

Competitive Interactions and Rhizome Reproductive Capacity of an Invasive Plant, Garden  
Loosestrife (*Lysimachia vulgaris* L.)

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requirements for the degree of

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University of Washington

**Abstract**

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Chair of the Supervisory Committee:

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Garden loosestrife (*Lysimachia vulgaris* L.) is an invasive wetland plant that has spread throughout King County and Washington State. While garden loosestrife populations are limited, it is of concern to land managers because it is difficult to distinguish among other vegetation when not it bloom and it spreads quickly and aggressively through vegetative reproduction. A trial was conducted to determine the ability of a vigorous native perennial species, small-fruited bulrush (*Scirpus microcarpus* J. Presl & C. Presl), to compete with garden loosestrife. Small-fruited bulrush growth was not negatively impacted by garden loosestrife. Conversely, garden loosestrife shoot weight, root weight, and total weight were reduced by small-fruited bulrush, although its vegetative rhizome growth was not affected by competition. Since rhizome growth is the primary method by which garden loosestrife colonizes new sites, a second study tested three rhizome segment sizes (1, 2, and 5 cm) transplanted at three depths (0, 4 and 8 cm) to determine the ability of this species to establish from fragmented rhizomes. These trials were begun either in early- or mid-summer, and plants were allowed to grow for six weeks in each. While shoots were produced by rhizome segments of all lengths, the only rhizomes that produced shoots were those

on the soil surface. There were no significant differences in growth based on fragment length, but rhizomes grew more when cut and grown in early-summer versus mid-summer. These findings will allow invasive plant managers to better plan for garden loosestrife control in sensitive wetland and riparian habitats.

## **Acknowledgements**

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A heartfelt thanks to all of the volunteers that helped to make these projects happen. Thanks especially to Sam Payne, who was a dedicated volunteer and supporter.

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## First Introduction

### Taxonomy

**Kingdom:** Plantae—Plants

**Subkingdom:** Tracheobionta – Vascular plants

**Superdivision:** Spermatophyta – Seed plants

**Division:** Magnoliophyta – Flowering plants

**Class:** Magnoliopsida – Dicotyledons

**Subclass:** Dilleniidae

**Order:** Primulales

**Family:** Primulaceae – Primrose family

**Genus:** *Lysimachia* L.

**Species:** *Lysimachia vulgaris* L. – garden loosestrife

(United States Department of Agriculture: Plant Database)

### Noxious Weed Designation

Garden loosestrife is classified as a Class B designate species in Washington State (King County Noxious Weed Control Program, 2010). The Washington State Noxious Weed Law (RCW 17.10) mandates control of Class B designate species on specified public and private lands within the state (King County Noxious Weed Control Program, 2010). It is also considered potentially invasive and is banned from sale in Connecticut (United States Department of Agriculture 2017). Oregon has a classified garden loosestrife as an A listed weed as a precaution to prevent spread from Washington State (Oregon Department of Agriculture 2017). An Oregon A listed noxious weed is defined as “A weed of known economic importance which occurs in the state in small enough infestations to make eradication or containment possible; or is not known to occur, but its presence in neighboring states make future occurrence in Oregon seem imminent” (Oregon Department of Agriculture 2017). The recommended action for A listed noxious weeds is the “eradication or intensive control when and where found” (Oregon Department of Agriculture 2017).

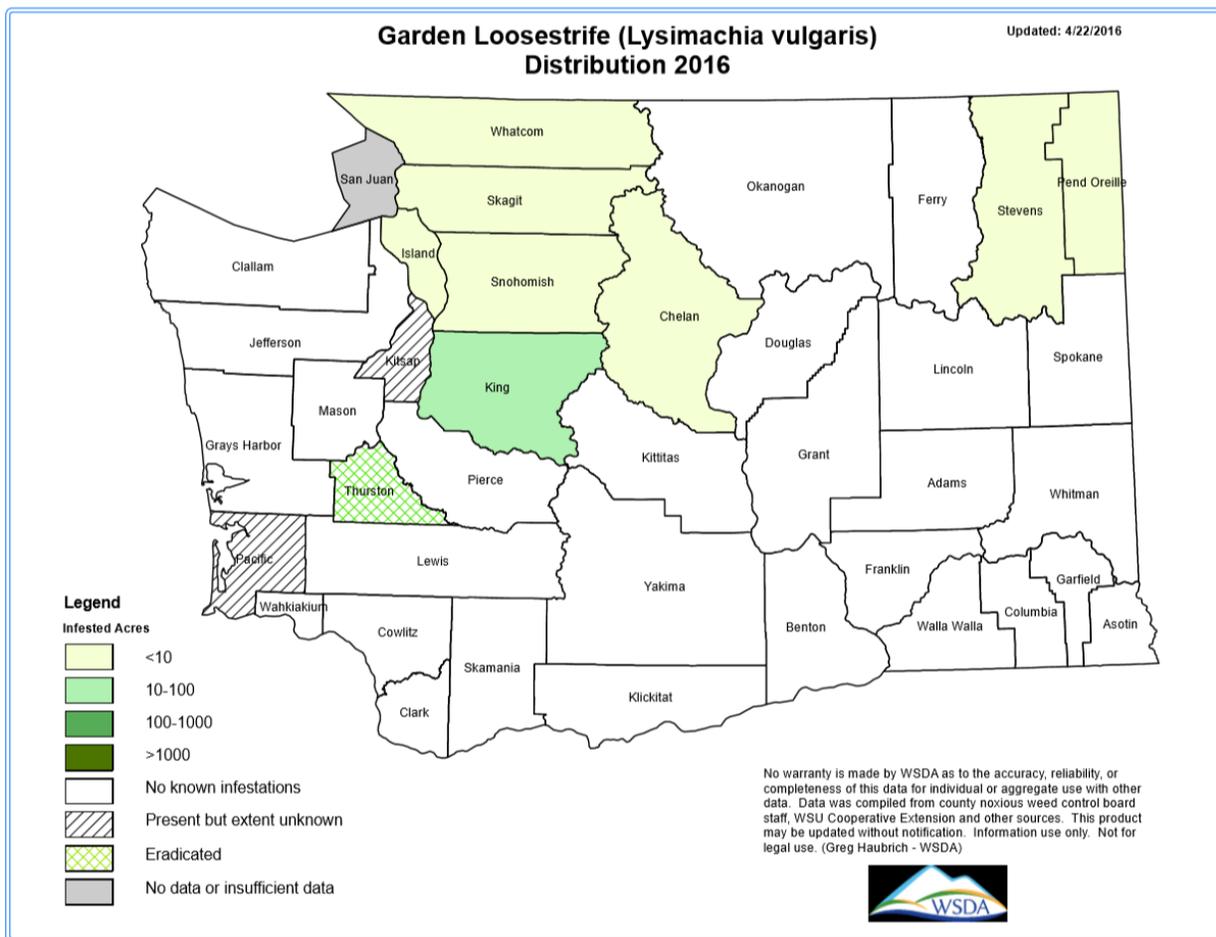
## **Description**

Garden loosestrife has a round, erect, and softly hairy stem, with long ovate leaves arranged in whorls of two to four. The leaves are hairy and tend to be 3-5 inches in length, with black or orange glands on the bottom of the leaf. Blooms are similar to other primrose-like flowers: they have showy, vibrant yellow petals with orange centers and have a five petal arrangement. The stamens range from yellow to orange-red. Flowering occurs from July through the end of August, but can extend through September in Washington state latitudes. Garden loosestrife is a perennial and often remains in a vegetative state for years before blooming (King County Noxious Weed Control Program 2010, United States Department of Agriculture 2017). Garden loosestrife is found in fens, wet woodland areas, lakeshores, river banks, and stream banks (King County Noxious Weed Control Program 2010). Garden loosestrife is often confused with yellow (or spotted or dotted) loosestrife (*Lysimachia punctata* L.), another non-native perennial species that grows in similar wetland and riparian habitats as garden loosestrife. Garden loosestrife can be distinguished from yellow loosestrife in that garden loosestrife flowers tend to cluster at the top of the plant with some flowers growing from the base of the upper leaves, whereas yellow loosestrife has flowers situated primarily in the leaf axils. Yellow loosestrife petals are also more pointed than garden loosestrife. Garden loosestrife is native to Eurasia and northern Africa, where it is sometimes used medicinally to lower blood pressure (Washington State Department of Ecology 2017).

