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Effect of Soil Conditions on Invasive Purple Loosestrife Compared to Native Species

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Abstract

Purple loosestrife (*Lythrum salicaria*) is an invasive plant that has degraded many wetland habitats since its initial introduction to the United States in the early 19th century. To aid in managing purple loosestrife, it is important to study how purple loosestrife grows under variable environmental conditions compared to common native species, which can help managers understand which habitats are most at-risk of invasion. High or low salinity and pH are often barriers to plant growth, and the stress caused by these environmental conditions can cause plants to deplete their starch reserves. If native species are more negatively affected by stressful environmental conditions than the invasive purple loosestrife, then habitats with those environmental conditions would likely experience a decline in native plant populations and an increasing population of purple loosestrife following establishment. To test the different responses of native and invasive plants, I grew purple loosestrife, native winged loosestrife (*Lythrum alatum*), and cattail (*Typha latifolia*) in soil with different salt concentrations (low, medium, and high), as well as soil with modified pH (acidic, neutral, and alkaline). The low-salt and neutral pH soils were considered control growth conditions. Purple loosestrife and winged loosestrife had stunted growth in alkaline conditions and in high soil salinity treatments, and did not produce as much biomass or starch when compared to the control soil treatments. Cattails were smaller in the high salinity and acidic treatments and did not produce as much biomass or starch compared to the control treatments. These results indicate that purple loosestrife and winged loosestrife respond similarly to stressful soil conditions, which has important management implications.

MONTCLAIR STATE UNIVERSITY

Effect of Soil Conditions on Invasive Purple Loosestrife Compared to Native Species

by

Madeline Ruth Baum

A Master's Thesis Submitted to the Faculty of

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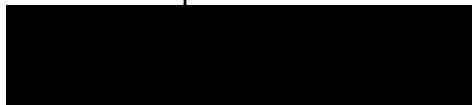
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EFFECT OF SOIL CONDITIONS ON INVASIVE PURPLE LOOSESTRIFE COMPARED TO
NATIVE SPECIES

A THESIS

Submitted in partial fulfillment of the requirements

For the degree of Master of Science

by

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Montclair State University

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Introduction

Invasive plants in the United States

The transport and establishment of invasive species is one of the greatest threats to biodiversity (Allendorf and Lundquist, 2003). For example, an approximate 50,000 invasive exotics established in the US cause extensive environmental damage and cost the country a total of over \$125 billion in economic losses per year (Pimentel, et al., 2000, Allendorf and Lundquist, 2003). It is estimated that 5,000 introduced plant species now live in the natural ecosystems of the US, a very large number compared to the approximately 17,000 native plant species (Pimentel, et al., 2000). Invasive plants have a multitude of impacts on plant communities by affecting soil chemistry and ecosystem function (Weidenhamer and Callaway, 2010). The long-term impact of leaf litter and root exudates can affect soil structure and mobilization of nutrients by modifying soil nutrient pools, which in turn alters nutrient cycles (Weidenhamer and Callaway, 2010). Some invasive species gain a competitive advantage by releasing compounds that are unique to the invaded community and negatively affect native plants (Weidenhamer and Callaway, 2010). Invasive plants can also indirectly affect native plant communities through the herbicides used to control them (Weidenhamer and Callaway, 2010). Herbicide drift can reduce the growth of non-target plants and can affect their secondary chemistry at sublethal doses (Weidenhamer and Callaway, 2010).

Oftentimes a time lag observed between the initial introduction and the development of the invasive species (Houghton-Thompson, et al., 2005). An exotic species' evolutionary processes often dictate whether that species can become invasive (Houghton-Thompson, et al., 2005). Researchers have demonstrated that some invasive species evolve in more competitive environments so they are intrinsically better competitors and can push out native species or better

hold on to an area (Allendorf and Lundquist, 2003). For example, exotic weeds are often much more difficult to get rid of than native weeds (Pimentel, et al., 2000). It is estimated that \$500 million is spent to control exotic weeds in residential areas and another \$1 billion is spent to control non-native weeds in golf courses alone (Pimentel, et al., 2000). Also, the absence of any predators allows invasive species to spread, obtain resources, and reproduce without the risk of being eaten (Allendorf and Lundquist, 2003). There are some studies that propose that non-native plants can invest more energy into growth because they don't need to spend energy on creating defensive compounds and thus have an advantage over native plants (Vanderklein, et al., 2015). Finally, plants can overcome the risk of inbreeding, which leads to genetic depression (Allendorf and Lundquist, 2003). In some cases, population growth is delayed until the invasive species evolves new adaptations through reassortment of existing genetic variety (Houghton-Thompson, et al., 2005). Often times, multiple introductions may provide sufficient genetic variability to allow this differentiation to occur (Houghton-Thompson, et al., 2005). Also, hybridization occurs between invasive and native species, which has often led to the creation of new taxa (Houghton-Thompson, et al., 2005).

