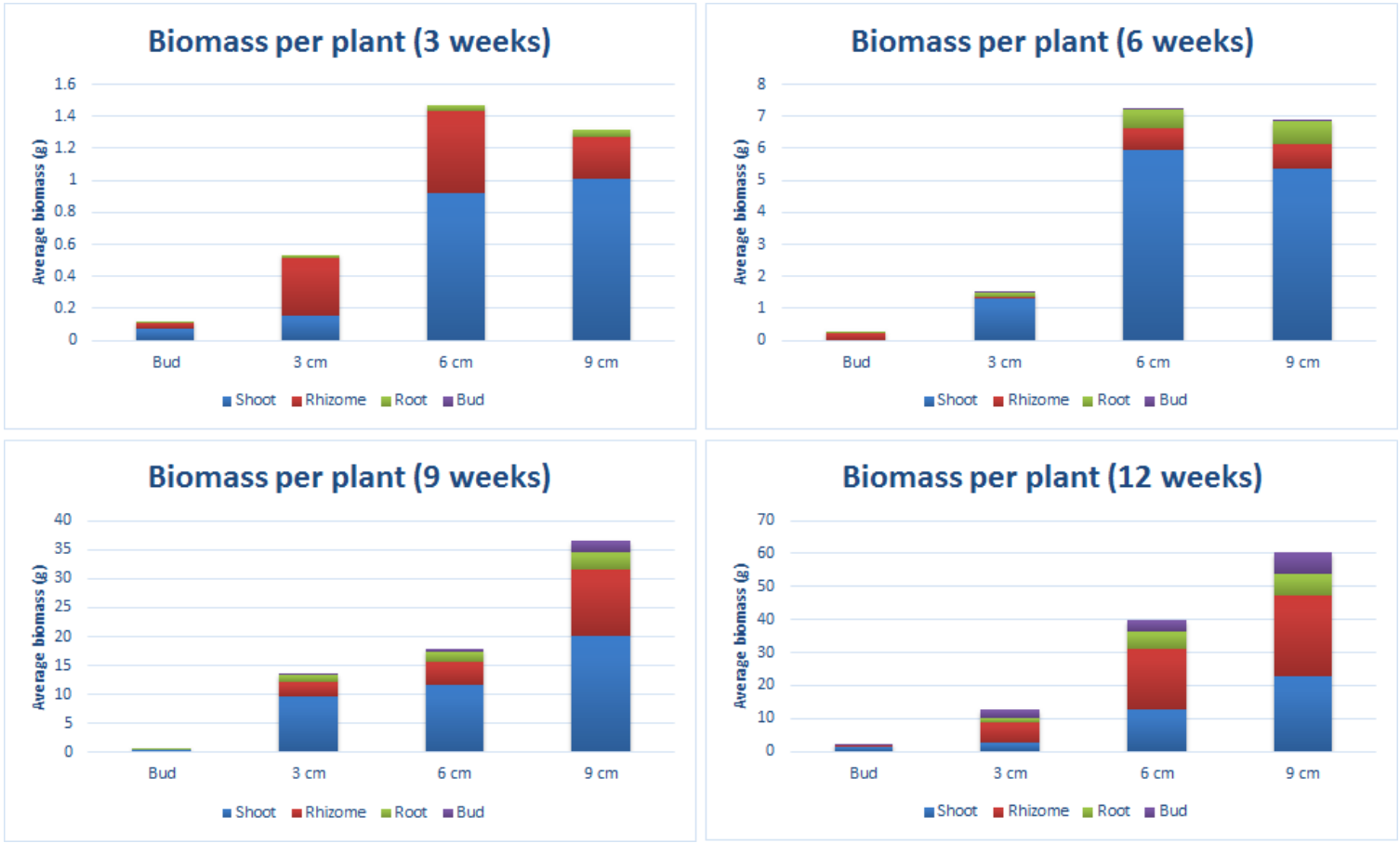

Figure 2.2 Plant Biomass

Average biomass of flowering rush propagules grown in mesocosms at R. R. Foil Plant Research Center, Mississippi State University.

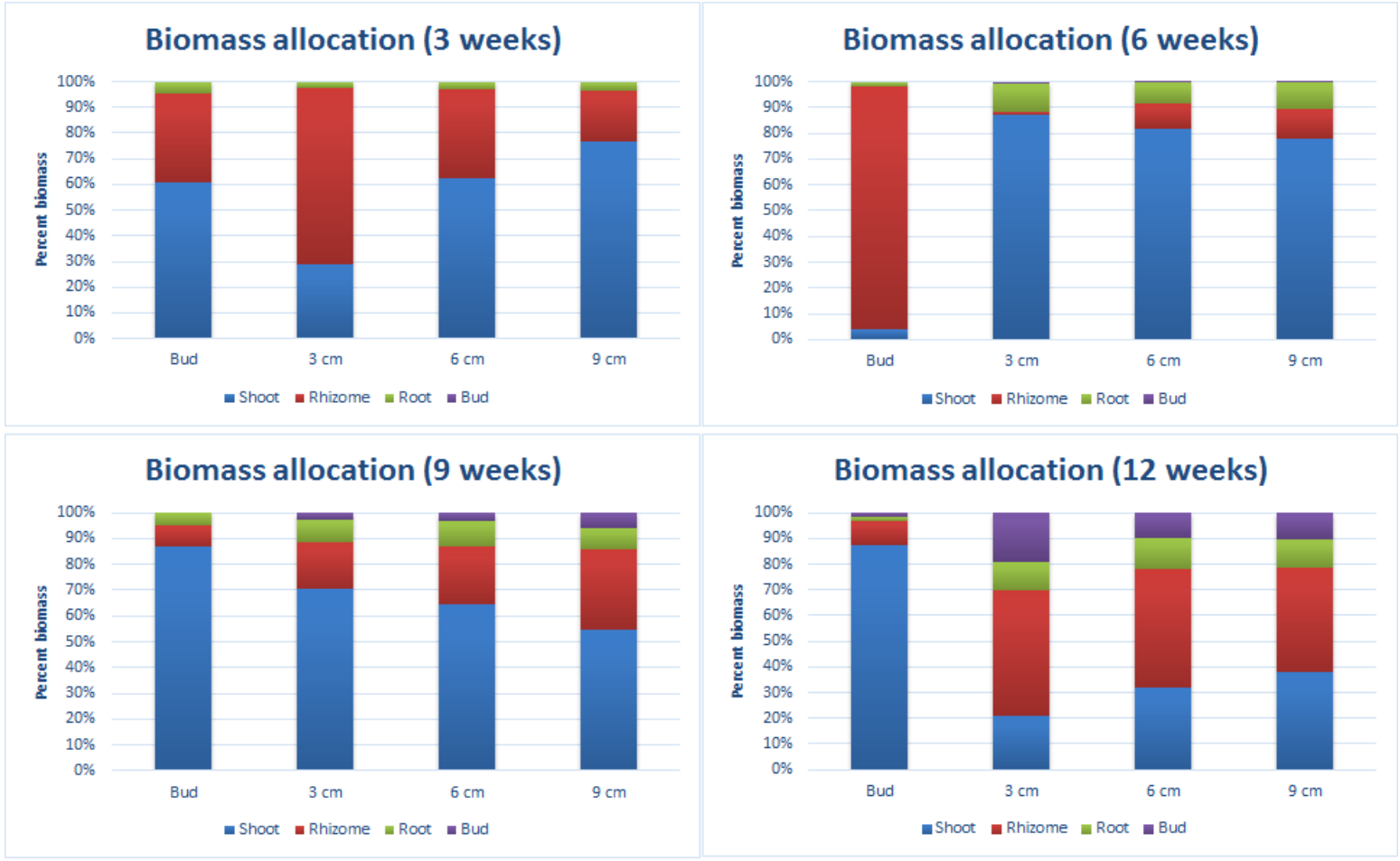

Figure 2.3 Biomass Allocation

Biomass allocation of flowering rush propagules grown in mesocosms at R. R. Foil Plant Research Center, Mississippi State University

CHAPTER III

FLOWERING RUSH GROWTH AND SPREAD IN MONOCULTURE

3.1 Introduction

Flowering rush is known to form monotypic stands which degrade native wetlands, potentially lowering native biodiversity. Flowering rush is a "phalanx" type clonal species, which expands with short rhizome internodes. (Fischer and Kleunen 2001, Oborny et al. 2012). This species type typically forms dense monoclonal stands that are not favorable for high species diversity within them. Local expansion of flowering rush typically takes place via rhizome expansion and dispersal occurs mainly through vegetative buds and rhizome fragmentation. Rhizome fragmentation can occur through mechanical disturbance or through autofragmentation, which has been shown to occur in triploid flowering rush plants after five growing seasons (Hroudová 1989).