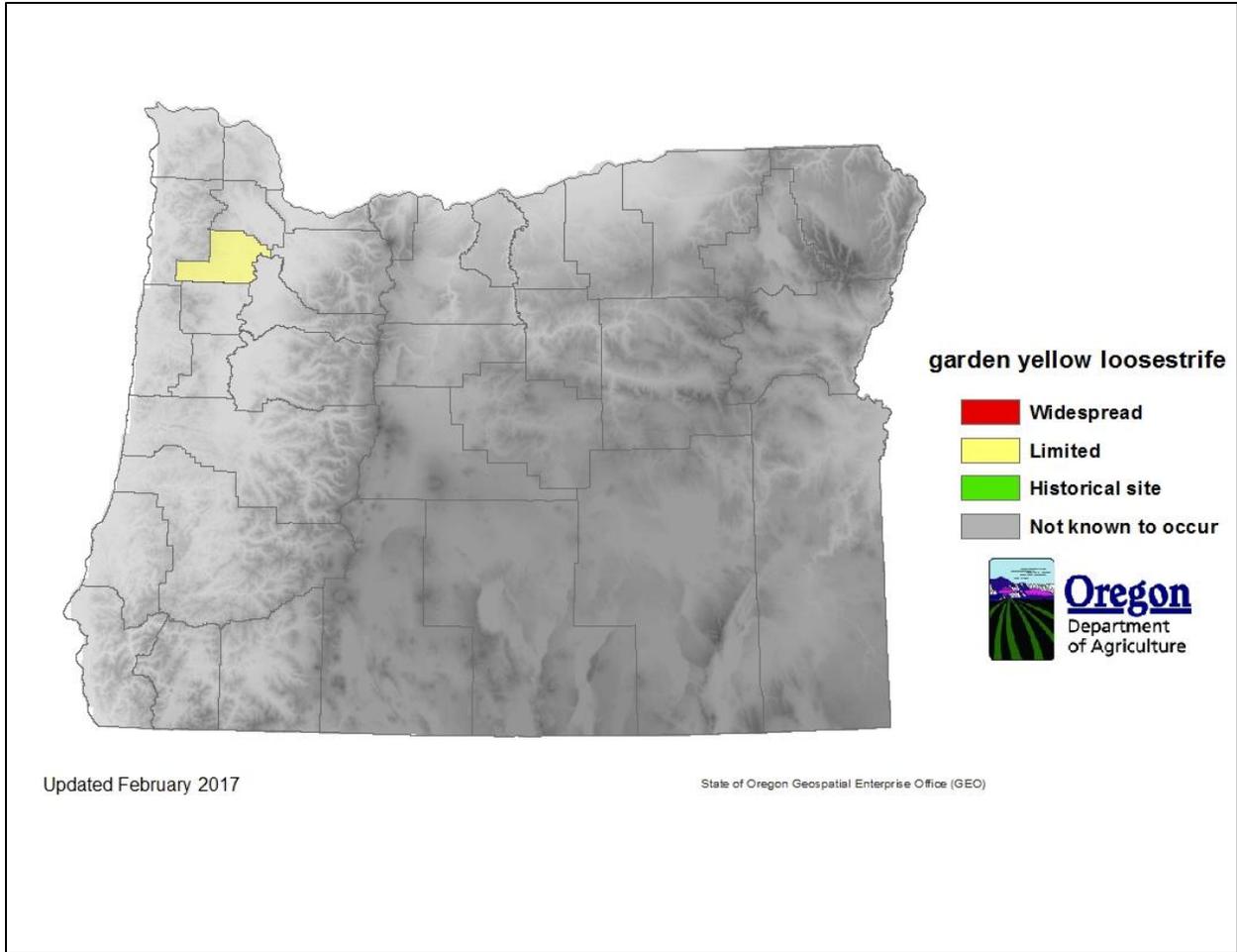
## **History and Distribution**

While garden loosestrife was originally brought to Washington State as an ornamental, it quickly escaped onto native landscapes and established in wetland habitats (Washington State Department of Ecology 2017). Garden loosestrife was first documented in Washington State with a single herbarium collection made by Dr. Bastiaan Meeuse curated at the University of Washington in 1978 (Washington State Department of Ecology 2017). The specimen was collected at the northeastern shore of Lake Washington near Juanita Junction (Washington State Department of Ecology 2017). In the early 1990s garden loosestrife began to appear in larger numbers, particularly on Lake Washington and Lake Sammamish (Messick and Kerr 2007). By the late 1990s it had spread to Lake Burien, south of Seattle, and Rutherford Slough in the Snoqualmie River Basin (Messick and Kerr 2007). It now resides in western counties Whatcom, Skagit, Island, Snohomish, King, Kitsap, and Pacific and eastern counties Chelan, Stevens, and Pend Oreille (Washington State Department of Agriculture 2017).

Washington is just one state impacted by garden loosestrife. Sections of the Northeastern and Mid-Atlantic States (including Maine, New Hampshire, Vermont, New York, Massachusetts, Connecticut, Rhode Island, Pennsylvania, New Jersey, Maryland, Ohio, and West Virginia), northern Midwest states (including Ohio, Kentucky, Indiana, Michigan, Wisconsin, Illinois, and Minnesota), and Western States (including Colorado and Montana), have also reported garden loosestrife populations (United States Department of Agriculture 2017). Canadian provinces including Quebec, Ontario, and British Columbia have also had garden loosestrife populations introduced to the region.



**Figure 1. Washington State Department of Agriculture 2016 map of garden loosestrife distribution in Washington State. Compared to the 2011 distribution map (not shown), garden loosestrife has been eradicated from Thurston County, but has spread to Pend Oreille, and Kitsap Counties.**



**Figure 2. Oregon State Department of Agriculture 2017 map of counties containing garden loosestrife populations. Garden loosestrife populations have only been observed in Yamhill County, and only in small patches, and efforts are underway to eliminate these populations to prevent further spread in Oregon (Beth Myers-Shenai, personal communication, 2017).**

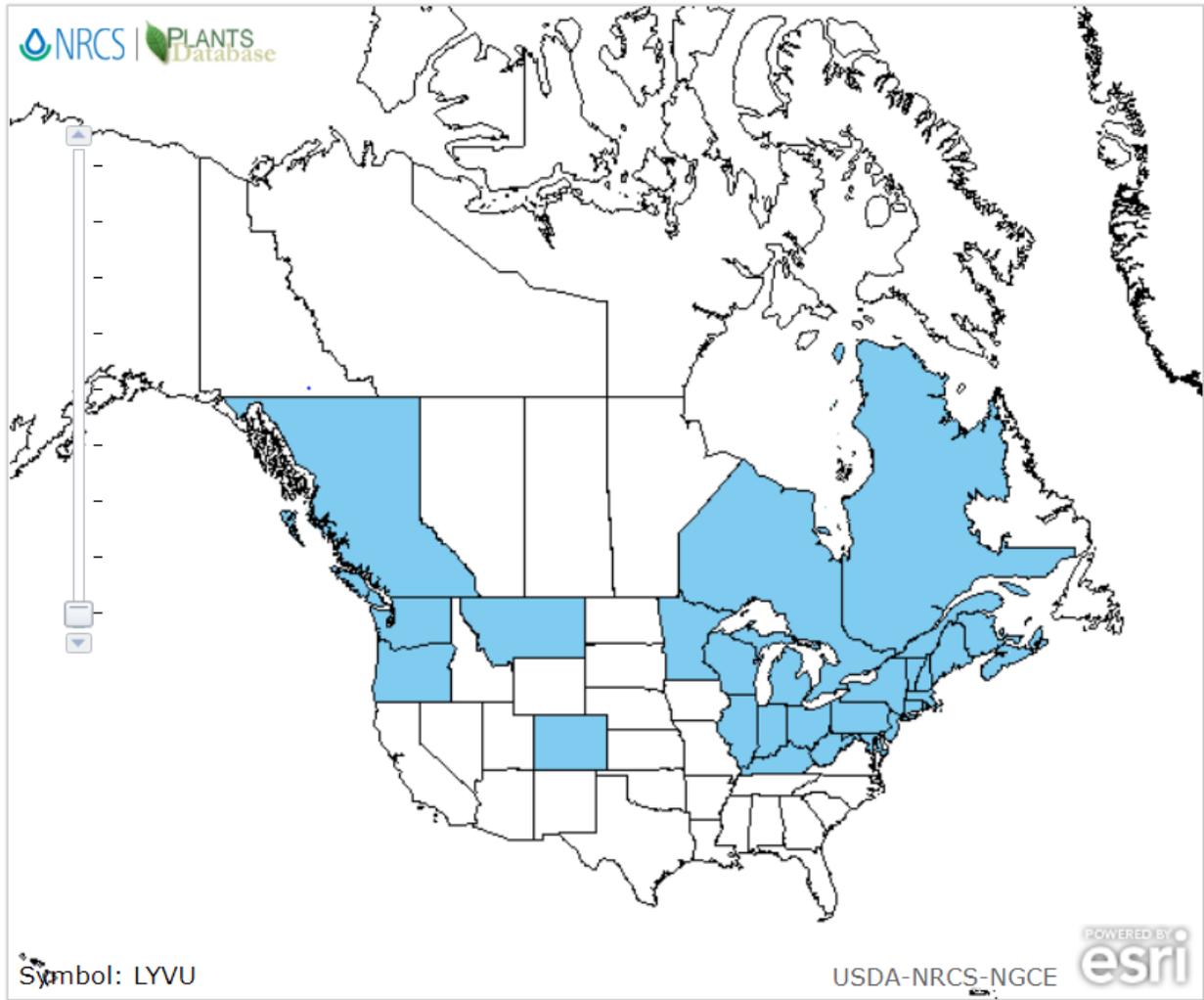


Figure 3. Washington State Department of Agriculture PLANTS Database 2017 map of garden loosestrife populations in the United States of America and Canada.

# Part I

## Introduction

Non-native plant invasions are responsible for long-term changes to ecosystems on a local and global scale, particularly due to their negative impact on biodiversity (Vilà and Weiner 2004). Invasive species can be superior competitors to their native counterparts (Bakker and Wilson 2001, Mincheva et al. 2016) and are predicted to maintain their dominant advantage in the wake of anthropogenic disturbance, as there is often an interactive effect of habitat loss and disturbance on native species decline (Fenesi et al. 2015). This pattern of dominant growth in disturbed sites is true for *Lysimachia vulgaris* L., or garden loosestrife, where it grows in King County and other areas of Washington State. Garden loosestrife populations have increasingly spread over the past two decades and have gained traction in one of the most developed counties in Washington State (Messick and Kerr 2007, King County Noxious Weed Control Program 2010). Much of this invasion is in disturbed habitats that are already dominated by other invasive plants (King County Noxious Weed Control Program 2010). While garden loosestrife grows in both low impact and high impact wetlands, it has been known to grow directly in contact with, or even outcompete, other aggressive non-natives including purple loosestrife (*Lythrum salicaria* L.) and yellow flag iris (*Iris pseudacorus* L.) and weedy natives like spiraea (*Spiraea douglasii* Hook.) and cattail (*Typha latifolia* L.) (Messick and Kerr 2007).

Another growing concern with garden loosestrife is the difficulty of its control. While aquatically labelled herbicides such as triclopyr-TEA have selectively reduced garden loosestrife populations, glyphosate and imazapyr are not selective and can leave exposed soil that allows non-native species to recolonize from nearby populations or via the seed bank (Messick and Kerr 2007). Soil residuals of imazapyr may also prevent immediate planting of natives (Messick and Kerr, 2007). Garden loosestrife has a high seed germination rate, up to 89% under ideal growing conditions (Dillon and Reichard 2014), and also spreads effectively from rhizomes that can reroot through fragmentation. It is also difficult to identify when not flowering, often growing in inconspicuous but dense monotypic stands that are identified as serious infestations when in bloom (Cusick 1986). While studies are in progress to improve the control of garden loosestrife (T. Miller and B. Peterson, personal communication), there is very little literature on garden loosestrife control.

Planting native wetland species to replace garden loosestrife will be an important part of restoration in these vital but delicate wetland habitats. To date, no studies have suggested which species might best compete with garden loosestrife and successfully recolonize former garden loosestrife infestations. One species of interest is small-fruited bulrush, a vigorous native sedge that has been considered weedy in certain environments (Sarah Spear Cooke, personal communication, 2017). Like most sedges, small-fruited bulrush is rhizomatous and spreads quickly through vegetative propagation. It is also an attractive sedge bearing delicate white flower heads. In this study, I tested the hypothesis that small-fruited bulrush, and by extension other fast growing and rhizomatous sedges, may serve as a viable native competitor to recommend for restoration efforts focused on recovering areas where garden loosestrife is prevalent. I hypothesized that competition from small-fruited bulrush would decrease shoot, belowground, and total biomass of garden loosestrife. In addition, I tested whether small-fruited bulrush competition would affect garden loosestrife shoot to root ratio, thereby favoring either shoot or belowground growth. Knowing how garden loosestrife partitions carbon when under stress will assist managers to develop effective control strategies. I hypothesize that plants under stress will produce more shoot growth in an attempt to gain light resources and grow more quickly.

## Methods

To test the hypothesis that competition with small-fruited bulrush negatively affects garden loosestrife growth, I used a two-way density matrix, an additive competition design (Hamilton 1994, Snaydon 1991). The matrix combines one or two plants each of garden loosestrife and small-fruited bulrush to fulfill all possible combinations. Both garden loosestrife and small-fruited bulrush were planted individually with one plant per pot representing the control (no competition) and two plants per plot providing intraspecific competition between plants of the same species (See Figure 4). The combination treatments represent 33%, 50% and 67% garden loosestrife presence, for a total of eight treatments.