Invasive species are very persistent and invasive plants are especially difficult to control. However, native and invaded habitats will never be exactly alike, but in the case of invasive plants, their native habitat and the habitats they've found may be close enough for them to thrive (Rejmanek, et al., 2005). Habitat compatibility is very important for any invasive species and knowledge of what kind of environment a species prefers can help in knowing how to exclude and prevent it from spreading (Rejmanek, et al., 2005).

Invasive purple loosestrife (*Lythrum salicaria* L.)

Purple loosestrife (*Lythrum salicaria*) was originally introduced from Eurasia in the 1800s and continues to spread throughout the northeast United States and the southeast portion of Canada (Malecki, et al., 1993). Purple is an herbaceous wetland perennial plant that typically grows to be 1-2m tall (Rogers, 2019). The fruit is an elongated oval capsule with two valves and contains millions of seeds (Mullin, 1998). The seeds are extremely small, but once the seedlings sprout, the plants can grow at a rate exceeding 1 cm/day and quickly outcompete native seedlings (Malecki, et al., 1993). The erect stems are tough and the base often appears to be wood-like, while the rest of the stem can appear to be smooth or hairy (Mullin, 1998). The leaves are cordate (heart-shaped), spear-shaped, and stalk-less meaning that the leaf blade is directly attached to the stem (Mullin, 1998). The flowers grow from a spike that is 15-30cm long and each flower has five to seven petals. They typically remain open from July through September or October (Mullin, 1998). The petals can vary from deep purple to pink to white and sometimes red, but are typically a purplish-magenta color (Mullin, 1998). The aboveground shoots die in autumn, but the dead stalks can survive for one to two years and create dense stands (Mullin, 1998). New shoots sprout from the root collar and from hibernating root buds during the following spring (Mullin, 1998). They possess a strong root stock, which serves as a storage organ for growth in spring and regrowth if the above ground portion is cut, burned, or killed by herbicides (Malecki, et al., 1993). Purple loosestrife can grow in many different soil types, it is usually found in cattail marshes, bogs, or in roadside ditches along waterways (Rogers, 2019). Due to the robustness of this plant, it can produce dense stands and will begin to replace native vegetation (Rogers, 2019). These attributes, coupled with the lack of suppressing herbivores and

pathogens, are thought to allow purple loosestrife to outcompete native species in the US (Malecki, et al., 1993).

Biological control has been an effective tool in controlling invasive plants, but the practice is often considered controversial (Louis, et al., 2020). Introducing novel species into an ecosystem can have unpredictable impacts and predicting the overall effectiveness is difficult (Louis, et al., 2020). One study observed the effects *Neogalerucella californiensis* and *Neogalerucella pusilla*, two species of beetles, on purple loosestrife populations 20 years after their initial release (Louis, et al., 2020). Louis et al. (2020) discovered that most of the signs consistent with effective long term biological control were not present including reduced fruit production, lower plant density, reduced biomass, and/or the return of native species to the site (Louis, et al., 2020). These findings agree with records that biological control methods often result in various outcomes, including research on purple loosestrife (Louis, et al., 2020).

Invasive plant species compete with native species for resources including sunlight, nutrients, water, and potential pollinators. Purple loosestrife's main ecological function is to uptake nutrients in wetland ecosystems and displace most other wetland plants, such as cattails (Rogers, 2019). When purple loosestrife invades a wetland, it degrades natural habitats for fish, amphibians, waterfowl, and other animals (Rogers, 2019). This has resulted in reductions in wetland plant diversity, reduced pollination and seed output of the native winged loosestrife, and reduced habitat compatibility for specialized species of wetland birds including black terns and marsh wrens (Blossey, et al., 2001). Although there are a number of generalist insect and bird species that have found ways to benefit from the purple loosestrife, specialized species are being threatened by the spread (Blossey, et al., 2001). In one study, it was found that purple loosestrife significantly reduced the pollinator visitation and seed set in the native winged loosestrife