Propagule pressure is thought to be one of the three major factors influencing species invasions, the others being the abiotic conditions of the new range and life history characteristics of the invading species (Catford et al. 2009). High propagule pressure in an invaded range can potentially increase genetic diversity available, conceivably allowing the species to adapt to an environment through reproduction and natural selection, but in a sterile organism such as triploid flowering rush, this is of no consequence. High propagule pressure also increases the chances of successful introduction events (Catford et al. 2009). Propagule pressure could also assist a species which experiences an Allee Effect, in which the presence

of conspecifics incurs individual fitness, and a minimum number of individuals is required for positive net population growth (Odum 1959). Flowering rush has been found to be as dense as 400 ramets per m^2 in the field (Marko et al. 2012). The present study examined the behavior of triploid flowering rush propagules colonizing bare patches at two densities, in part to better understand if propagule pressure plays an important role in colonization. I hypothesized that less dense propagules will produce more biomass than more tightly arranged propagules over the course of a growing season.

3.2 Methods

This study was conducted in 378.5L (100 gallon) Rubbermaid commercial stock tanks (53"L x 31"W x 25"H). The experiment ran for twelve weeks beginning May 30, 2013. Ten tanks were filled with 15 cm of sand, then tanks were filled to the top with water and refilled as needed to replace loss from evaporation. A grid overlay was constructed that, when placed over the tank, divided the surface into 10×10 cm grid cells. Eight flowering rush rhizome fragments, each approximately 10 cm long, were planted per tank in one of two spatial arrangements. Five tanks were randomly assigned to the "aggregate" treatment, which consisted of eight fragments planted into each of the central eight cells in a 2×4 arrangement (Figure 3.1). Five tanks were randomly assigned to the "dispersed" treatment, consisting of eight fragments evenly distributed within the central 16 cells (4×4 arrangement Figure 3.2).

Each tank was examined weekly, and presence of aboveground ramets in each grid cell was recorded. After twelve weeks, all biomass was removed from the tank. Biomass collected was divided into shoot, root, rhizome, and rhizome buds, and then

placed in a drying oven at 70° C until no mass change was observed in a 24 hour period (Figures 3.3, 3.4).

3.3 Results

Both tank and core total biomass varied significantly with arrangement (t-test: tank biomass t = -2.373, df = 4, P= 0.038,). Total grid cells occupied at week twelve also varied significantly with arrangement (t-test: t= -2.157, df= 4, P=0.049- Figure 3.5). Density of occupied cells, measured as total biomass of a tank divided by the number of occupied cells did not differ significantly between planting densities (t-test: t= -0.087, df =4, P= 0.47 , mean = 12.1 g/cell), nor did buds produced per gram of rhizome (t-test: P=0.34, mean = 1.04 buds/g rhizome). Rate of spread, measured as final area of cells occupied minus initial area of cells planted divided by time was over twice that in the dispersed treatment when compared to the aggregate treatment (0.15 cm^2 per day in dispersed treatment, 0.07 cm^2 per day in the aggregate treatment).

3.4 Discussion

Overall, triploid flowering rush propagules produced more biomass in the less dense, dispersed treatment. However, measures which could be attributed to relative performance or production, such as biomass per occupied cell or rhizome buds produced per gram of rhizome, did not differ between treatments. This suggests that the increased biomass in the less dense treatment could simply be an artifact of the spatial arrangement. This is, the propagules in the dispersed treatment had more empty cells around them to colonize than fragments in the aggregate treatment and thus were able to accumulate more colonized cells, at lower plant densities, and produce greater overall biomass.

Monospecific stands of *Potamogeton perfoliatus* showed an increase of allocation to above-ground biomass with an increase in density (Wolfer and Straile 2004). Biomass allocation remained almost constant in this experiment (Fig. 3.4), both densities analyzed in this experiment may have been too low to see such an effect of density dependent biomass allocation here, as flowering rush has been found as dense as 400 ramets per m^2 in the field (Marko et al. 2012)

It is important to note that this study had some inherent flaws. First, the grid which divided the surface had to be made large to accommodate variation in tank dimensions resulting from deformation once they were filled with water. This resulted in the grids not having a snug, uniform fit among tanks. Secondly, acquiring a proper top-down view was also difficult, and this likely led to imprecise readings of grid cell occupancy. Dense crowding of ramets during later weeks also made viewing the interface between the substrate and water problematic, which complicated assignment of ramets to individual grid cells.

Here, flowering rush was grown at two initial densities. Although the less dense treatment grew more biomass per tank, biomass per cell occupied, rhizome buds produced per gram rhizome, and biomass allocation did not differ between treatments. The difference in biomass produced per tank can be interpreted as an artifact of the increased amount of free space to invade for initial plantings in the less dense treatment. Flowering rush has been found to be as dense as 400 ramets per square meter in the field (Marko et al. 2012); the densities examined here may have been too low to see an effect on the variables measured.

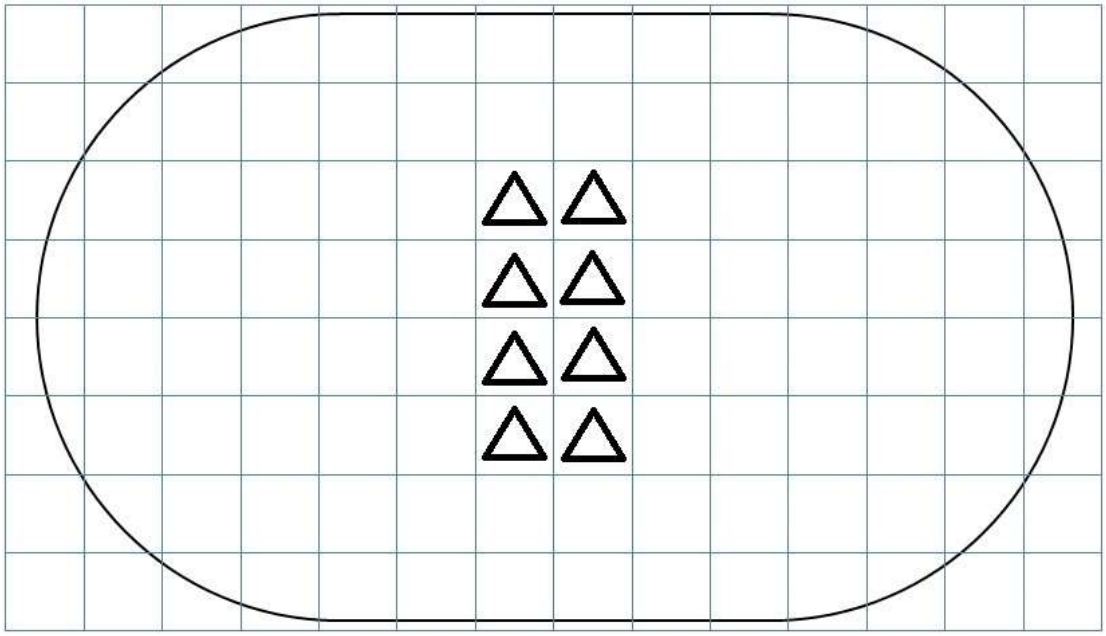

Figure 3.1 Aggregate Layout

Rhizome fragments, approximately 10cm in length, were planted one per cell in the central most eight grid cells of each tank.