On May 18-20, 2016, I collected whole small-fruited bulrush plants in Yesler Swamp at the Center for Urban Horticulture, Seattle, WA, and placed them in standing water to prevent desiccation prior to their being transplanted into pots on May 22. I collected whole garden loosestrife plants on May 21 at Timberlake Park in Issaquah, WA, and placed them in standing water for about a month to continue to grow as the small-fruited bulrush established in appropriate pots. I cut the small-fruited bulrush into equal lengths of root and shoot, ensuring that cuttings were of similar size, although they differed somewhat in length depending on the stem diameter of each plant. Cuttings were weighed and further trimmed to fall within a range of 18-22g per cutting ( $\pm 10\%$  from the 20g target weight). Each cutting was allowed to bear a single rhizome, although not all cuttings had rhizomes. Some of these cuttings died in the first week after planting, due to transplant stress or bird interference. I therefore collected more small-fruited bulrush on May 29 and, using the same methods for making uniform cuttings, planted new cuttings in the pots where the failing small-fruited bulrush starts had died. I made garden loosestrife cuttings on June 18, keeping root and shoot segments similar and falling in a weight range which of 9-11g ( $\pm 10\%$  from the 10g target weight). All rhizomes were removed from the garden loosestrife cuttings prior to weighing. The garden loosestrife cuttings were then transplanted. However, additional small-fruited bulrush cuttings failed to establish, and died during the experiment. To minimize the impact of these losses, I collected more small-fruited bulrush from Yesler Swamp on July 6, and on July 8, I made new cuttings using the average height of the tallest leaf from surviving potted bulrush cuttings. Again, these new cuttings were trimmed so roots and shoots were of equal length, and each cutting bore a maximum of one rhizome. Weights of these cuttings varied and were recorded to include in analyses. Ten garden loosestrife also performed poorly after transplanting and were replaced by garden loosestrife cuttings that had been planted in the same soil within separate pots on June 18. Therefore, no approximation of growth was necessary.

Pools were watered using sub-irrigation 3-4 days a week depending on weekly temperature and growing needs. Miracle Gro® Liqua Feed® (12N-4P-8K) fertilizer (The Scotts Company LLC, Marysville, OH, USA) was applied using a Miracle Gro® Garden Feeder® hose adaptation. Fertilizer was applied directly into the pots for a one-second spray in each pot (about 75mL). Applications occurred on July 24, August 7 and 21, and September 4. To prevent intervention by wildlife, netting was hung around the experiment on May 26. An insecticide (Safer® Brand Caterpillar Killer) was applied to all of garden loosestrife plants in the summer to reduce predation from non-native sawfly larvae (*Monostegia abdominalis* Fabricius) (Looney et al. 2016). I also hand weeded as needed to maintain weed-free pots, usually after watering.

All plants were destructively harvested from October 26-November 3, placed in paper bags and stored at 6°C until measured. From December 6, 2016-January 4, 2017 I recorded shoot number and longest leaf blade for both species, as well as number of rhizomes of small-fruited bulrush and the length and number of rhizomes for garden loosestrife. Rhizome/root and shoot material was separated, dried for one week at 65°C (Hotpack® oven, Philadelphia, PA, USA) and above and belowground biomass was weighted and recorded.

#### Data Analysis

To better understand the relationship of biological outcomes from interspecific competition, I used Analysis of Variance (ANOVA) to determine if any of the measurements was significantly affected by the various levels of competition severity. I removed an outlier from the data analysis, a LV2, SM0 treatment (LV= garden loosestrife, SM= small-fruited bulrush). Skewness and kurtosis tests were performed to check the normal distribution of the data, and transformations were applied where necessary to meet normality assumptions for ANOVA (square root and logarithmic transformations). Significant results were subjected to mean separations using Tukey's Honestly Significant Difference (HSD) Post-Hoc test to determine directionality of the variance and determine treatment differences.

Small-Fruited Bulrush (*Scirpus microcarpus*)

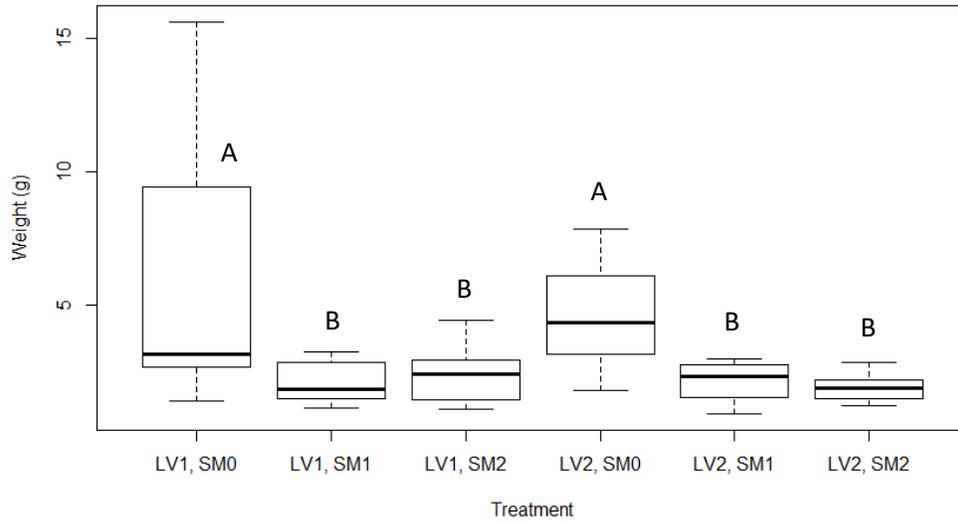
Garden Loosestrife ( <i>Lysimachia vulgaris</i> )		0 LV 1 SM	0 LV 2 SM
	1 LV 0 SM	1 LV 1 SM	1 LV 2 SM
	2 LV 0 SM	2 LV 1 SM	2 LV 2 SM

Figure 4. Two way density matrix of garden loosestrife (LV) and small-fruited bulrush (SM).

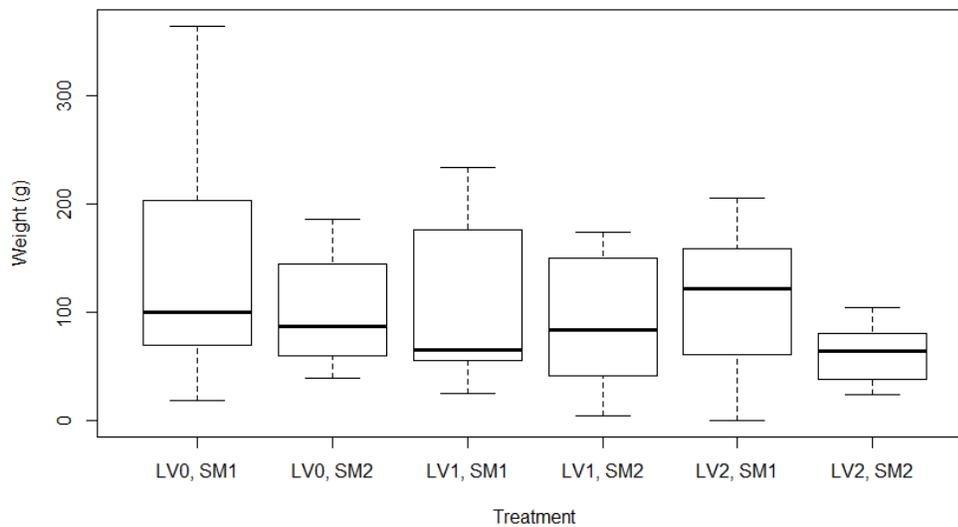
## Results

Garden loosestrife total weight, shoot weight, root weight, and root to shoot ratio (root:shoot) was significantly decreased by the presence of bulrush in many of the mixed treatments (See Table 2). To increase normality in the dataset I used a log transformation on the data, although it did not change the result of the test. The results of the Tukey HSD Post-Hoc test confirmed a significant difference between treatments. Total garden loosestrife dry weight, root:shoot, and root weight was greater in the LV1, SM 0 treatment than the LV1, SM1 and LV1, SM2 treatments ( $p=0.007$ ,  $p=0.021$  for total dry weight, respectively;  $p=0.034$ ,  $p=0.013$  for root:shoot, respectively;  $p=0.014$ ,  $p=0.043$  for root weight, respectively). LV1, SM 0 also had a significantly larger shoot weight than LV1, SM1 ( $p=0.041$ ). Similarly, the LV 2, SM0 treatment had a significantly higher total weight, root:shoot, and root weight than LV2, SM1, and LV2, SM2 treatments ( $p=0.036$ ,  $p=0.014$  for total weight, respectively;  $p=0.023$ ,  $p=0.011$  for root:shoot, respectively;  $p=0.025$ ,  $p=0.012$  for root weight, respectively). The presence of small fruited-bulrush did not significantly affect the number of rhizomes or rhizome length in garden loosestrife (See Table 4).

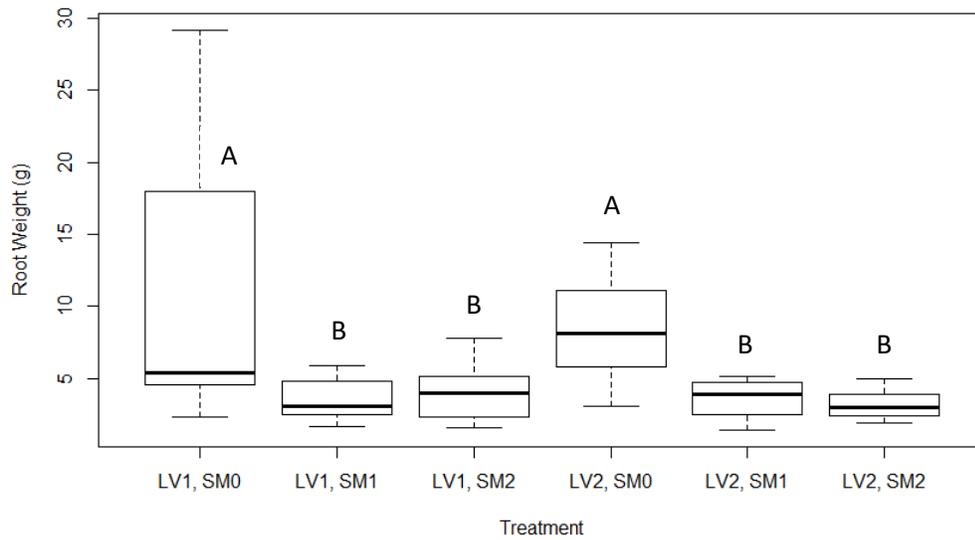
Except in one case, small-fruited bulrush total weight, shoot weight, root weight, and root:shoot was not significantly affected by the presence of garden loosestrife, regardless of density (See Table 1). The LV1, SM1 treatment produced significantly more rhizomes per individual than the LV1, SM2 treatment ( $p=0.027$ ). Therefore, in this case, intraspecific competition was more impactful to small-fruited bulrush than interspecific competition with garden loosestrife. Shoot number and shoot length did not differ significantly by treatment, and while root number did differ in one case it was due to intraspecific competition, not due to competition between garden loosestrife and small-fruited bulrush (See Table 3).