(Brown, et al., 2002). Furthermore, the pollinators were found to move frequently between the two species, which may cause pollen transfer that can result in a reduction of both pollen quantity and quality for the winged loosestrife (Brown, et al., 2002). One study has found evidence of hybridization between the two species, but very few of the winged loosestrife genes were retained in the hybrids (Houghton-Thompson, et al., 2005). This is very concerning as invasive species may cause a drop in the production of seeds in native plant species as well as create hybrids. The purple loosestrife is damaging the wetland systems of North America, which is why it is necessary to research what environment they prefer and how to prevent them from spreading. In this experiment, I am focusing on the compatibility of purple loosestrife in different salinity and pH soil conditions. Salinity and pH are known for being barriers to plant growth and may be limiting the growth of the purple loosestrife in the US (Chapman, 1975, Kidd and Proctor, 2000, Neina, 2019). However, very little is known about what salinity and pH conditions purple loosestrife is able to survive in or how well it thrives compared to native species.

Plants' response to Salinity

Salinity becomes a problem when the concentration of salts, such as sodium chloride, sodium sulphate, sodium carbonate, or other salts, exceed levels where plants can tolerate them (Chapman, 1975). However, we do not know how high the salinity levels must be to pose a problem for plant life. Many different communities study the responses of plants to saline environments, including agriculture and industrial development (Chapman, 1975). Botanists and plant physiologists study the changes caused by saline environments in hopes of identifying how it affects plants' form, growth, metabolism, and response to stimuli (Chapman, 1975).

In North America, particularly in the US, saline soils exist all along the Atlantic seaboard down to Florida where the saline soils extend along the Gulf of Mexico (Chapman, 1975). There are mainly two types of primary saline environments, wet and dry (Poljakoff-Mayber and Lerner, 1999). Wet saline environments are usually salt marshes bordering the sea and are periodically inundated by tidal action which results in salinity fluctuations (Poljakoff-Mayber and Lerner, 1999). Dry saline habitats are located inland or on the borders of deserts, these mostly consist of salt lakes (Poljakoff-Mayber and Lerner, 1999). The soil in salt marshes and mangroves is considered to be very rich in nutrients compared to areas inland (Chapman, 1975). The soil is created through the movement of rivers or sea erosion and it is supplied with a great amount of nutrients through the mixing of seawater and freshwater in estuaries (Chapman, 1975). This creates areas with high biological activity, which is sometimes further enriched by runoff from human settlements (Chapman, 1975). So long as a plant can tolerate the saline soils, they can easily thrive given the rich, steady supply of nutrients. However, not all plants can handle saline soils and even those adapted to saline environments have a limit on how much salinity they can handle. Salt-tolerant invasive species are especially of concern, since areas such as salt marshes are so nutrient rich, invasive species gaining a foothold have the opportunity to establish and spread.

Secondary salinization can be caused by improper irrigation or other agricultural practices, examples include overgrazing, sea water intrusion, dryland salinity, and irrigation salinity (Zhou, et al., 2013, Safdar, et al., 2019). Secondary salinization differs from primary salinization which occurs when a change in the soil or groundwater naturally brings salt to the surface (Zhou, et al., 2013). Overgrazing causes the natural vegetation to become sparse, which allows the water table to rise and thus progressive salinization develops (Safdar, et al., 2019).

Sea water intrusion occurs in coastal aquifer systems when the groundwater has been over-exploited and is replaced by sea water (Safdar, et al., 2019). Dryland salinity is mainly caused when native vegetation is replaced with shallow rooted crops, which causes the groundwater levels to rise (Zhou, et al., 2013). Irrigation salinity is caused by excessive irrigation, which results in increased groundwater levels, and a lack of adequate drainage that can remove salts (Zhou, et al., 2013). The hydrologic processes that mobilize the salt are similar in all these forms, but the management options are different (Zhou, et al., 2013). Dryland salinity and overgrazing can be mitigated through replanting deep-rooted plants and revegetation (Zhou, et al., 2013). Irrigation salinity and sea water intrusion can be mitigated by improving irrigation management systems (Zhou, et al., 2013).