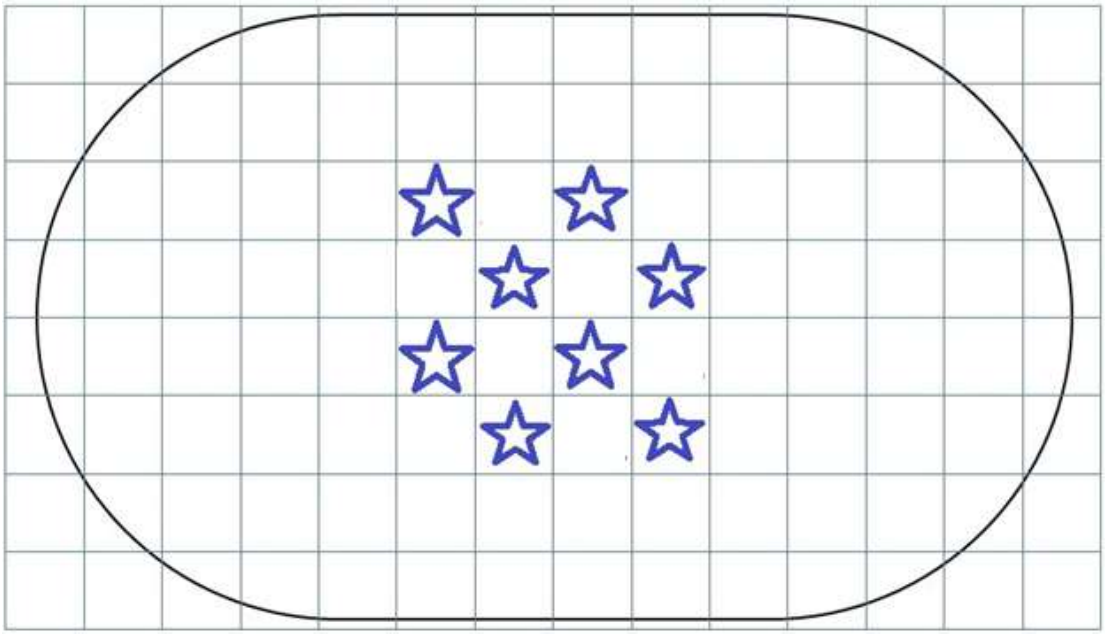

Figure 3.2 Dispersed Layout

Rhizome fragments, approximately 10cm in length, were planted one per cell, evenly spaced across the central most sixteen grid cells of each tank.

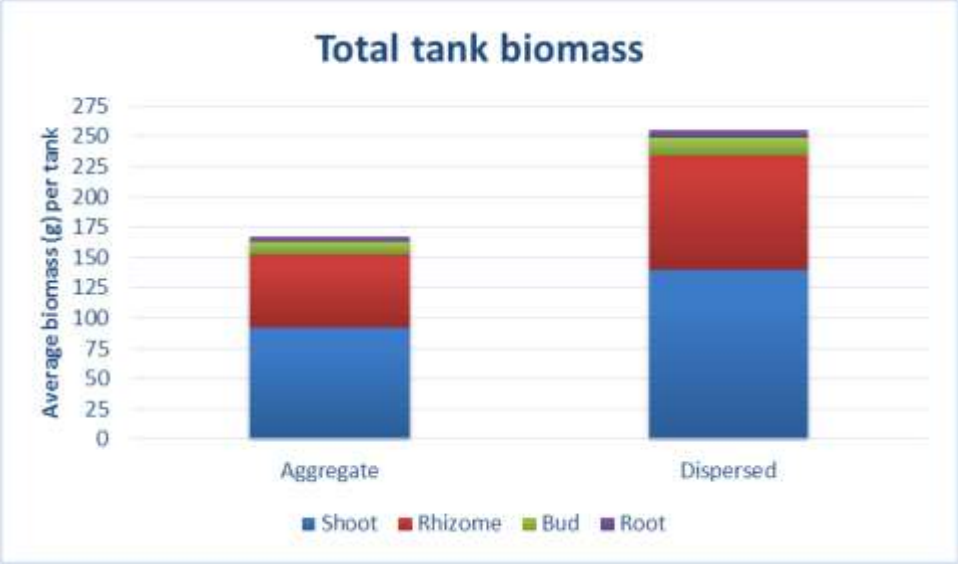

Figure 3.3 Average Biomass per Tank

Average biomass of flowering rush fragments grown in mesocosms at R. R. Foil Plant Research Center, Mississippi State University.

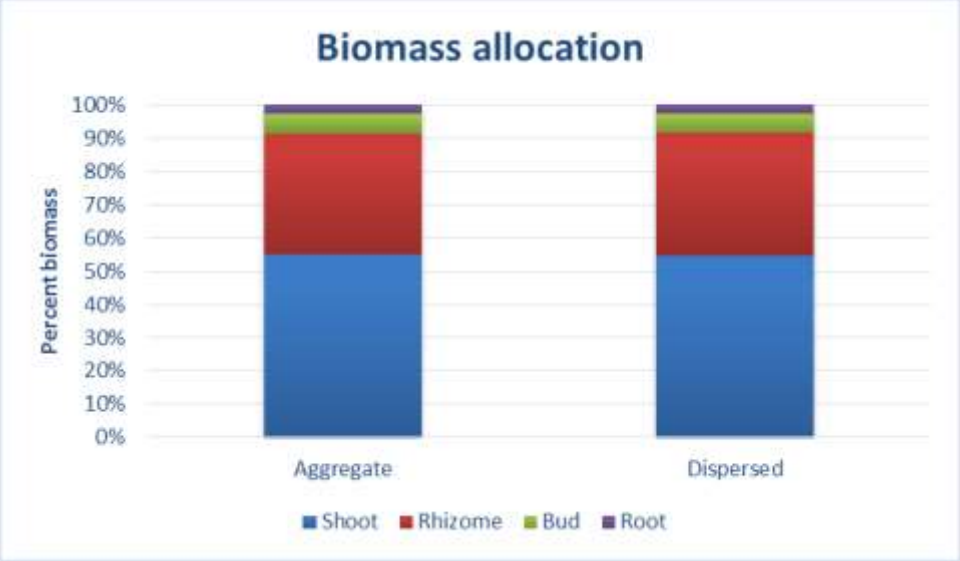

Figure 3.4 Biomass Allocation by Treatment

Percent biomass of flowering rush fragments grown in mesocosms at R. R. Foil Plant Research Center, Mississippi State University.

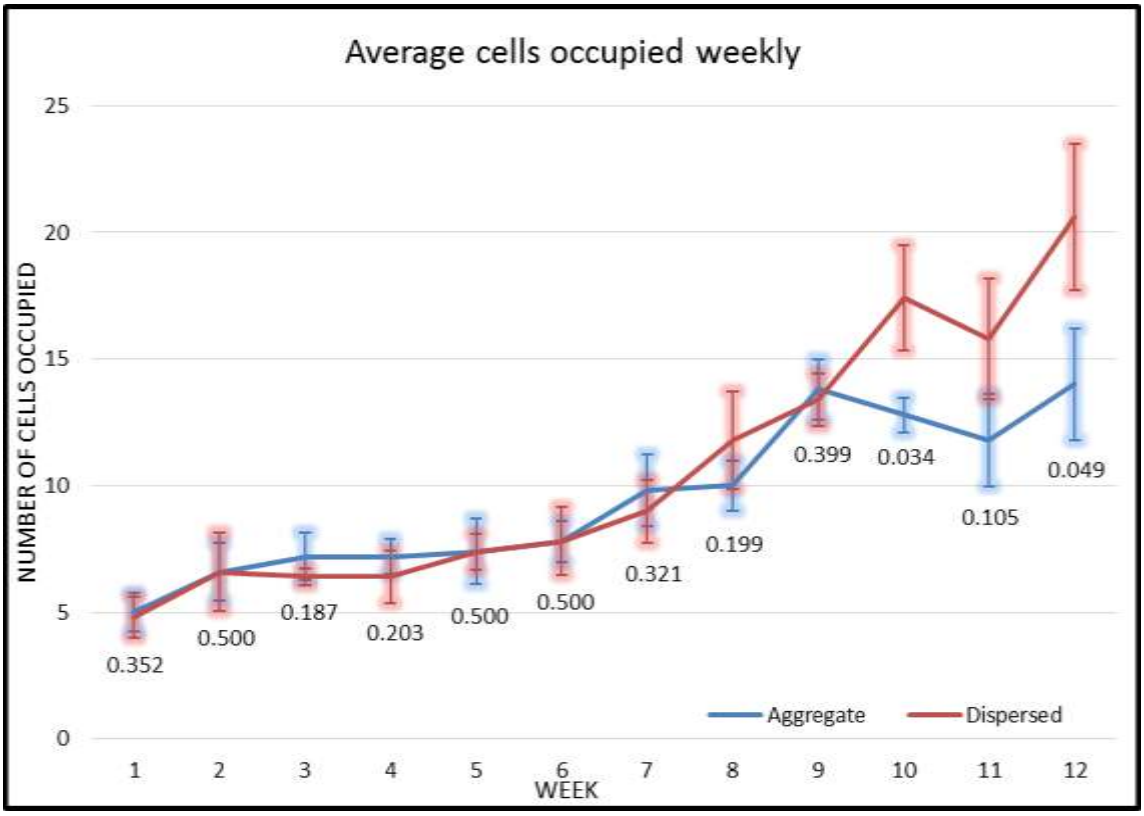

Figure 3.5 Grid Cells Occupied by Week

Average grid cells occupied by flowering rush weekly. Error bars represent 95% confidence intervals of a homoscedastic t-test corresponding to each week, P values of each test are shown below data.