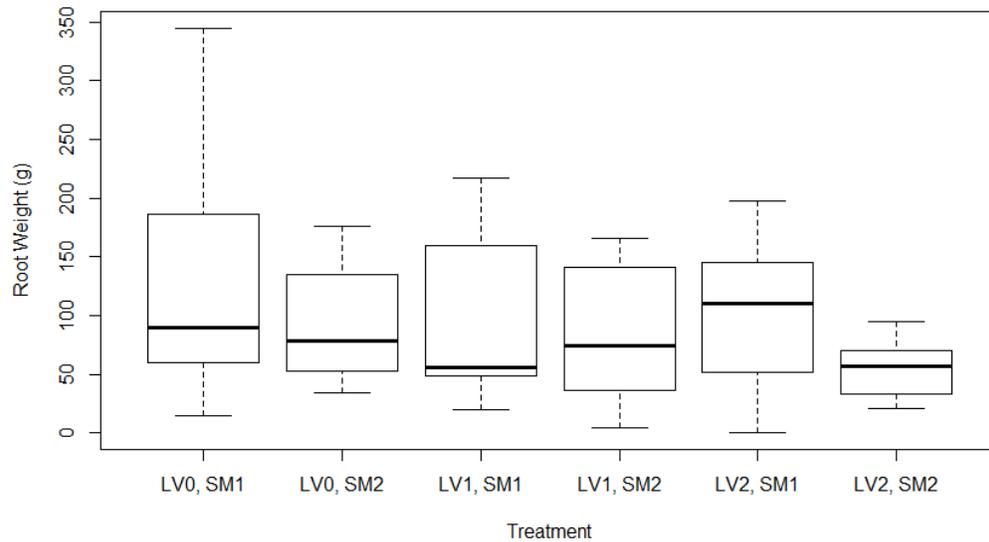
**Figure 5. Boxplot of total dry weight for garden loosestrife individuals within each treatment group. Means for each treatment are represented by a solid black line show highest and lowest values, box ends show first and third quartiles. Letters group treatments that are not significantly different from one another.**



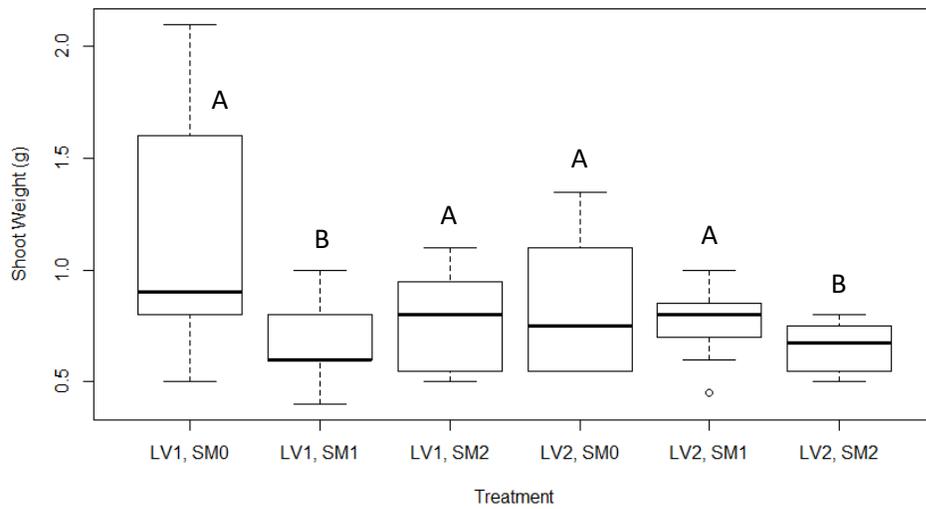
**Figure 6. Boxplot of total dry weight for small-fruited bulrush individuals within each treatment group. Means for each treatment are represented by a solid black line. Bars show highest and lowest values, box ends show first and third quartiles.**



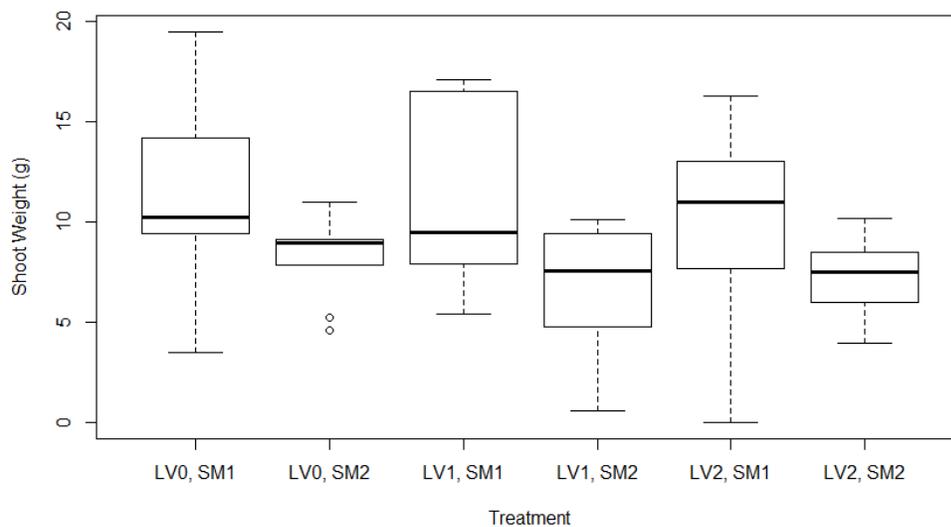
**Figure 7. Boxplot of dry root weight for garden loosestrife individuals in each treatment. Means for each treatment are represented by a solid black line. Bars show highest and lowest values, box ends show first and third quartiles. LV = garden loosestrife, SM = Small-fruited bulrush. Letters group treatments that are not significantly different from one another.**



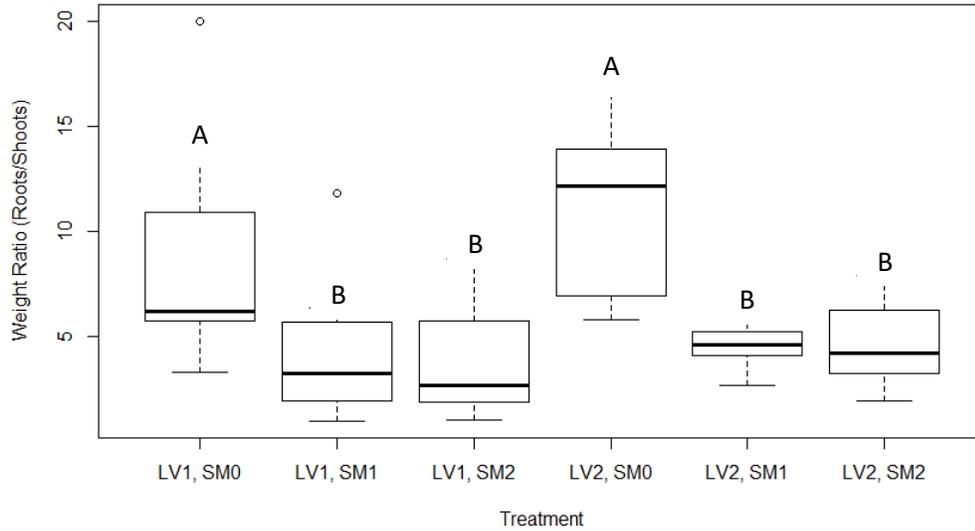
**Figure 8. Boxplot of dry root weight for small-fruited bulrush individuals in each treatment. Means for each treatment are represented by a solid black line. Bars show highest and lowest values, box ends show first and third quartiles. LV = garden loosestrife, SM = Small-fruited bulrush.**



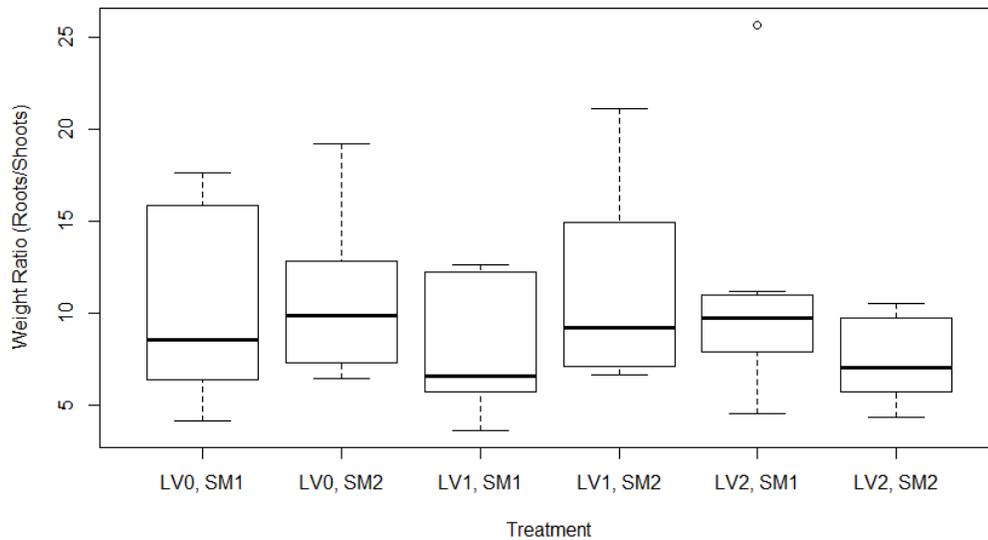
**Figure 9.** Boxplot of dry shoot weight for garden loosestrife individuals in each treatment. Means for each treatment are represented by a solid black line, and the open circles indicate outliers. Bars show highest and lowest values, box ends show first and third quartiles. LV = garden loosestrife, SM = Small-fruited bulrush. Letters group treatments that are not significantly different from one another.



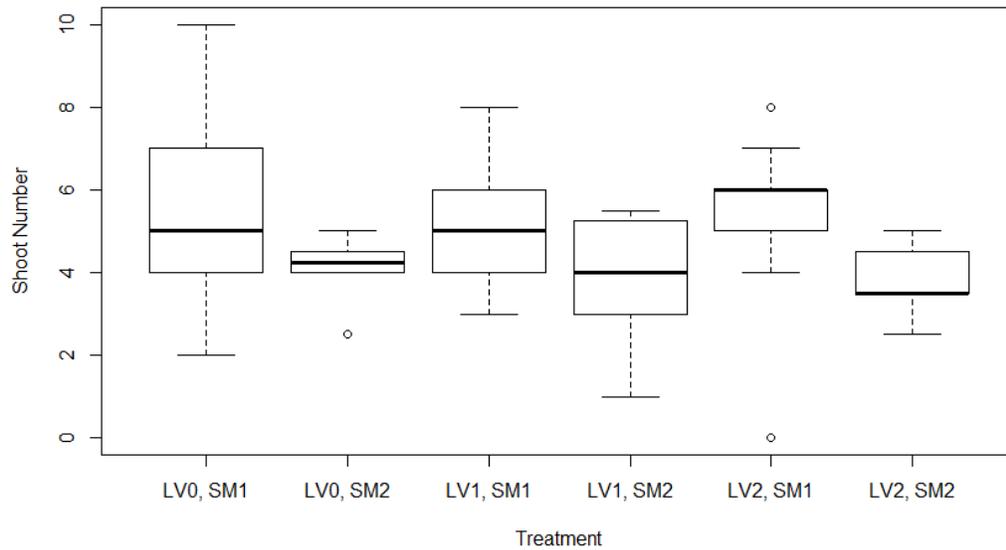
**Figure 10.** Boxplot of dry shoot weight for small-fruited bulrush individuals in each treatment. Means for each treatment are represented by a solid black line, and the open circles indicate outliers. Bars show highest and lowest values, box ends show first and third quartiles. LV = garden loosestrife, SM = Small-fruited bulrush.