Road salts are a nonpoint source pollutant and are considered a long-term source of salt in wetlands, streams, and riparian zones (Cooper, et al., 2014). Road salts can persist beyond when they were implemented by lingering in groundwater and surface water (Cooper et al., 2014). An increase in chloride concentrations could negatively impact the aquatic ecosystems located in ponds, streams, lakes, and wetlands which will harm freshwater biota (Jones, et al., 2017). One study found that salinity levels persisted in an urban stream throughout the year and exceeded levels found in a stream that was not intersected by a highway (Cooper, et al., 2014). This suggests that road salts can accumulate long term in groundwater reservoirs (Cooper, et al., 2014). It was also found that road salt application led to chronic toxicity levels of salt in groundwater and surface water in New Hampshire, which caused the groundwater to exchange a significant amount of cations (Daley et al., 2009). In a natural setting, chronic salinity levels could lead to damaged vegetation and peaks in salinity could affect other aquatic biota (Cooper et al., 2014). One study discussed that increased concentrations of road salts led to a reduced

zooplankton abundance and reduced pH (Jones, et al., 2017). These same researchers also found that increased concentrations of NaCl reduced American toad tadpole activity and caused a trophic cascade that resulted in more phytoplankton (Jones, et al., 2017). Therefore, road salt may represent an environmental risk that could affect aquatic biota as well as limit the effectiveness of resource management and restoration efforts (Cooper, et al., 2014). Purple loosestrife, winged loosestrife, and cattails all live in wetlands, meaning that road salts can affect their populations as well. It is worth studying the effects of road salts on the biota of rivers and wetlands as well as ways to mitigate the damage.

In one study conducted in the Mediterranean, scientists studied the responses of two related plant species in areas of different salinity levels within three salt marshes (Al Hassan, et al., 2016). One of the species was a halophyte native to the salt marshes (*Inula crithmoides*), while the other was a taxonomically related invasive species (*Dittrichia viscosa*) (Al Hassan, et al., 2016). Al Hassan et al., (2016) found that both species responded by activating the same physiological stress tolerance systems, based essentially on the accumulation of specific osmolytes needed for osmotic adjustment and transporting toxic ions to the leaves where they are presumably compartmentalized in vacuoles (Al Hassan, et al., 2016). However, the native species was more efficient compared to the invasive species and the osmolytes accumulated at a much higher level (Al Hassan, et al., 2016). Thus, the native species had a slightly higher salinity stress tolerance compared to the invasive species (Al Hassan, et al., 2016). However, just because the invasive species could not directly compete with the native species, that does not mean it was incapable of surviving in saline environments. It was nevertheless able to tolerate the stress and continues to present a threat for Mediterranean salt marshes (Al Hassan, et al., 2016).

Plants' response to Acidity and Alkalinity

Soil acidity is considered to be a major growth-limiting variable for plants across the globe (Kidd and Proctor, 2000). The significance of each chemical constraint or interaction relies heavily on soil type and horizon, soil pH, organic matter, concentration and plant species, parent material, species of nutrient/element, soil physical properties, and climate (Clark and Baligar, 2000). Hydrogen, aluminum, and iron are all common acid-forming cations (Gondal, et al., 2021). The solubility of aluminum, iron, and manganese increases to maximum levels at low pH (Gondal, et al., 2021). However, these can become toxic for plants when their concentrations reach certain levels (Gondal, et al., 2021). An excessive amount of soluble aluminum in soil solution restricts root growth, reduces the supply of macronutrients, and affects microbial development (Gondal, et al., 2021). Higher concentrations of H⁺ ions also dissolve basic cations at a lower pH level and will remove them from exchange sites, release them into the soil solution, and a very small concentration of these nutrients are utilized by plants and the remaining is lost through leaching (Gondal, et al., 2021). Under acidic conditions, microbes are responsible for transforming nitrogen, phosphorus, and sulfur into usable forms for plants, thus reducing the concentration of certain minerals involved in these processes, such as molybdenum (Gondal, et al., 2021). However, the availability of magnesium, calcium, nitrate ions, boron, phosphorus, and molybdenum, which is a metal in an enzyme microbes use to catalyze nitrogen fixation, is reduced in low pH which makes it more difficult for microbes to obtain the necessary minerals (Gondal, et al., 2021).

Usually, the hydrogen ion itself is rarely cited as the cause of poor growth, but high concentrations can decrease nodule growth of leguminous plant roots as well as the activity, multiplication, and survival of *Rhizobia* and other advantageous microorganisms in acidic