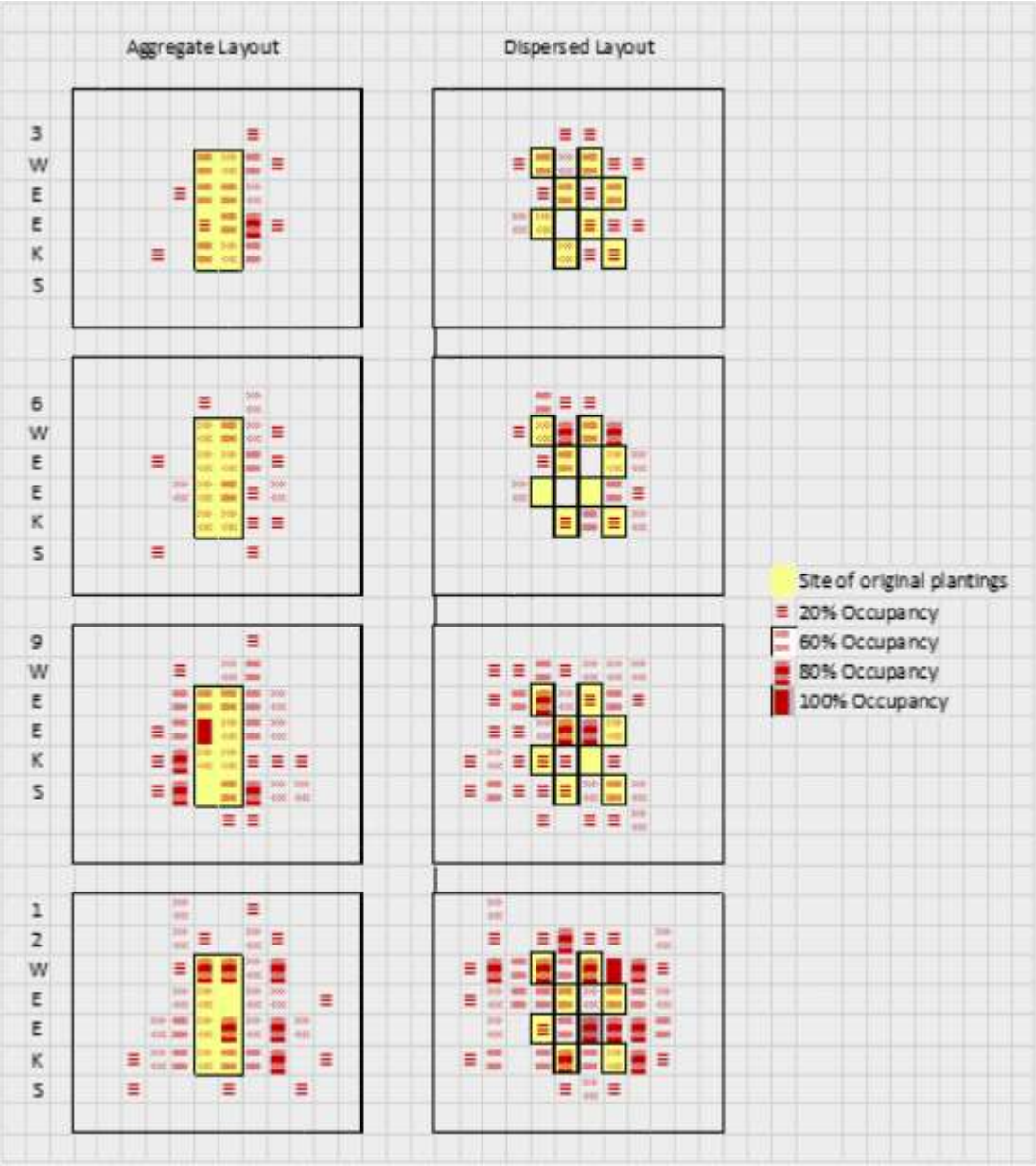

Figure 3.6 Overhead View of Grid Cells Occupied

Shown here is occupied cells by flowering rush at time periods of three weeks (averaged across tanks) of plants grown in mesocosms at R. R. Foil Plant Research Center, Mississippi State University.

CHAPTER IV

FLOWERING RUSH GROWTH RESPONSE ALONG A DEPTH GRADIENT

4.1 Introduction

Flowering rush exhibits extreme morphological plasticity across environmental gradients (Sarbu et al. 2009). The plant is capable of existing fully submerged, as well as with an emergent habit in exposed soils. This plasticity is accomplished without heterophylly. The leaves are able to exist as thin ribbons when submerged and become turgid and stiff when emerged, without substantial alterations in general leaf morphology (Sarbu et al. 2009). The ability to exist along a large water depth gradient only adds to the invasibility of flowering rush, as plants in deep water may be difficult to detect or to treat with herbicide. Field observations at Detroit Lakes, Minnesota showed an increase in attributes associated with production (height, biomass, and density) to a depth of 1.2 m, beyond which, the measures began to decline, and virtually all plants at depths greater than 1.5 m were completely submerged. The present study experimentally isolated depth as a factor to examine the performance of flowering rush propagules along a depth gradient from zero to 1.37 meters. Here, I tested the hypothesis that flowering rush growth would increase with depth, as observed in previous studies.

4.2 Methods

This experiment was conducted in 28 1900-L tanks (137 cm diameter, 157 cm height). Two flowering rush rhizome segments approximately 10 cm in length were placed in a one gallon pot filled with top soil and capped with pea gravel to prevent water loss. Ten such pots were planted in each of the 28 tanks. Tanks were arranged 4 × 7 with the rows aligned east to west. Tanks were randomly assigned to one of seven depth treatments: 0 cm, 22 cm, 44, cm, 66 cm, 88 cm, 110 cm, and 132 cm above the soil surface. The tanks were wrapped in black visqueen to prevent light from penetrating the sides of tanks. Water was added as needed to replace loss due to evaporation. Light intensity was recorded weekly from the water's surface and at 22 cm intervals until reaching the soil level of the pots. The experiment was concluded after twelve weeks. All biomass collected was divided into shoot, root, rhizome, and rhizome buds, and then placed in a drying oven at 70° C until no mass change was observed in a 24 hour period.

4.3 Results

Total biomass, root:shoot ratio (root, rhizome, and bud mass divided by shoot mass), and rhizome buds produced all declined linearly with depth. A one-way ANOVA found total biomass ($P < 0.001$), root:shoot ratio ($P < 0.001$), and rhizome buds produced ($P < 0.001$) all varied significantly with depth. A subsequent regression analysis found total biomass (R^2=0.81), root:shoot ratio (R^2= 0.80), and rhizome buds produced (R^2= 0.73) were found to decline fairly linearly with depth Figures (4.1-4.3). Only one pot out of the 280 failed to exhibit any plant growth.

4.4 Discussion

Total biomass, as well as rhizome buds produced, declined with depth (Figs. 4.1, 4.3). As depth increased, biomass allocation also shifted from below ground material to above ground material (Fig 4.2), as would be expected following allocation models (Hunt and Nicholls 1986), which predict the ratio of above ground material to increase as light availability decreases. The average root:shoot ratio of flowering rush was over four times greater at zero depth than that of the 132 cm treatment. A study of four emergent species found similar shifts in biomass allocation over a 1 m depth gradient, with gramineous species (*Phragmites australis* and *Phalaris arundinacea*) exhibiting an abrupt shift in allocation, and sedges (*Scirpus maritimus* and *Scirpus lacustris*) exhibiting a steady shift across depths similar to those in this work (Coops et al. 1996). In a study of two species of *Typha*, *T. latifolia* and *T. domingensis*, the less water tolerant species , *T. latifolia*, showed an increase in aboveground biomass allocation with increasing depth, while the more tolerant species, *T. domingensis*, increased in biomass with depth, and had a fixed rate of biomass allocation (Grace 1989).