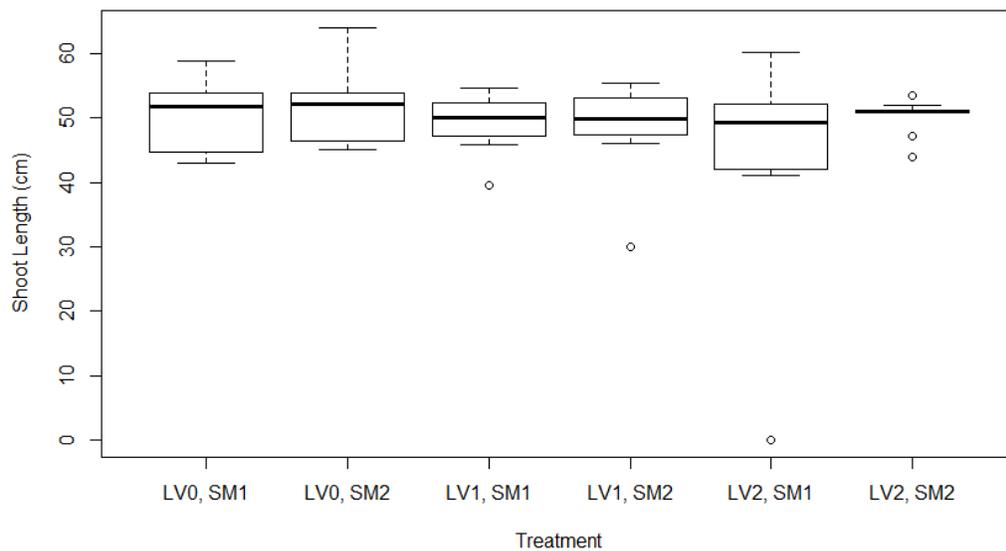
**Figure 11. Root to shoot ratio for garden loosestrife individuals in each treatment. Means for each treatment are represented by a solid black line, and the open circles indicate outliers. Bars show highest and lowest values, box ends show first and third quartiles. LV = garden loosestrife, SM = Small-fruited bulrush. Letters group treatments that are not significantly different from one another.**



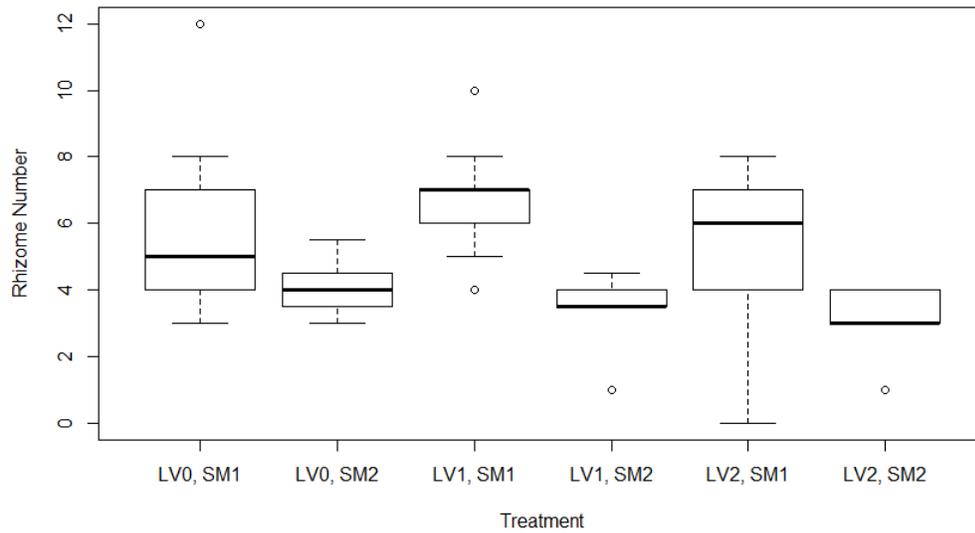
**Figure 12. Root to shoot ratio for small-fruited bulrush individuals in each treatment. Means for each treatment are represented by a solid black line, and the open circles indicate outliers. Bars show highest and lowest values, box ends show first and third quartiles. LV = garden loosestrife, SM = Small-fruited bulrush.**



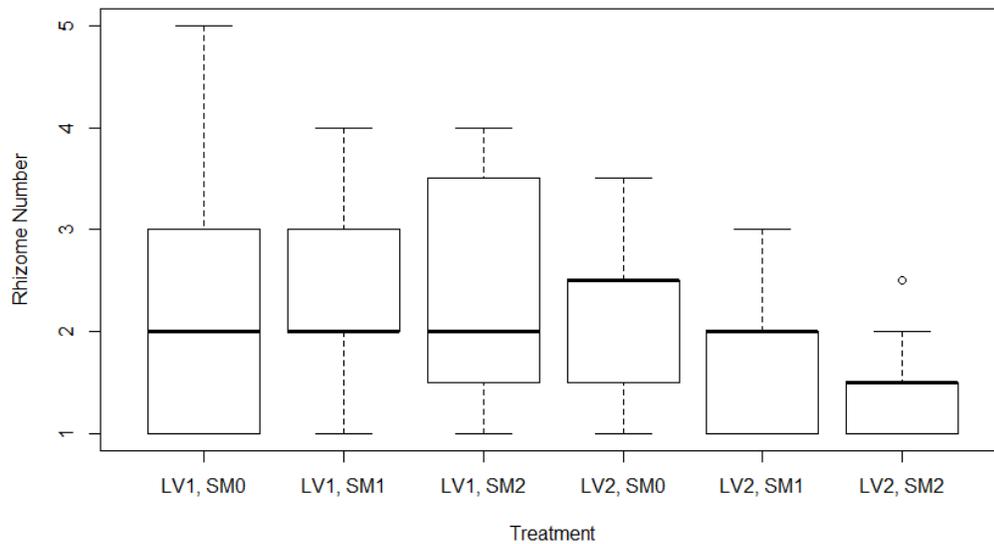
**Figure 13. Shoot number, or number of shoots resulting in clonal individuals, for small-fruited bulrush individuals in each treatment. Means for each treatment are represented by a solid black line, and the open circles indicate outliers. Bars show highest and lowest values, box ends show first and third quartiles. LV = garden loosestrife, SM = Small-fruited bulrush.**



**Figure 14. Shoot length in centimeters for small-fruited bulrush individuals in each treatment. Means for each treatment are represented by a solid black line, and the open circles indicate outliers. Bars show highest and lowest values, box ends show first and third quartiles. LV = garden loosestrife, SM = Small-fruited bulrush.**



**Figure 15.** Number of rhizomes for small-fruited bulrush (including clonal offspring) individuals in each treatment. Means for each treatment are represented by a solid black line, and the open circles indicate outliers. Bars show highest and lowest values, box ends show first and third quartiles. LV = garden loosestrife, SM = Small-fruited bulrush.



**Figure 16.** Number of rhizomes for small-fruited bulrush individuals in each treatment. Means for each treatment are represented by a solid black line, and the open circles indicate outliers. Bars show highest and lowest values, box ends show first and third quartiles. LV = garden loosestrife, SM = Small-fruited bulrush.

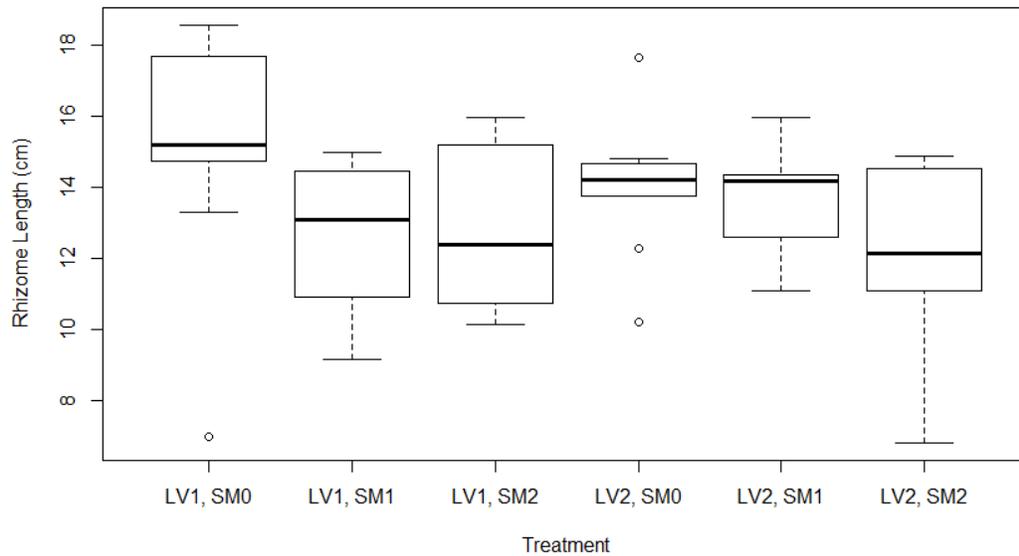


Figure 17. Rhizome length in centimeters for garden loosestrife individuals in each treatment. Means for each treatment are represented by a solid black line, and the open circles indicate outliers. Bars show highest and lowest values, box ends show first and third quartiles. LV = garden loosestrife, SM = Small-fruited bulrush.

Table 1. Results of ANOVA testing for small-fruited bulrush dry weights, where  $p=0.05$  is significant. \*, \*\*, and \*\*\* indicate degree of significance.

	DF	Sum Sq	Mean Sq	F value	P value
Total Dry Weight	5	17244	3349	0.823	0.536
Dry Weight Shoots	5	169.7	33.95	2.355	0.0541
Dry Weight Roots	5	28612	5722	1.232	0.309
Root/Shoot Ratio	5	110.3	22.05	1.061	0.349

Table 2. Results of ANOVA testing for garden loosestrife dry weights, where  $p=0.05$  is significant. \*, \*\*, and \*\*\* indicate degree of significance.