conditions (Clark and Baligar, 2000). Kidd and Proctor (2000) found that hydrogen ion induced pH levels of 4.0 or lower produced lower root elongations, root numbers, and shoot elongations in *Holcus lanatus*. In another species, *Betula pendula*, there was a reduction in total leaf area, as well as a reduction in root elongation and root number (Kidd and Proctor, 2000). Excess hydrogen ions (H⁺) will compete with other cations for root absorption sites, interfere with ion transport and uptake, and cause root membranes to become “leaky” (Kidd and Proctor, 2000). These observations have led to the conclusion that alterations in nutrient uptake, specifically nutrients such as phosphorus, calcium, magnesium, and iron, play an important part in hydrogen ion toxicity (Kidd and Proctor, 2000). However, in some cases there are adaptations that allow plants to have a better tolerance for hydrogen ion toxicity (Kidd and Proctor, 2000). For example, a hydrogen tolerant cultivar of maize has been recorded to increase the amount of calcium in its roots at low pH levels (Kidd and Proctor, 2000). Calcium regulates plasma membrane integrity and the functioning of the proton effluent pump, therefore maintaining higher levels of calcium at low pH levels may contribute to ion tolerance (Kidd and Proctor, 2000). However, further research must still be done as the study of the effect of direct hydrogen ion toxicity in acidic soils has been historically under-considered (Kidd and Proctor, 2000).

The effect of alkaline soil on plants is very different compared to acidic soils. For instance, negative ions are dominant in high pH where positive ions are dominant in low pH (Neina, 2019). Also, the solubility of most trace elements decreases which leads to low concentrations as the pH increases (Neina, 2019). Soil pH also increases the solubility of organic matter through the separation of acid functional groups and by separating the bonds between organic elements and clays, thus as the pH increases, the quantity of dissolved organic matter also increases and in turn the amount of mineralizable carbon and nitrogen (Neina, 2019).

This is why alkaline soil conditions have strong effects on the filtering out of dissolved organic carbon and nitrogen in soils with high quantities of organic matter (Neina, 2019). A high pH environment can also cause metal ions and phosphorus to precipitate, greatly affecting the absorption of inorganic ions, and can disrupt the ionic balance and pH homeostasis in the root tissues (Zhang and Mu, 2009). This means that plants in alkaline soil must manage ion toxicity, while maintaining intracellular ionic balance (Zhang and Mu, 2009). Intracellular ion balance produces a stable pH, which is necessary for plants to maintain normal metabolism (Zhang and Mu, 2009). Independent of the surrounding pH changes, the pH within the tissue should not change so long as the plant can continue to acclimate (Zhang and Mu, 2009). However, alkaline environments lead to an influx of sodium ions which causes ionic imbalance (Zhang and Mu, 2009). To combat this, plants usually take up more inorganic anions and organic anions to maintain balance, but under alkaline stress the concentrations of inorganic anions are significantly reduced (Zhang and Mu, 2009). This suggests that the uptake of inorganic anions may be inhibited by high pH caused by alkaline stress and cause the plants to become more reliant on organic anions which may prevent them from regaining ionic balance (Zhang and Mu, 2009). It has been thought that organic anion accumulation sometimes seen in plants may therefore be a central adaptive mechanism to maintain balance under alkaline stress (Zhang and Mu, 2009). The metabolic regulation of organic anions may involve enzymes that take part in metabolic pathways such as the glyoxylate cycle, glycolysis, the tricarboxylic acid cycle, or other pathways (Zhang and Mu, 2009).

Importance of Starch in Plants

Starch is an insoluble, non-structural carbohydrate composed of α -glucose polymers. Plants and algae synthesize it in order to store energy in a dense, osmotically inert form (Pfister

and Zeeman, 2016). Starch is often categorized into two types based on its biological function: transitory starch and storage starch (Pfister and Zeeman, 2016). Starch that is synthesized from photosynthesis directly in the leaves throughout the day is classified as transitory starch (Pfister and Zeeman, 2016). This is because the transitory starch is degraded and used up during nighttime to maintain metabolic processes, biosynthesis, and energy production once photosynthesis ceases for the night (Pfister and Zeeman, 2016). If there was a reduction in transitory starch production, such as if a mutation impaired starch synthesis, then the plant's growth would be impeded and it would experience severe starvation (Pfister and Zeeman, 2016). Storage starch is defined as starch which is stored for long periods of time in non-photosynthetic structures such as roots, stems, seeds, or tubers (Pfister and Zeeman, 2016). Remobilization of the stored starch occurs when seeds germinate, photosynthesis cannot meet energy demands for biosynthesis, and during periods of sprouting or repair (Pfister and Zeeman, 2016). The ability to store starch is especially important to perennial plants, which need those extra starch reserves in the event of grazing, cutting back, and to withstand die-back and regrowth after winter (Vriet, et al., 2014). In perennial plants, such as the perennial forage legume alfalfa, there are positive correlations between the starch contents of the roots during winter and the amount of regrowth in spring (Vriet, et al., 2014). In a study conducted on *Lotus japonicus*, it was found that starch reserves in the roots, rather than transitory starch in the leaves, were essential for regrowth, especially in the event of cutting back and in a natural environment where grazing and winter die-back occurs (Vriet, et al., 2014).