A meta-analysis of wetland plant studies analyzing water regime found an increase in depth to decrease belowground biomass, reproductive output, and shoot density; and to increase shoot length consistently across studies (Webb et al. 2012).These trends agree with the findings of this experiment, and while neither shoot density nor length was measured in this study, maximum shoot length was unmistakably higher in the 132 cm treatment than any other, as shoots not only reached the water's surface but emerged slightly (1-2 cm).

Solving the biomass linear regression equation for zero indicates biomass production would be expected to bottom out at around 1.7m. This finding does not correspond to field observations of triploid flowering rush existing at depths out to 3m (Hroudová 1989) and 4.86 m (Madsen et al. 2013).

The discrepancy between field observations and the results of this experiment can, at least in part, be ascribed to the fact that the experiment examined the growth of newly establishing fragments, in their first growing season, and may not directly reflect the demography and ecology of established clonal patches (Hay and Kelly 2007). Field sampling is likely to only rarely sample plants growing from lone, unconnected rhizome fragments, so it would be reasonable to assume most of the field samples were taken from large interconnected networks that have been established for more than one growing season.

Integrated clonal patches, interconnected by either rhizomes or stolons have been shown to transfer water, minerals, and carbohydrates between ramets (Ikegami et al. 2008, Pennings and Callaway 2000, Stuefer et al. 2004), They also have the capability to share information about the surrounding environment, allowing them to actively "forage" for particular resources (Fischer and Kleunen 2001, Oborny et al. 2012, Stuefer et al. 2004). Clonal networks have also been shown to share defense cues, inducing herbivore resistance in surrounding and newly forming ramets (Gómez and Stuefer 2006). The ability of interconnected ramets to share resources and information allows the genet to take advantage of heterogeneous resources, such as light and nutrients and share these along the network, providing a type of "post-natal" care to newly forming ramets (Stuefer et al. 2004).

The invasive grass *Phalaris arundinacea* was found to colonize areas of heavy shade when connected to an unshaded ramet, and if this shaded area was high in nutrients, vegetative expansion in these areas was increased (Maurer and Zedler 2002). In the submerged macrophyte *Vallisneria spiralis*, shading apical ramets of connected clones not only increased biomass of the shaded ramet but of the entire plant in comparison to plants grown in homogenous light conditions (Xiao et al. 2007). A plant such as flowering rush, which is capable of great morphological plasticity, can shift biomass allocation to establish ramets in what would be unsuitable patches to a lone plant. The sharing of resources between ramets could possibly explain establishment and successful growth in the field that does not match the findings of this experiment.

As an invasive species, triploid flowering rush was capable of positive net growth across all depth gradients examined. The plant's ability to shift biomass allocation to this environmental gradient is likely to contribute to the invasibility of the organism, granting it access to a wide range of depths. The ability to exist in deeper waters could provide a refugia from management practices, as well as from competition, as wetland plant diversity is thought to decrease with depth (Webb et al. 2012).

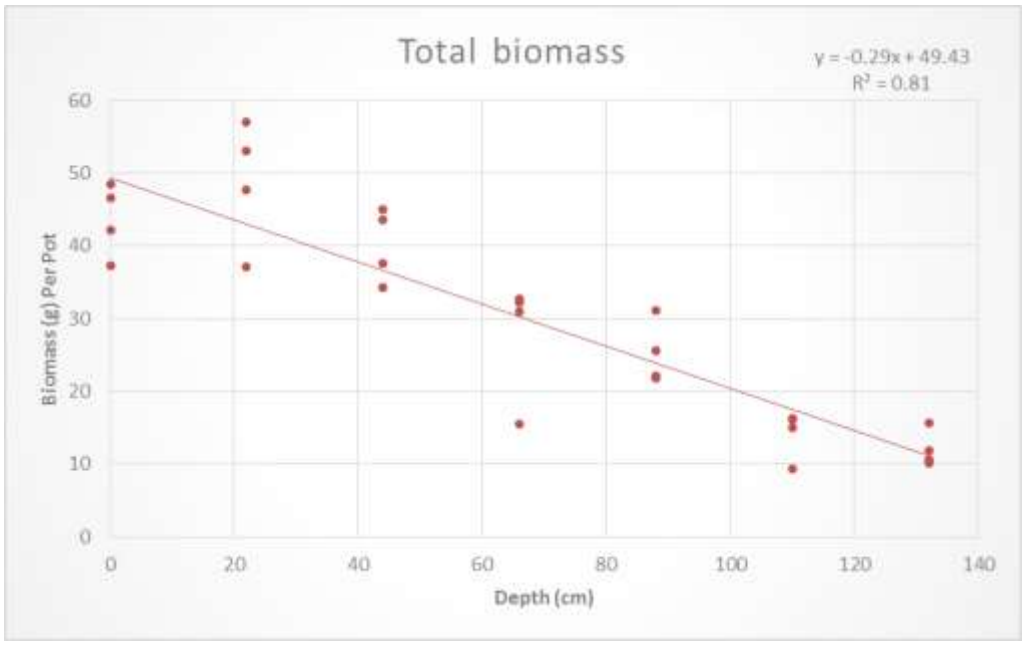

Figure 4.1 Biomass Production

Linear regression of biomass produced by flowering rush plants grown in mesocosms at R. R. Foil Plant Research Center, Mississippi State University.

Figure 4.2 Root-Shoot Ratio

Linear regression of root-shoot ratio of flowering rush plants grown in mesocosms at R. R. Foil Plant Research Center, Mississippi State University.

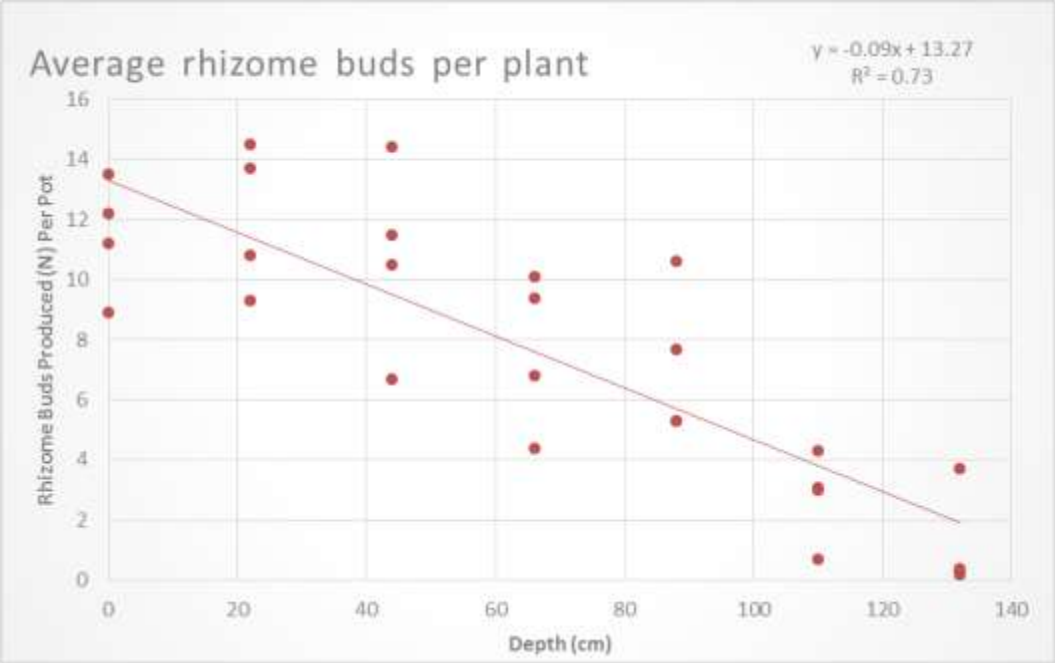

Figure 4.3 Rhizome Buds Produced

Linear regression of rhizome buds produced by flowering rush plants grown in mesocosms at R. R. Foil Plant Research Center, Mississippi State University.