	DF	Sum Sq	Mean Sq	F value	P value
Total Dry Weight	5	7.474	1.4908	5.971	0.000218 ***
Dry Weight Shoots	5	1.423	0.2846	2.831	0.0253 *
Dry Weight Roots	5	9.028	1.8057	6.011	0.000206 ***
Root/Shoot Ratio	5	10.1	2.019	6.632	8.7 e -5 ***

**Table 3. Results of ANOVA testing for small-fruited bulrush morphological features, where  $p=0.05$  is significant. \*, \*\*, and \*\*\* indicate degree of significance.**

	DF	Sum Sq	Mean Sq	F value	<i>P</i> value
Shoot #	5	24.44	4.888	1.679	0.157
Shoot Length	5	369	73.8	0.993	0.431
Rhizome #	5	5.164	1.0328	4.328	0.00238**

**Table 4. Results of ANOVA testing for garden loosestrife morphological features, where  $p=0.05$  is significant.**

	DF	Sum Sq	Mean Sq	F value	<i>P</i> value
Rhizome #	5	5.61	1.1212	1.248	0.302
Rhizome Length	5	56.79	11.359	1.992	0.0963

## Discussion

The study was designed to simulate an invasion of garden loosestrife into an already established small-fruited bulrush population, which limits the comparisons we can make to areas where garden loosestrife already exists. Still, it was clear that late-planted garden loosestrife individuals were negatively affected by competition with previously-established small-fruited bulrush. All garden loosestrife dry weight measurements showed a marked decrease compared to garden loosestrife plants grown without competition or grown under less stress from competition caused by fewer small-flowered bulrush plants per pot. Even with no competition, however, none of the garden loosestrife plants regrew from their apical meristem once cut, so while the plants produced additional leaves and stems, they remained at only a small fraction of their potential height. This may have been caused by the already-established small-fruited bulrush plants overgrowing garden loosestrife plants, resulting in plants that would likely have grown taller under field conditions. Preferential feeding of garden loosestrife leaves by *Monostegia abdominalis* larvae may also have limited plant growth after transplanting. Prior to their mid-summer control with insecticide, these caterpillars caused substantial damage to garden loosestrife, but did not feed on small-fruited bulrush. While the observed lack of upward garden loosestrife growth obscures the surety of these results, these findings may have implications for control of small garden loosestrife infestations. Even if cutting or mowing the stems of field-grown garden loosestrife did not result in the death of the plant, cutting should limit the final height of garden loosestrife during that season. The only morphological feature of small-fruited bulrush that was negatively impacted by competition was number of rhizomes, although this apparently occurred due to increased competition from other small-fruited bulrush plants rather than from garden loosestrife. This indicates that mature small-fruited bulrush could be strong competitor where garden loosestrife has invaded but is still relatively small and has limited establishment.

Importantly, while garden loosestrife root and shoot growth was negatively impacted by the presence of small-fruited bulrush, rhizome number and length were not significantly different regardless of competition. Garden loosestrife, moves distances through seed propagation but colonized close areas through vegetative propagation, in this case using rhizomes (King County Noxious Weed Control Program 2010). All garden loosestrife individuals grew rhizomes, though none of the individuals started the experiment with rhizomes. Clearly rhizomes are an integral part of the garden loosestrife life strategy, and merits further research. In future studies, I would start the experiment earlier in the year,

shortly after the loosestrife emerged, and harvest the study in September before the plants begin to senesce. Although growing the plants outside was a better approximation of natural conditions, the other variables that come with growing plants in a less controlled environment ultimately impacted the experiment in a negative way, which could have been avoided by using a greenhouse. This study would have benefitted from using whole plants instead of cuttings, to improve establishment of both species and to allow garden loosestrife to grow to its full potential height. Ultimately, it is not possible to say on the basis of this study whether small-fruited bulrush would be a good competitor to replant in areas where garden loosestrife is prevalent.

Garden loosestrife has a competitive advantage compared to many natives in Washington State. Part of the reason this is the case is that there are not many native herbaceous perennials that can tolerate wetland conditions. This leaves a niche open in the ecosystem, which is one possible explanation for the large number of wetland invasives that are also herbaceous perennials (purple loosestrife, yellow flag iris, etc.). Wetland themselves are prone to invasion; while less than 6% of the earth's land mass is wetland, 24% of the world's most invasive plant species are also considered wetland species (Zedler and Kercher 2004). Zedler and Kercher pose that wetlands act as landscape sinks, which accumulate debris, water, sediments, and nutrients, which feed and facilitate invasions through regular disturbance and nutrient accumulation. While no single plant attribute or disturbance event can explain wetland plant invasions, the regular propagule influx, salt influx, and hydroperiod alteration likely contribute to the high occurrence of invasion (Zedler and Kercher 2004).

## Part II

### Introduction

Garden loosestrife reproduces primarily through vegetative propagation when establishing at a site (King County Noxious Weed Control Program 2010). Anecdotal observation from staff at the King County Noxious Weed Control Board and other land managers suggest that garden loosestrife also establishes populations primarily through rhizome propagation. Japanese knotweed (*Fallopia japonica* (Houtt.) Ronse Decr.), another noxious weed in Washington State found primarily on shorelines, was shown to establish more from rhizome growth (85%) than from seed (3%) or stem fragments (16%) in a riparian forest understory (Gowton et al. 2016). Giant reed (*Arundo donax* L.), an invasive species found in riparian habitats, relies on factors such as vegetative propagation and abiotic site conditions for establishment and was unaffected by the composition of the native community at a site (Quinn and Holt 2008). Bohemian (*Fallopia x bohemica* (Chrték & Chrtková) J.P. Bailey) and Japanese knotweed can spread downstream and over land through vegetative propagation (Duquette et al. 2015). Russian knapweed (*Rhaponticum repens* (L.) Hidalgo), another knapweed, has also been recorded to spread rapidly in small areas close to the point of origin from vegetative propagation (Gaskin and Littlefield 2017).

While producing vegetative propagules can be costly in terms of resources, benefits are substantial if a plant can obtain more resources from a larger area with a clonal offspring (Lopp and Sammul 2016). In some circumstances, it may be even more advantageous to produce through vegetative reproduction than sexual reproduction of seeds (Atwater et al. 2017). Johnsongrass (*Sorghum halepense* L.) propagates more efficiently from seed than by rhizomes on a per unit of carbon basis, but rhizomes were more efficient for establishment of a new plant on a per propagule basis (Atwater et al. 2017). While seeds may be a preferred method of reproduction when plants are under stress from interspecific competition and increased propagule densities, rhizomes are used for colonization of areas that are inhospitable to seeds, and that the cost and benefit to the plant utilizing rhizomes is complex (Atwater et al. 2017).

Propagule growth from rhizomes is a concern to land managers who work to control invasive species. Propagule pressure is considered a primary factor for invasive plant establishment and spread,

especially in regard to propagule size and condition (Estrada et al. 2016). In a rhizome propagule study of cogongrass (*Imperata cylindrica* (L.) P. Beauv.), researchers found that rhizome length of at least three nodes significantly enhanced establishment (Estrada et al. 2016). The depth at which rhizomes are grown in the soil is another factor which could indicate rhizome viability. A study of two invasive *Solidago* clonal species used both length of rhizome propagules and depth planted in soil to determine their “resprouting ability,” and found that rhizomes could resprout from depths of 10-20cm depending on the species (Weber 2011). The authors concluded that managers of these invasive plants should avoid any activity that would cause disturbance in the soil or create rhizome fragmentation (Weber 2011).

The length of garden loosestrife rhizome section required for resprouting and the depth at which shoots may emerge from buried rhizome fragments has not been reported. This study seeks to answer two main questions in control of garden loosestrife populations. The first is to investigate the viability and vitality of garden loosestrife rhizome sections of varying lengths and transplanted at varying depths detachment from the parent plant. Results as to the minimum length and maximum depth rhizomes will successfully produce clonal offspring will help managers determine if mechanical removal of garden loosestrife is a feasible option for removal of small populations. The second question centers on the seasonality of sprouting, when rhizomes may be more likely to produce shoots and flowers. If so, managers may time their removal of garden loosestrife to reduce the likelihood of recolonization through lowering the incidence of rhizome sprouting.



**Figure 18. Garden loosestrife rhizome fragment collected in 2016. This fragment is not representative of the size of rhizomes used in this study, but illustrates the condition of the rhizomes that were collected for this experiment. Rhizomes were excluded from the study if they had already begun leafing out.**

## Methods

To test the hypothesis that garden loosestrife rhizomes are viable at a range of small sizes and shallow soil depths, and at different times during the summer growing season, I designed a study that planted and observed the success of garden loosestrife rhizome fragments. The study was conducted over the course of 15 weeks and examined the full extent of regeneration of garden loosestrife rhizomes. On May 6, 2017 I collected whole garden loosestrife plants from Timberlake Park in Issaquah, WA, located on the south side of Lake Sammamish. I collected over one hundred plants and transported them to the Center for Urban Horticulture at the University of Washington and placed in a 5-ft diameter pool of water to grow out their rhizomes for five weeks. 100mL of Miracle Gro® Liqua Feed® (The Scotts Company LLC, Marysville, OH, USA) was applied to the water on the same day to stimulate rhizome growth. An additional 200mL of fertilizer was applied one week before the start of the experiment to ensure there would be enough rhizomes for the study.

On June 12, I removed the growing apical portion of garden loosestrife rhizomes and placed them in a container of water for holding. Rhizomes fragments were cut into segments of 1, 2, or 5cm and planted at depths of 0 cm (surface), 4, or 8cm in the soil. One centimeter segments had one node, 2cm segments had one or two nodes, and 5cm segments typically had two or three nodes. There were therefore nine treatments per replicate, planted into 16 replicates (planting blocks). After cutting to the appropriate length, segments were weighed using a Sartorius three decimal scale (Sartorius Corporation, Edgewood, NY, USA) and planted immediately into a Deepot cell (model D40-H, Stuewe and Sons. Inc., Tangent, OR, USA). To ensuring that there was minimal movement of the depth of the rhizome after planting, pots were filled with potting soil (Sunshine #4 potting soil, SunGro, Bellevue, WA, USA) and firmed to the appropriate level before planting. Rhizome sections were then placed on top of the potting soil and additional potting medium applied to achieve the proper burial depth. Pots were then irrigated for one minute using a nozzle on mist setting, and allowed to drain. After an adjustment period of two days, the study officially started on June 14. Pots were maintained in the Douglas Conservatory Greenhouse at the Center for Urban Horticulture, University of Washington. The experiment was maintained at 68-72°F with a 14-hour photoperiod. Pots were watered 6-7 times a week, for about 30 seconds for each planting block using a mist setting (~ 1 L) to maintain moist potting soil for optimal rhizome sprouting conditions. To help minimize the effect of common greenhouse pests on the rhizome growth (fungus

gnats, house flies, etc.), fly cards were distributed throughout the trays to catch insects and prevent them from interacting with the rhizomes or burrowing into the bare soil of the pots.

Each week the surface rhizomes were observed for emergence, appearance, and growth, for a total of six weeks. At the end of six weeks (August 2), rhizomes were photographed and harvested for measurements. Stem height, root length, and leaf number were measured, then root and shoot material was excised, separately bagged, dried at 95° C for 24 hours (Hotpack® oven, Philadelphia, PA, USA), and dry weights were recorded.

In order to determine whether the date of rhizome removal (early summer versus late summer) influenced shoot production, a second trial was conducted. Garden loosestrife plants collected on May 6 for the first trial (hereafter the “June trial”) were kept alive in the outside pool and were used for this second trial (hereafter, the “July trial”). I used 100ml of the same fertilizer used in the June trial was applied to the water on June 19 to encourage rhizome growth, and rhizome cuttings were taken on July 14 as previously described. The watering procedure remained the same, but due to hotter greenhouse conditions, the length of time required for pot irrigation ranged from 30 seconds to one minute (1-2L). Six weeks later (August 28), garden loosestrife rhizomes and shoots were harvested, morphological data were collected, and dry weights determined as previously described. Finally, at the end of the experiment I dug up some of the rhizomes that had been buried at 4 and 8cm in the July trial and put them on the surface of the potting soil to observe what would happen to previously covered rhizomes.