Starch is also very important for allowing plants to recover from herbivory. Foliage herbivory reduces leaf area and subsequently reduces photosynthesis which in turn can deplete the plant's storage reserves (Hultine, et al., 2021). A certain species of woody shrub, *Tamarix*,

evolved under intense herbivory in its native range and is now a very successful invasive species in the US (Hultine, et al., 2021). As a result, a species of herbivorous beetle from its native range was introduced in the US, these beetles cause repeated episodic defoliation which depletes their starch reserves, eventually resulting in significant dieback and mortality (Hultine, et al., 2021). However, the dieback can vary from near 0% to more than 80%, which raises questions about their starch allocation and regulation (Hultine, et al., 2021). To study this, a series of defoliation treatments were performed, and it was found that defoliation disproportionately affects starch vs. soluble sugar storage (Hultine, et al., 2021). Soluble sugars perform immediate functions in plants such as osmoregulation, phloem transport, and molecular synthesis, thus a depletion in soluble sugars impacts metabolism and hydraulic function (Hultine, et al., 2021). During times of episodic disturbance, soluble sugars are depleted, and starch is needed for defense and recovery (Hultine, et al., 2021). In the case of repeated defoliation, the starch reserves are exhausted and the plant will likely die (Hultine, et al., 2021). Learning how different carbohydrates are allocated can be very helpful in learning how to control invasive plant species as it may tell us which structures to target to best kill the plant.

Another study conducted on Japanese barberry similarly studied the effects of defoliation treatments on starch content, biomass, and physiology and found the defoliation had virtually no effect on any of these factors (Vanderklein, et al., 2015). They also found that stomatal gas exchange and transpiration had increased in new leaves compared to the old leaves, but this did not affect the rate of photosynthesis, amount of biomass, or starch concentration (Vanderklein, et al., 2015). This finding was surprising to them as plants often display some sort of compensatory response. They believe this result may be because leaf loss did not cause a great enough affect to change the plants' behavior (Vanderklein, et al., 2015). The leaves on the larger plants have a

high rate of photosynthesis and a low carbon investment which produces a low carbon loss per return rate (Vanderklein, et al., 2015). The smaller plants compensated for the loss by increasing leaf size, compared to the larger plants and other species, the smaller plants had a lower leaf mass per unit area (LMA) suggesting that leaves are cheap to construct (Vanderklein, et al., 2015). Invasive species often have a low LMA, which is part of an ensemble of traits that are advantageous over native species (Leishman, et al., 2007). Therefore, it is possible the small Japanese barberry were able to increase the size of their leaves without decreasing their starch content or increasing the carbon input through photosynthesis (Vanderklein, et al., 2015). Taken together, the data suggests that, even in the presence of herbivory, defoliation has almost no impact on Japanese barberries and they are well adapted to succeed in North America (Vanderklein, et al., 2015).

One study conducted on invasive Chinese tallow trees (*Triadica sebifera*) examined the concentrations of different carbohydrates, such as starch, soluble sugar, fructose, sucrose, and cellulose as well as the mass of the stems, roots, and leaves (Li, et al., 2020). Li et al. (2020) also studied the arbuscular mycorrhizal fungi (AMF) colonization and the trees' root water potential. Carbohydrates like cellulose are used to defend the plant from predators and other attackers, but low levels of cellulose can increase AMF colonization which allows symbiosis to occur (Li, et al., 2020). The AMF forms a large network of root hyphae to assist the plant in collecting water and nutrients (Li, et al., 2020). Other carbohydrates like soluble sugars (glucose, fructose, and sucrose) are used in the short term for physiological functions, while starch is stored for later use (Li, et al., 2020). They found that the invasive plants had more stem and leaf mass compared to native populations, but there was no difference in their root masses, which resulted in a lower root/shoot ratio (Li, et al., 2020). The invasive populations also had higher soluble sugar

concentrations, negative root potentials, and AMF colonization, but their leaves, roots, and stems had low concentrations of cellulose and starch (Li, et al., 2020). These results suggest that the invasive plants focus less on long term starch storage and more on producing faster and greater aboveground growth (Li, et al., 2020). Differences in how the invasive species allocated resources suggests that the population shifted their strategy towards rapid growth and away from defense and energy storage in the invaded area (Li, et al., 2020). Less starch was synthesized because the invasive plants had more of a demand to grow and more sugars were produced to increase metabolic energy, which may have proven advantageous to collect more resources in a new environment (Li, et al., 2020). Therefore, in order to get rid of the invasive species, since the starch reserves usually used for defense are focused on growth, targeting the roots with some form of chemical agent could kill them.