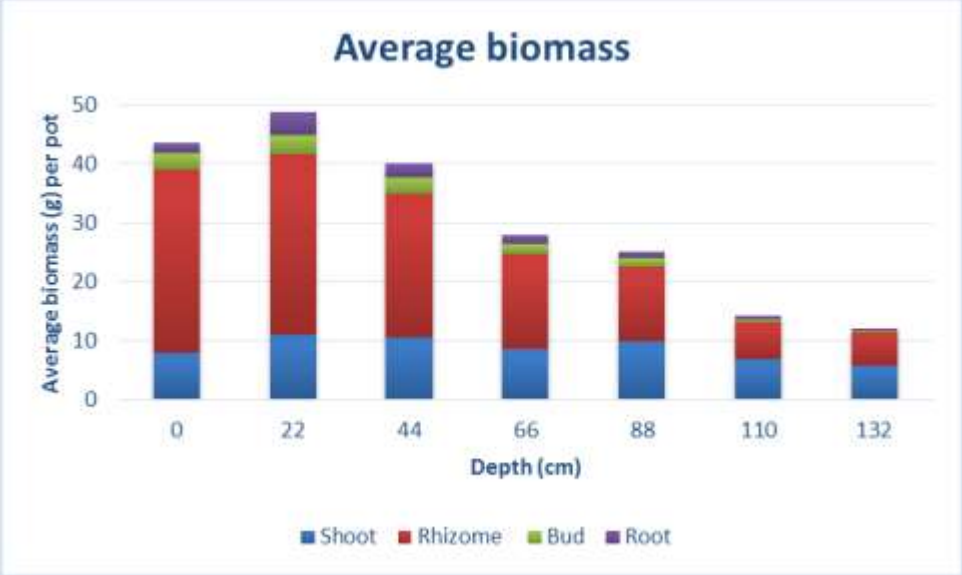

Figure 4.4 Average Biomass

Average biomass of flowering rush fragments grown in mesocosms at R. R. Foil Plant Research Center, Mississippi State University.

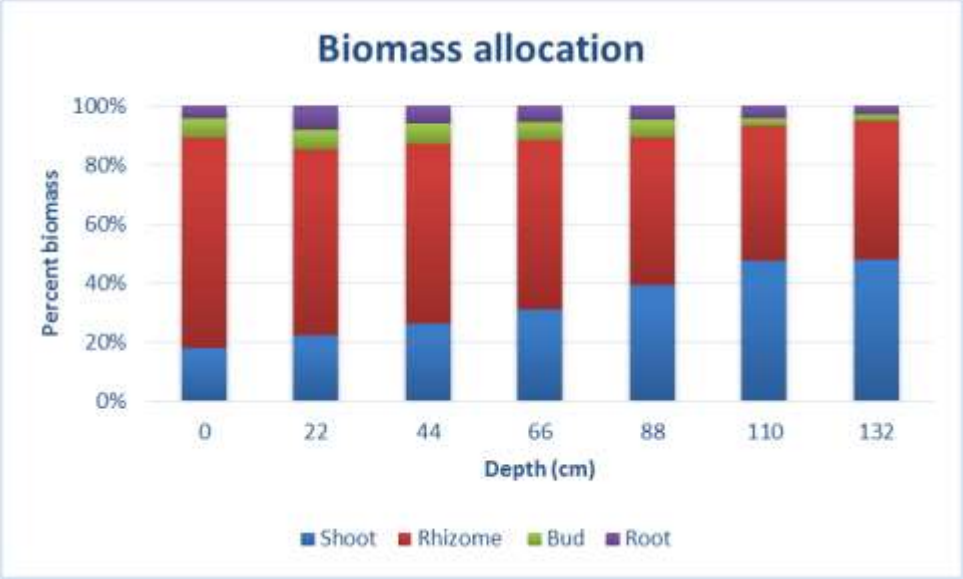

Figure 4.5 Biomass Allocation

Percent biomass of flowering rush fragments grown in mesocosms at R. R. Foil Plant Research Center, Mississippi State University.

CHAPTER V

CONCLUSIONS

Triploid flowering rush invasion is a unique and interesting system because the plant exhibits extreme morphological plasticity, vigorous clonal growth, and the ability to exist across diverse environmental gradients. Clonal networks allow the genet to support ramets undergoing physical stress, competition, herbivory, and to take advantage of heterogeneous resources, such as light and nutrients. The morphological plasticity of flowering rush grants ramets the capacity to exist in potentially unsuitable patches when supported by the clonal network. Once established in patches other plants might not be able to occupy, the genet may then begin to displace nearby established patches of native species (Shumway 1995).

While these studies shed some light on the ecology of flowering rush, the system has many uncommon traits which deserve further study. Mechanisms of clonal network resource and information sharing, specifically response to herbivory and defense signaling, are poorly explored phenomena. The degree to which flowering rush can reallocate biomass in response to environmental gradients is also a very interesting area of research. Finally, the invasion potential of single propagules in contrast to established genets is also a fascinating subject.

Management of flowering rush is a difficult problem, given its intense clonal growth with no need for a minimum population size for sexual reproduction. Typically,

plant diversity decreases with depth (Webb et al. 2012), and deeper waters could be viewed as a refugia from competition and management practices. This plant's ability to exist in deep waters could allow cryptic invasions to evade management and allow regeneration of propagules in a body of water. On the other hand, while the clonality of the plant gives it many advantages as an invasive species, management can focus solely on vegetative dispersal (Okada et al. 2009). Here, the possibility of a highly virulent pathogen as a biocontrol agent is one vulnerability that management could take advantage of, as the lack of any genetic diversity and high connectivity of ramets would likely lead to the devastation of a population (Mölken and Stuefer 2008).

While these studies elucidate some ecological traits of flowering rush, all of these experiments took place across short time spans involving only one growing season. Flowering rush invasions involve much larger clonal networks of ramets, which develop over many growing seasons. The results of this investigation provide empirical evidence that flowering rush asexual propagules are viable at various sizes, including that of a single bud, and along a depth gradient out to 132 cm.

REFERENCES

Anderson, L. C., C. D. Zeis, and S. F. Alam. 1974. Phytogeography and possible origins of *Butomus* in North America. Bulletin of the Torrey Botanical Club 101:292.

Bailey, J. P., K. Bímová, and B. Mandák. 2009. Asexual spread versus sexual reproduction and evolution in Japanese Knotweed s.l. sets the stage for the "Battle of the Clones". Biological Invasions 11:1189–1203.

Bailey, J. P., and C. D. Preston. 2011. Spatial separation of diploid and triploid *Butomus umbellatus* in Britain and Ireland. New Journal of Botany 1:28–32.

Barrat-Segretain, M.-H., G. Bornette, and A. Hering-Vilas-Bôas. 1998. Comparative abilities of vegetative regeneration among aquatic plants growing in disturbed habitats. Aquatic Botany 60:201–211.

Blackburn, T. M., P. Pyšek, S. Bacher, J. T. Carlton, R. P. Duncan, V. Jarošík, J. R. U. Wilson, and D. M. Richardson. 2011. A proposed unified framework for biological invasions. Trends in Ecology & Evolution 26:333–339.

Boutwell, J. E. 1990. Flowering-rush: a plant worth watching. Aquatics, 12: 8-11

Catford, J. A., R. Jansson, and C. Nilsson. 2009. Reducing redundancy in invasion ecology by integrating hypotheses into a single theoretical framework. Diversity and Distributions 15:22–40.

Coops, H., F. W. B. van den Brink, and G. van der Velde. 1996. Growth and morphological responses of four helophyte species in an experimental water-depth gradient. Aquatic Botany 54:11–24.

Core, E. L. 1941. *Butomus umbellatus* in America. Ohio Journal of Science 41:79–85.

Costanza, R., R. d' Arge, R. de Groot, S. Farber, M. Grasso, B. Hannon, K. Limburg, S. Naeem, R. V. O'Neill, J. Paruelo, R. G. Raskin, P. Sutton, and M. van den Belt. 1998. The value of the world's ecosystem services and natural capital. Ecological Economics 25:3–15.

Countryman, W. D. 1970. The history, spread and present distribution of some immigrant aquatic weeds in New England. Hyacinth Control Journal 8:50–2.

Cuenca, A., G. Petersen, and O. Seberg. 2013. The complete sequence of the mitochondrial genome of *Butomus umbellatus* – A Member of an Early Branching Lineage of Monocotyledons. PLoS ONE 8:e61552.