## Data Analysis

To determine which factors are important to garden loosestrife survival and growth, I used a Hurdle Model to analyze the binary and continuous datasets. A Hurdle Model is a “two-part model that specifies one process for zero counts and another process for positive counts. The idea is that positive counts occur once a threshold is crossed, or put another way, a hurdle is cleared” (University of Virginia Library). This class of model is ideal for handling excess zeros and over dispersion (University of Virginia Library). The model functions by taking into account binary data first, whether a condition has been met or not, and if that condition has been satisfied then compares the data that is available. This model was

used to compare biological data including rhizome shoot height, number of leaves, shoot weight, length of roots, and root weight.

$$E[y|x] = \frac{1 - f_1(0|x)}{1 - f_2(0|x)} \mu_2(x)$$

In order to analyze the end result of survival and growth of rhizomes I used a simple proportions test, a two way sample test for equality of proportions with continuity correction. To visualize the change in growth and survival over the course of six weeks I used a Kaplan-Meier curve to capture the horizontal change over the course of six weeks. Kaplan-Meier curves are an excellent option to visualize the fraction of “subjects” living for a certain time after treatment (Goel et al. 2010).

## Results

Rhizomes planted at depths of 4cm and 8cm did not sprout over the course of the study. Only rhizomes that were placed on the surface of the soil (the 0cm treatment) grew during the six weeks after transplanting for both the June and July trials. At the end of six weeks, the proportion of living rhizomes to dead rhizomes was much higher in June than in July start dates. This was also true for the proportion of growing rhizomes to non-growing rhizomes (See Tables 5-6). Using a proportions test, I determined that the rhizomes in the June trial had significantly more rhizomes alive and growing than rhizomes in the July trial (See Table 7). Some of the buried rhizomes were dug up and placed on the surface of the soil, and after a few weeks on the surface, when watered through a timed mist bench, a few of the rhizomes grew.

Kaplan-Meier curves of the trends in growth and survival over the course of the six week trials revealed little for growth. No clear patterns were shown to exist between either rhizome lengths or start date of experiment. However, there was a loose association of growth groupings between June and July experiment start dates, further supported by the significant difference in proportion of growing rhizomes at the end of six weeks. The Kaplan-Meier curves describing survivorship showed one clear pattern, however: the majority of surface rhizome deaths occurred after one week, and all subsequent deaths did not vary perceptibly from that point through week six (See Figures 19-20).

Rhizome length did not significantly affect any of the biological parameters, including height, root length, number of leaves, shoot weight, or root weight. However, the onset of vegetative growth from rhizome sections was significantly affected by the start date of the experiment. June trial rhizomes were more likely to grow than July trial rhizomes, and when signs of growth were present, shoot height, number of leaves, and shoot weight were greater in the June trial than July trial. Although the presence of root growth, including root length and root weight, was impacted by the starting date of the experiment, the root growth thereafter did not differ based on start date. None of the growth parameters were impacted by starting weight of the rhizomes.

**Table 5. Proportion of alive to dead rhizomes at the end of six weeks for June and July experiment start dates.**

	Dead	Alive
June	29	19
July	41	7

**Table 6. Proportion of growing to non-growing rhizomes at the end of six weeks for June and July experiment start dates.**

	Not Growing	Growing
June	35	13
July	44	4

**Table 7. Proportion of growing/non-growing and alive/dead (survival) rhizomes for June and July experiment start dates. The proportions test confirmed that there was a significant difference between the proportions for June and July experiment start dates for both growth and survival.  $p = 0.05$ .**

Test	July	June	X squared	DF	<i>P</i> value
Growth	0.916667	0.7291667	4.5748	1	0.03244*
Survival	0.854167	0.6041667	6.3824	1	0.01153*

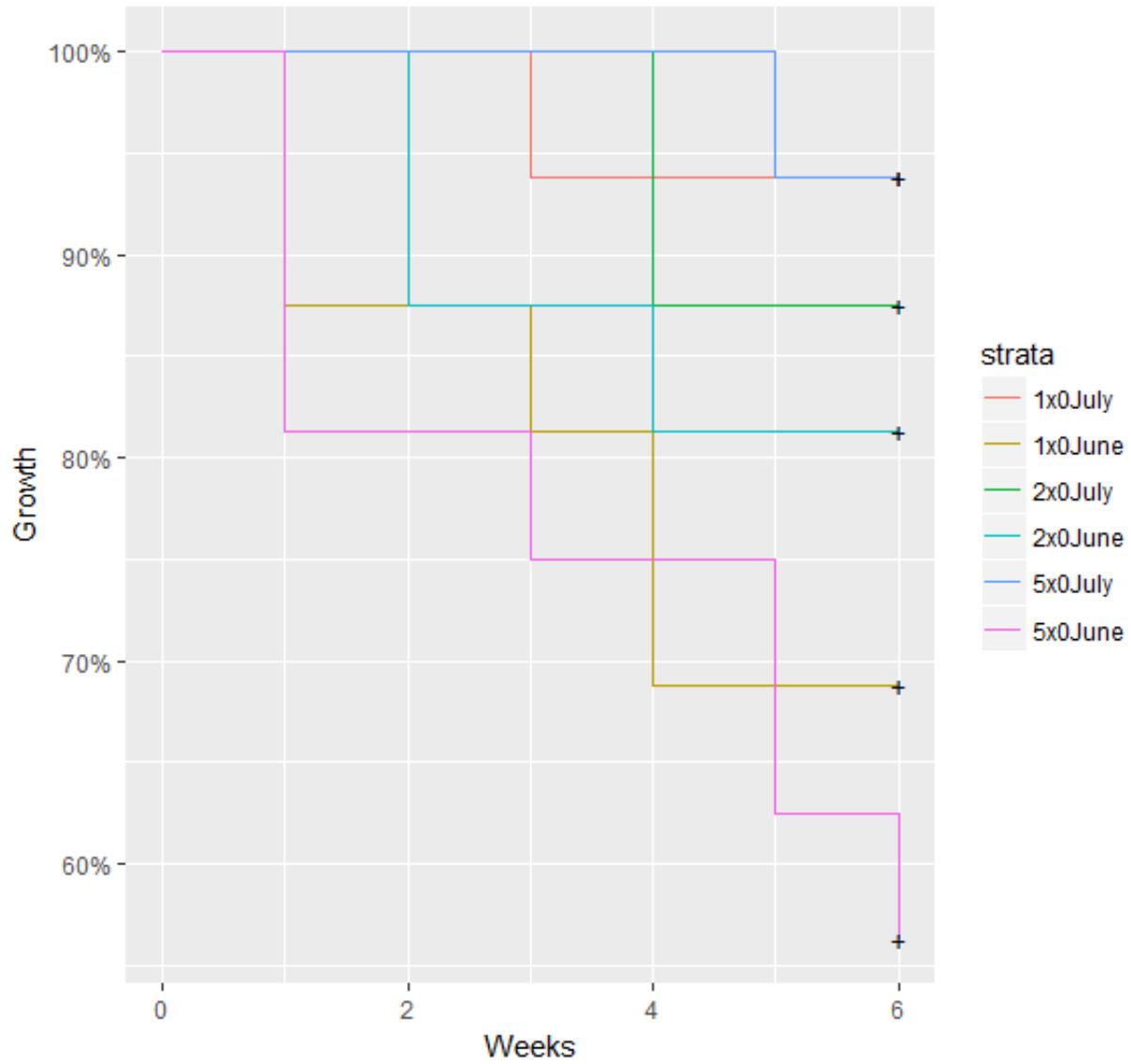


Figure 19. Kaplan-Meier curve of growth in rhizomes organized by rhizome length and month of experiment start date using binary data. The graph describes the percentage of rhizomes that have not achieved growth, decreasing as more rhizomes grow throughout the six week trial.

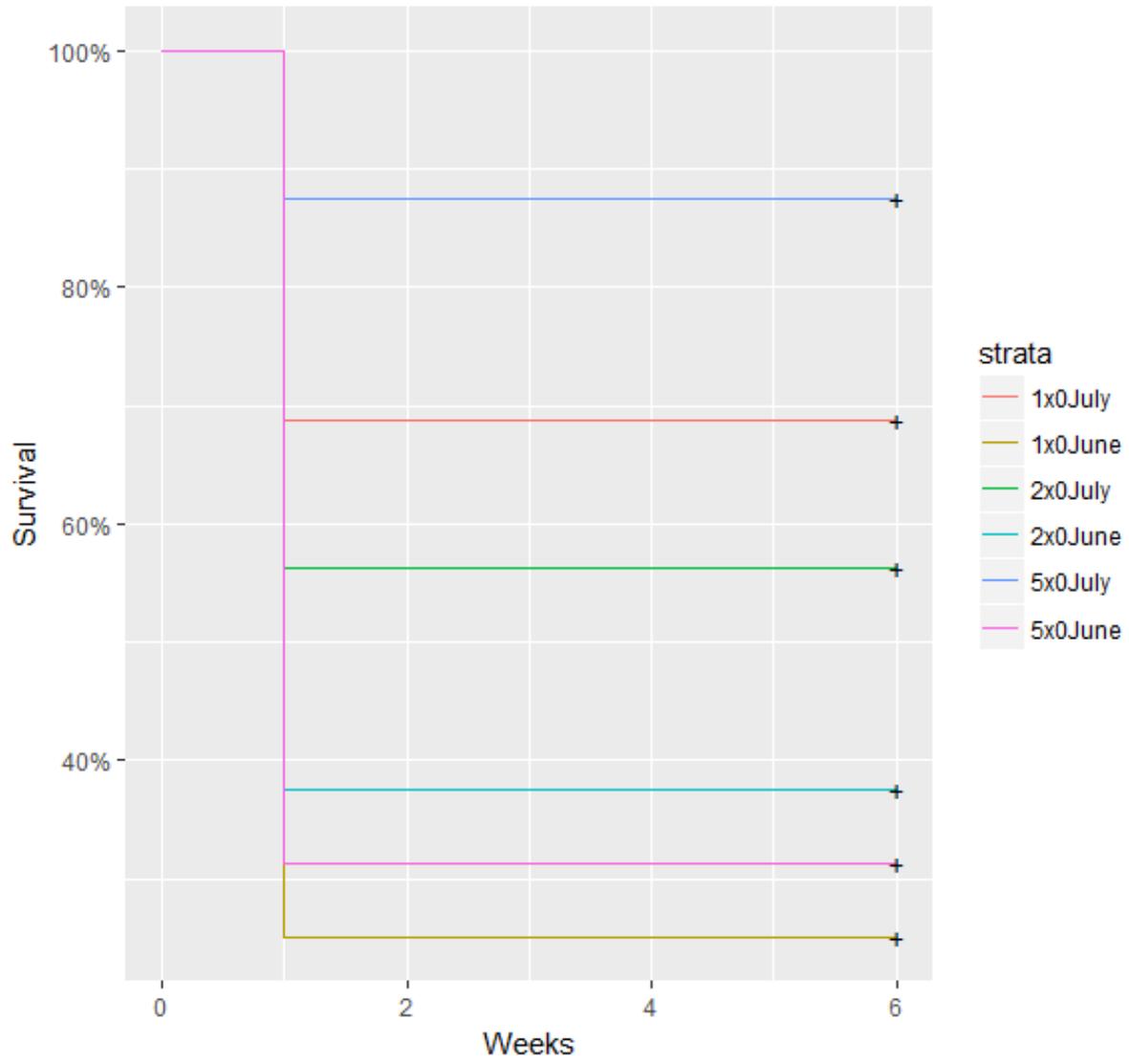


Figure 20. Kaplan-Meier curve of survival in rhizomes organized by rhizome length and month of experiment start date using binary data. The graph describes the percentage of rhizomes that do not appear alive, decreasing as rhizomes show signs of life throughout the six week trial. Signs of life include a round, succulent looking fragment and green (chlorophyll) color present in the rhizome.