Starch also has many other roles aside from just an energy source including making osmoprotectants, which protect the cells from osmotic pressure, and making cryoprotectants, which protect cells from freezing (Ribeiro, et al., 2022). Starch is also an energy source used in rapid opening of the stomata, scavenging free radicals and signals, and reverting embolized vessels (Ribeiro, et al., 2022). Also, storage starch acts as an alternative source of sugar when photosynthesis is not enough or unavailable, such as during the night, when seeds germinate, during tuber sprouting, tissue regeneration, or when the plant is living in stressful conditions (Ribeiro, et al., 2022). Biotic and abiotic stresses may vary in terms of their cause, influence, duration, developmental stage of the plant, time of day, and how quickly conditions change (Ribeiro, et al., 2022). The impact of stress on starch metabolism depends on many factors, such as the influence on photosynthesis, which structures are affected, how the stress alters carbon allocation, and the energy requirements necessary to mitigate the stress (Ribeiro, et al., 2022).

Under abiotic stresses, starch reserves are depleted, but starch may reaccumulate when the rate of growth is decreased to the point that it is lower than the rate of photosynthesis (Ribeiro, et al., 2022). Under biotic stresses, starch accumulates, but researchers are still unclear as to what molecular mechanisms are involved (Ribeiro, et al., 2022). In the case of the purple loosestrife, there is likely a similar importance placed on starch reserves in order to survive in less than optimal circumstances. They are necessary for the plant to store energy required for times of new growth and repair during times of stress.

Hypotheses

It is important to understand where the line is between thriving, tolerating, and suffering. To gauge how well the purple loosestrife thrives, I looked at how large they grow, how much biomass they can produce, and how much starch they can accumulate under stressful conditions. My initial hypothesis was that if the purple loosestrife has a higher tolerance towards salinity, then it should have more growth, dry weight, and a higher starch content compared to the native species. Additionally, differences in pH may also cause a lack of growth or damage to the root tissues that require the plants to draw upon their starch reserves to survive. The next hypothesis was that if the purple loosestrife thrives in acidic and alkaline soils, then it will grow larger, have more biomass, and have higher mean starch content in the root tissues compared to the other plant species. Finally, plants draw on starch reserves stored in their roots in order to survive under stressful conditions such as salinity or high or low pH. Therefore, how much starch the purple loosestrife is able to conserve can be used to judge how well it thrives under certain conditions or how badly it was stressed and had to deplete its starch reserves. My final hypothesis was that if the purple loosestrife thrived under the different conditions, then it will have a higher average starch content stored in its roots compared to the winged loosestrife and

cattails. These two species were chosen because winged loosestrife is related to purple loosestrife and is native to the US. The cattails are very common wetland plants and compete with purple loosestrife for resources in cattail marshes.

Methods

Preparation

In order to test the influence of different soil conditions, I grew plants of each species; purple loosestrife (*Lythrum salicaria*, L.), winged loosestrife (*Lythrum alatum*, Pursh), and common cattail (*Typha latifolia*, L.), in a greenhouse and separated the plants into two control groups, two salinity treatments, and two pH treatments. At least a dozen seeds of each species were planted in 180, 4-inch pots. Each pot had been prepared by placing coffee filter material in the bottom and about 80-100g of potting soil on top. The pots were then placed in separate plastic cups. The plastic cups had two drainage holes cut on either side about 2cm from the rim and were then filled with water. The plants were labeled with their species, a letter, and what treatment the plants were given. There were 10 plants for each individual treatment, 30 plants of each species were in the pH treatments and 30 were in the salinity treatment. If more than one seed germinated then as many of the smaller plants as possible were removed without disturbing the largest plant. The salinity and pH treatments each had their own control group, so that they could be analyzed separately at the end of the experiment, the 0ppt and 6.5pH groups, respectively. There were 60 plants of each species for a total of 180 plants. The pots were separated into their treatment groups and placed in boxes according to what treatment they were receiving. I placed the plants in a greenhouse, haphazardly placed the boxes in groups of 6 on a table to minimize the potential for gradients and watered the seeds regularly (4 times per week) so that the soil remained moist. To prevent the plants from dying before they had a chance to

sprout, I waited until the sprouts had reached at least 3cm tall before adding any of the treatment solutions. Once the treatments started, I rotated the boxes around the table each week to give them equal light levels.