Eckert, C. G., B. Massonnet, and J. J. Thomas. 2000. Variation in sexual and clonal reproduction among introduced populations of flowering rush, *Butomus umbellatus* (Butomaceae). Canadian Journal of Botany 78:437–446.

Fischer, M., and M. V. Kleunen. 2001. On the evolution of clonal plant life histories. Evolutionary Ecology 15:565–582.

Funnell, E., M. Heaton, F. MacDonald, and B. Brownson. 2009. The aquarium and horticultural industry as a pathway for the introduction of aquatic invasive species—outreach initiatives within the Great Lakes basin. Biodiversity 10:104–112.

Gómez, S., and J. F. Stuefer. 2006. Members only: induced systemic resistance to herbivory in a clonal plant network. Oecologia 147:461–468.

Grace, J. B. 1989. Effects of water depth on *Typha latifolia* and *Typha domingensis*. American Journal of Botany 76:762.

Hay, M. J. M., and C. K. Kelly. 2007. Have clonal plant biologists got it wrong? The case for changing the emphasis to disintegration. Evolutionary Ecology 22:461–465.

Hroudová, Z. 1989. Growth of *Butomus umbellatus* at a stable water level. Folia Geobotanica et Phytotaxonomica 24:371–385.

Hroudová, Z., A. Krahulcová, P. Zákravský, and V. Jarolímová. 1996. The biology of *Butomus umbellatus* in shallow waters with fluctuating water level. Pages 27–30 *in* J. M. Caffrey, P. R. F. Barrett, K. J. Murphy, and P. M. Wade, editors. Management and Ecology of Freshwater Plants. Springer Netherlands.

Hroudová, Z., and P. Zákravský. 1993a. Ecology of two cytotypes of *Butomus umbellatus* III. Distribution and habitat differentiation in the Czech and Slovak Republics. Folia Geobotanica et Phytotaxonomica 28:425–435.

Hroudová, Z., and P. Zákravský. 1993b. Ecology of two cytotypes of *Butomus umbellatus* II. Reproduction, growth and biomass production. Folia Geobotanica et Phytotaxonomica 28:413–424.

Huang, S.-Q., and X.-X. Tang. 2008. Discovery of gynoecium color polymorphism in an aquatic plant. Journal of Integrative Plant Biology 50:1178–1182.

Hunt, R., and A. O. Nicholls. 1986. Stress and the coarse control of growth and root-shoot partitioning in herbaceous plants. Oikos 47:149–158.

Ikegami, M., D. F. Whigham, and M. J. A. Werger. 2008. Optimal biomass allocation in heterogeneous environments in a clonal plant—Spatial division of labor. Ecological Modelling 213:156–164.

Kliber, A., and C. G. Eckert. 2005. Interaction between founder effect and selection during biological invasion in an aquatic plant. Evolution 59:1900–1913.

Krahulcová, A., and V. Jarolímová. 1993. Ecology of two cytotypes of *Butomus umbellatus* I. Karyology and breeding behaviour. Folia Geobotanica et Phytotaxonomica 28:385–411.

Les, D. H., and L. J. Mehrhoff. 1999. Introduction of nonindigenous aquatic vascular plants in southern New England: A Historical Perspective. Biological Invasions 1:281–300.

Lieu, S. M. 1979. Growth forms in the Alismatales. II. Two rhizomatous species: *Sagittaria lancifolia* and *Butomus umbellatus*. Canadian Journal of Botany 57:2353–2373.

Lockwood, J. L., M. F. Hoopes, and M. P. Marchetti. 2013. Invasion Ecology. John Wiley & Sons.

Lui, K., F. L. Thompson, and C. G. Eckert. 2005. Causes and consequences of extreme variation in reproductive strategy and vegetative growth among invasive populations of a clonal aquatic plant, *Butomus umbellatus* L. (Butomaceae). Biological Invasions 7:427–444.

Madsen, J. D., K. D. Getsinger, R. M. Stewart, J. G. Skogerboe, D. R. Honnell, and C. S. Owens. 1999. Evaluation of transparency and light attenuation by Aquashade™. Lake and Reservoir Management 15:142–147.

Madsen, J.D., B. Sartain, G. Turnage, and M. Marko. 2013. Herbicide trials for management of flowering rush in Detroit Lakes, Minnesota for 2012. Geosystems Research Institute Report 5059, Geosystems Research Institute, Mississippi State University, Mississippi State, MS. April 2013.

Madsen, J.D., R. Wersah, M. Marko, and J. Skogerboe. 2012. Ecology and management of flowering rush (*Butomus umbellatus*) in the Detroit Lakes, Minnesota. Geosystems Research Institute Report 5054, Geosystems Research Institute, Mississippi State University, Mississippi State, MS. July 2012.

Maki, K., and S. Galatowitsch. 2004. Movement of invasive aquatic plants into Minnesota (USA) through horticultural trade. Biological Conservation 118:389–396.

Marko, M., J. Madsen, C. Olson and R. Smith. 2012. Ecology of flowering rush (*Butomus umbellatus*) in Detroit Lakes. Report for Pelican River Watershed District

Maurer, D. A., and J. B. Zedler. 2002. Differential invasion of a wetland grass explained by tests of nutrients and light availability on establishment and clonal growth. Oecologia 131:279–288.

Mitchell, D. S. 1974. Aquatic vegetation and its use and control, edited by D. S. Mitchell. Paris, Unesco, 1974.

Mölken, T. van, and J. F. Stuefer. 2008. Virulence in clonal plants: conflicting selection pressures at work? Evolutionary Ecology 22:467–470.

Odum, E. P. 1959. Fundamentals of ecology [by] Eugene P. Odum in collaboration with Howard T. Odum. Philadelphia, Saunders, 1959.

Pieterse, A. H., and K. J. Murphy. 1990. Aquatic weeds : the ecology and management of nuisance aquatic vegetation, edited by Arnold H. Pieterse and Kevin J. Murphy. Oxford [England] ; New York : Oxford University Press, 1990.

Oborny, B., C. Mony, and T. Herben. 2012. From virtual plants to real communities: A review of modelling clonal growth. Ecological Modelling 234:3–19.

Okada, M., B. J. Grewell, and M. Jasieniuk. 2009. Clonal spread of invasive *Ludwigia hexapetala* and *L. grandiflora* in freshwater wetlands of California. Aquatic Botany 91:123–129.

Pennings, S. C., and R. M. Callaway. 2000. The advantages of clonal integration under different ecological conditions: a community-wide test. Ecology 81:709–716.

Roberts, M. 1972. *Butomus umbellatus* in the Mississippi watershed. Castanea 37:83–85.

Sarbu, A., D. Smarandache, A. Paraschiv, and D. Mihai. 2009. *Butomus umbellatus* morpho-structural considerations on adaptive plasticity. Scientific Annals of "Alexandru Ioan Cuza" University of Iasi, New Series, Section II a. Vegetal Biology.

Shumway, S. W. 1995. Physiological Integration among Clonal ramets during invasion of disturbance patches in a new england salt marsh. Annals of Botany 76:225–233.

Simberloff, D. 2011. How common are invasion-induced ecosystem impacts? Biological Invasions 13:1255–1268.

Singh, V., and R. Sattler. 1974. Floral development of *Butomus umbellatus*. Canadian Journal of Botany 52:223–230.

Spencer, D. F. 1987. Tuber size and planting depth influence growth of *Potamogeton pectinatus* L. American Midland Naturalist 118:77.

Stuefer, J. F., S. Gómez, and T. van Mölken. 2004. Clonal integration beyond resource sharing: implications for defence signalling and disease transmission in clonal plant networks. Evolutionary Ecology 18:647–667.

Webb, J. A., E. M. Wallis, and M. J. Stewardson. 2012. A systematic review of published evidence linking wetland plants to water regime components. Aquatic Botany 103:1–14.

Whitton, J., C. J. Sears, E. J. Baack, and S. P. Otto. 2008. The dynamic nature of apomixis in the angiosperms. International Journal of Plant Sciences 169:169–182.

Xiao, K., D. Yu, X. Xu, and W. Xiong. 2007. Benefits of clonal integration between interconnected ramets of *Vallisneria spiralis* in heterogeneous light environments. Aquatic Botany 86:76–82.

APPENDIX A

PICTURE OF VEGETATIVE BULBILS IN INFLORESCENCE

A.1 Vegetative bulbils

Figure A.1 Vegetative bulbils growing within a flowering rush inflorescence.