**Table 8.** The probability that measurable height is present due to the factors of rhizome length, month of experiment start date, and initial weight of rhizome fragments. The timing of the experiment start date has a significant effect on whether there will be a presence of height in the rhizome treatments ( $p = 0.05$ ). \*, \*\*, and \*\*\* account for the degree of significance.

Factor	Estimate	Standard Error	T value	P value
2 cm Rhizome	0.1097	0.7674	0.143	0.886296
5 cm Rhizome	1.2288	1.5428	0.796	0.425758
Month	1.5914	0.6455	2.465	0.013690 *
Initial Weight	-4.4268	5.1391	-0.861	0.389027

**Table 9.** The probability that the measurable height present in rhizome fragments is affected by rhizome length, month of experiment start date, and initial weight of rhizome fragments. The timing of the experimental start date has a significant effect on the height of growing rhizomes ( $p = 0.05$ ). \*, \*\*, and \*\*\* account for the degree of significance.

Factor	Estimate	Standard Error	T value	P value
2 cm Rhizome	0.332	0.5489	0.605	0.55651
5 cm Rhizome	-1.298	1.8865	-0.688	0.50451
Month	1.2665	0.3883	3.262	0.00681 **
Initial Weight	6.3463	6.344	1	0.33688

**Table 10.** The probability that leaves are present on the rhizome fragments due to rhizome length, month of experiment start date, and initial weight of rhizome fragments. The timing of the experiment start date has a significant effect on the presence of leaves on the rhizome fragments ( $p = 0.05$ ). \*, \*\*, and \*\*\* account for the degree of significance.

Factor	Estimate	Standard Error	T value	P value
2 cm Rhizome	0.1097	0.7674	0.143	0.886296
5 cm Rhizome	1.2288	1.5428	0.796	0.425758
Month	1.5914	0.6455	2.465	0.013690 *
Initial Weight	-4.4268	5.1391	-0.861	0.389027

**Table 11.** The probability that leaf number on the rhizome fragments are affected by rhizome length, month of experiment start date, and initial weight of rhizome fragments. The timing of the experiment start date has a significant effect on the number of leaves grown on the rhizome fragments ( $p = 0.05$ ). \*, \*\*, and \*\*\* account for the degree of significance.

Factor	Estimate	Standard Error	T value	P value
2 cm Rhizome	0.2574	0.309	0.833	0.42114
5 cm Rhizome	-0.2648	1.0622	-0.249	0.80736
Month	0.7302	0.2186	3.34	0.00589 **
Initial Weight	1.0124	3.5718	0.283	0.78166

**Table 12.** The probability that shoots have mass from initial rhizome fragments due to rhizome length, month of experimental start date, and initial weight of rhizome fragments. The timing of the experiment start date has a significant effect on the presence of shoot mass ( $p = 0.05$ ). \*, \*\*, and \*\*\* account for the degree of significance.

Factor	Estimate	Standard Error	T value	P value
2 cm Rhizome	-0.4587	0.673	-0.681	0.49556
5 cm Rhizome	0.3534	1.0077	0.351	0.72579
Month	1.5107	0.548	2.757	0.00583 **
Initial Weight	-1.2627	2.9431	-0.429	0.66788

**Table 13. The probability that shoot weight, where present, was impacted by length of rhizome, month of experimental start date, and initial weight of rhizome fragments. The timing of the experiment start date has a significant effect on the final weight of shoots ( $p = 0.05$ ). \*, \*\*, and \*\*\* account for the degree of significance.**

Factor	Estimate	Standard Error	T value	P value
2 cm Rhizome	0.0743	0.7672	0.097	0.9239
5 cm Rhizome	-2.6776	1.9435	-1.378	0.1843
Month	1.6311	0.6534	2.496	0.0219 *
Initial Weight	11.9628	6.0799	1.968	0.0639

**Table 14. The probability that the presence of root growth was due to length of rhizome, month of experimental start date, and initial weight of rhizome fragments. The timing of the experiment start date has a significant effect on presence of growing roots ( $p = 0.05$ ). \*, \*\*, and \*\*\* account for the degree of significance.**

Factor	Estimate	Standard Error	T value	P value
2 cm Rhizome	0.3303	0.7982	0.414	0.678991
5 cm Rhizome	1.1528	1.5946	0.723	0.469716
Month	1.7801	0.7104	2.506	0.012217 *
Initial Weight	-4.115	5.219	-0.788	0.430421

**Table 15. The probability that the root length from rhizome fragments was impacted by length of rhizome, month of experimental start date, and initial weight of rhizome fragments. None of the measured factors had a significant impact on the length of the roots ( $p = 0.05$ ).**

Factor	Estimate	Standard Error	T value	P value
2 cm Rhizome	-0.1031	0.30722	-0.336	0.744
5 cm Rhizome	0.21651	1.12869	0.192	0.852
Month	-0.08622	0.24812	-0.348	0.735
Initial Weight	0.07533	3.68848	0.02	0.984

**Table 16. The probability that the presence of root mass in rhizome fragment growth was due to the length of rhizome, month of experimental start date, and initial weight of rhizome fragments. The timing of the experiment start date has a significant effect on presence of roots with mass ( $p = 0.05$ ). \*, \*\*, and \*\*\* account for the degree of significance.**

Factor	Estimate	Standard Error	T value	P value
2 cm Rhizome	0.3303	0.7982	0.414	0.678991
5 cm Rhizome	1.1528	1.5946	0.723	0.469716
Month	1.7801	0.7104	2.506	0.012217 *
Initial Weight	-4.115	5.219	-0.788	0.430421

**Table 17. The probability that the root mass, when present, was affected by the length of rhizome, month of experimental start date, and initial weight of rhizome fragments. None of the measured factors had a significant impact on the final weight of the roots ( $p = 0.05$ ).**

Factor	Estimate	Standard Error	T value	<i>P</i> value
2 cm Rhizome	-0.9215	0.8864	-1.04	0.323
5 cm Rhizome	-4.7534	3.2564	-1.46	0.175
Month	1.2104	0.7158	1.691	0.122
Initial Weight	19.0172	10.6146	1.787	0.104

## Discussion

The complete lack of garden loosestrife shoot growth from rhizome burial depths of 4 to 8 cm suggests that manually removing plants from the soil may be a realistic option for land managers hoping to eliminate small populations. While garden loosestrife rhizomes required surface conditions to sprout in the first 6 weeks after transplanting, fragmented rhizomes from other invasive species such as giant goldenrod (*Solidago gigantea* Aiton.) can produce sprouts from depths of up to 20cm (Weber 2011). *Solidago gigantea* rhizome fragments 5-20cm in length had a regeneration rate of 85% (Weber 2011) compared to 27% regeneration in the garden loosestrife June trial and 8% for the July trial. Some of these discrepancies could be explained by the length of the rhizome used in the experiment. Weber (2011) used rhizome sections longer than most of the rhizomes in this study. Potentially, the *Solidago* cuttings held more nutrients and carbohydrates leading to greater sprouting even under conditions that were less than ideal. Some difference in rhizome sprouting between the goldenrod trial and this garden loosestrife trial may also be expected due to differences between species from Asteraceae (*Solidago*), and Primulaceae (*Lysimachia*). Because garden loosestrife grows in wet, often saturated soils, it is possible that those rhizomes will not tolerate anoxic conditions after detachment from the parent plant. In the goldenrod study (Weber 2011), Canada goldenrod (*Solidago canadensis* L.) had a regrowth rate of 19% and could be buried to depths of no more than 10cm, further illustrating that rhizome regeneration rates may vary widely between species as well as families. Rhizome regeneration at depth could have been underestimated due to hotter and dryer conditions in the greenhouse compared to the cool, shady soil of a natural wetland. While many rhizomes were unable to survive after weeks buried under the soil, some of them were clearly alive and viable even after six weeks. A future study could look at what factors limit growth under the surface of the soil, and whether absence of light, oxygen, or a combination of these factors may be the cause of dormancy.

There was no significant difference in survival and growth of 1, 2, and 5-cm rhizome segments, even though the number of nodes differed between segment lengths. This is similar to performance of vegetative propagules of Hottentot fig (*Carpobrotus edulis* (L.) N. E. Br.), an invasive succulent species (Souza-Alonso and González 2017). Using either one or two apical verticillies, researchers found no difference in the morphological, physiological, or biochemical deterioration rates of propagules stored at increasing lengths of time before planting and measuring growth, and that shoot growth did not differ by propagule size. However, fragmented cogongrass rhizomes (*Imperata cylindrical*), had higher

establishment rates if the fragments had three or more nodes (Estrata et al. 2016). Additionally, rhizome fragments up to 1cm in length were found to have equal vigor in growth than those from larger rhizome fragments in European beachgrass (*Ammophila arenaria* (L.) Link), an invasive coastal plant from southern New Zealand (Konlechner et al. 2016). These researchers also found that seasonality affected the sprouting ability of rhizome fragments, explained by the translocation of nitrogen or soluble carbohydrate reserves away from the rhizomes into support the first flush of shoots or the production of flowers and seeds. These findings are echoed with Japanese knotweed, where Early Detection, Rapid Response removal of vegetative propagules caused plants to prioritize shoot production over rhizome growth in the spring, and that viability of rhizomes did not extend into a second season (Colleran and Goodall 2015). While temperature differences would be a logical variation between the two trials, the average high temperature in Seattle from June 14 to August 2 was 77 °F, compared to 80 °F for July 14 through August 28. It seems unlikely that a temperature difference of 3 °F would cause a significant decrease in growth and survival. July trial garden loosestrife rhizomes were cut after the plants had flowered for the year, which could help explain the difference in rhizome survival and shoot production in June and July trial. Rhizomes can serve as storage organs in plants that hold reserves of non-structural carbohydrates and nitrogen (Kleijn 2005). Chaplin et al. (1990) state that carbohydrate reserves are important for supporting vegetative reproduction, especially for perennials. Garden loosestrife, using carbohydrate reserves for flowering, could have resulted in rhizomes that had fewer stores to grow from. However, in many perennial grasses and herbs, extent of reproduction had little or no influence on vegetative growth (Chaplin et al. 1990). It is difficult to say conclusively whether carbohydrates are redistributed from rhizomes to flowers during garden loosestrife reproduction.

In conclusion, the challenge of removing miniscule rhizome fragments from the surface of the soil is such that mechanical removal of garden loosestrife is not considered to be practical. Garden loosestrife rhizomes can regenerate from fragments as small as 1cm, and under ideal conditions 27% of rhizomes can successfully regrow a new individual. If mechanical removal is desirable, using a tarp on site to prevent rhizomes from being left behind and in contact with the ground is highly recommended.

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