APPENDIX B

LIGHT AND TEMPERATURE DATA

B.1 HOBO Data

HOBO data sondes (Onset Computer Corporation, Bourne, MA) were randomly placed in two tanks for the studies conducted in chapters two and three, and two per treatment in the depth study from chapter four to collect light and temperature data hourly. .

Table B.1 Average temperature and percent daylight

Spread Study		
Logger	Temp (°C)	% Daylight
1	28.3	41.5
2	27.9	42.4
Propagule Study		
Logger	Temp (°C)	% Daylight
1	28.6	40.9
2	28.1	49.7

Average temperature and percent daylight (number of hours sensor detected light divided by twenty-four) collected from HOBO data loggers for the studies from chapters two and three.

Table B.2 Average temperature and percent daylight

Depth Study			
Tank	Depth (cm)	Temp (°C)	% Daylight
3	132	28.5	50.2
16	132	27.6	47.0
13	110	27.8	46.8
22	110	28.1	47.1
2	88	28.1	48.4
9	88	28.1	45.1
19	66	27.5	47.1
28	66	27.6	51.1
12	44	26.9	46.7
17	44	27.3	45.7
8	22	26.7	47.3
23	22	26.8	44.2
14	0	26.0	44.2
27	0	26.2	45.2

Average temperature and percent daylight (number of hours sensor detected light divided by twenty-four) collected from HOBO data loggers for the studies from chapters two and three.

B.2 Photosynthetically Active Radiation

Light data were collected for the depth study (chapter four) using a LI COR light meter with aerial and submersible photosynthetically active radiation (PAR, 400-700 nm) sensors (LiCor Environmental, Lincoln, NE). Light was measured every 22 cm until the sensor reached the height of the soil surface. Data from each reading was averaged for each treatment. Percent light transmittance (Madsen et al. 1999), calculated as

$$Transmittance = {}^{I_{zn}}/_{I_{0n}} \times {}^{I_{zn-1}}/_{I_{0n-1}} \quad \text{(B.1)}$$

Where I_{zn} is the light intensity reading at depth n, I_{0n} is the light intensity reading from the aerial "deck cell" corresponding to I_{zn}, $I_{0n-1}$is the deck cell reading at from the previous depth, and $I_{zn-1}$is the light reading from the previous depth. The second factor serves as a correction factor for variable surface readings.

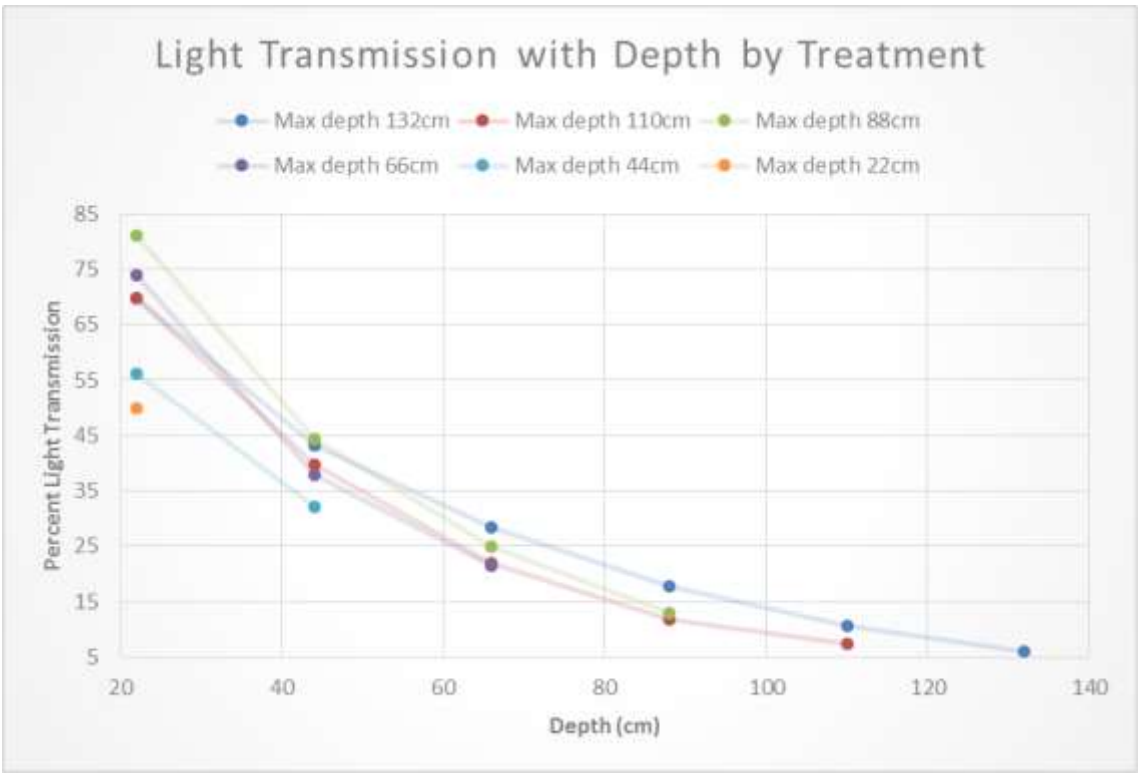

Figure B.1 Percent light transmission at each depth interval (22cm) below the surface for each treatment with at least one depth interval.

APPENDIX C

PROPAGULE STUDY DATA

Table C.1 Propagule Biomass Data

3 week results									
Propagule Size	Total	N w/Roots	N w/Shoots	N w/ buds	Root Mass	Shoot Mass	Rhizome Mass	Bud Mass	Total Mass
bud only	6	5	2	0	0.005±0.002	0.069±0.055	0.040±0.004	NA	0.113±0.061
3 cm	6	4	3	0	0.013±0.009	0.152±0.121	0.364±0.121	NA	0.529±0.250
6 cm	6	6	5	0	0.043±0.024	0.923±0.465	0.507±0.108	NA	1.472±0.598
9 cm	6	5	4	0	0.043±0.025	1.009±0.266	0.266±1.003	NA	2.056±0.410
6 week results									
Propagule Size	Total	N w/Roots	N w/Shoots	N w/ buds	Root Mass	Shoot Mass	Rhizome Mass	Bud Mass	Total Mass
bud only	6	2	1	0	0.005±0.004	0.102±NA	0.237±NA	NA	0.344±NA
3 cm	6	5	5	1	0.164±0.227	1.317±1.006	0.197±0.123	0.011±NA	1.689±1.357
6 cm	6	4	4	4	0.586±0.359	5.953±4.186	0.698±0.402	0.031±0.018	7.267±4.964
9 cm	6	5	5	3	0.714±0.691	5.366±1.515	0.790±0.181	0.019±0.011	6.888±2.397
9 week results									
Propagule Size	Total	N w/Roots	N w/Shoots	N w/ buds	Root Mass	Shoot Mass	Rhizome Mass	Bud Mass	Total Mass
bud only	6	4	4	0	0.023±0.026	0.426±0.312	0.041±0.026	NA	0.525±0.363
3 cm	6	4	4	4	1.159±0.851	9.648±3.427	2.494±2.208	0.396±0.360	14.057±6.846
6 cm	6	4	4	4	1.787±0.827	11.603±4.289	4.033±2.674	0.552±0.316	17.974±8.106
9 cm	6	4	4	4	2.995±1.381	20.040±5.530	11.419±7.054	2.092±1.161	36.546±15.126
12 week results									
Propagule Size	Total	N w/Roots	N w/Shoots	N w/ buds	Root Mass	Shoot Mass	Rhizome Mass	Bud Mass	Total Mass
bud only	6	4	4	2	0.0254±0.446	1.441±1.346	0.155±0.208	0.029±0.026	1.878±2.026
3 cm	6	3	3	3	1.383±1.518	2.652±2.179	6.142±9.039	2.395±2.979	12.572±15.714
6 cm	6	2	2	2	4.858±3.854	12.288±9.357	18.396±3.888	3.888±2.613	39.430±29.492
9 cm	6	3	3	3	6.666±2.749	22.744±18.458	24.619±10.307	6.223±4.992	60.252±36.506

Flowering rush biomass produced from a single bud or 3, 6, and 9 cm rhizome fragments grown in mesocosms at R. R. Foil Plant Research Center, Mississippi State University.