Salinity treatment

In order to test the effects of salinity on plants, they were watered 4 days a week with 50mL of either 0ppt, 5ppt, or 10ppt saline solution. In order to prevent the buildup of salt in the cups, water was dumped out of the collection cups every 4 weeks.

Also, in order to test the salinity of the soil and the cups of water, the conditions were replicated for 4 weeks without planting any seeds. Approximately 80g of soil was added to 6 cups, 2 cups were each given the same salinity treatments as the salinity groups and control group. After 4 weeks the pots were dried in the oven until all the moisture was gone. Using the final weight of the control groups, I estimated the amount of water loss in the oven, which was about 44.96g. I then subtracted the initial weight from the sum of the final weight and the water loss to get the weight of the salt. After 4 weeks the average amount of salt in the soil was 0g in 35.08g of soil for the 0ppt, 1.08g of salt in 36.2g of soil in the 5ppt group, and 2.15g of salt in 37.24g of soil for the 10ppt. Considering that each pot lost approximately 45g of water before being weighed, this means the salinity of the water in the soil was 0ppt, 2.22ppt, and 4.44ppt, respectively which would have put the salinity close to 0ppt, 5ppt, and 10ppt after 10 weeks assuming the soil retained the salt each week. In any case, the salinity of the water the plants were watered with each week was definitely 0ppt, 5ppt, and 10ppt, respectively.

pH treatment

To test the effects of high and low pH levels on the plants, I created two solutions, an acidic and an alkaline solution. The acidic group was called the 5.5pH group, the control group

was called the 6.5pH group and the alkaline group was called the 7.5pH group. Although the exact pH's of the solutions and the soil in the pots were not at these levels, these labels are used to differentiate between the different treatments. First, I prepared an alkaline solution by adding 14.40g of baking soda to a 3.785L of DI water which resulted in a pH of 8.0. Then I made an acidic solution by adding 0.237L of vinegar to a 3.785L of DI water which resulted in a pH of 3.0. In the 5.5pH group, I started with a small amount of vinegar water (5mL) along with 45mL of water to produce a 4.0 pH solution. The plants were watered 4 times a week and every week I slowly increased the amount of vinegar solution, while decreasing the amount of water in 5mL increments. This continued each week until the purple loosestrife showed signs of extreme stress (losing leaves, stunted growth, discolored leaves) at week 7 at 35mL of solution which had a pH of 3.5. Once the purple loosestrife started to wilt, I kept the amount of vinegar solution and water at 35mL and 15mL, respectively, for all the plants of all the species. For the 7.5pH group, I added 50mL of the baking soda solution. The control group was given tap water which had a pH of about 6.5-7. I dumped the cups every 4 weeks to prevent an accumulation of solutions that could lead to the pH being too high or low.

To find the pH of the soil and the water in the cup, the conditions were replicated for 4 weeks without planting any seeds. Approximately 80g of soil were added to 6 cups, 2 cups were each given the same solutions as the treatment groups and the control group. After 4 weeks, litmus strips were used to measure the pH of the soil and water. The average pH of the soil and water was 6 for the 6.5pH group, 4.75 for the 5.5pH group, and 8.125 for the 7.5pH group. It is assumed that since the cups were emptied and refilled with fresh water every 4 weeks, that these levels would have stayed the same. It should also be noted that the 7.5pH group had sodium

added due to the fact that baking soda (sodium bicarbonate) was used to change the pH, but we are choosing to focus solely on the change of pH in this treatment group.

Recording Growth and Harvesting

Height and dry weight were measured to provide information on how well the plant was able to grow and how much biomass they accumulated. Height was measured each week starting at the base of the stem and rounding to the nearest half cm. For the cattail, I measured the height from the base to the tip of the tallest leaf. The other plants were measured from the base to the tip of the stem. If a plant died, I harvested them immediately and stopped recording their height until the end of the experiment. Once the plants reached 10 weeks after their initial measurement, I harvested them within a few days. The plants were separated by stems, leaves, roots, and flowers into paper bags and labeled with the species, treatment, letter of the pot, and tissue component (e.g., purple loosestrife, 5ppt, pot D, stem). The roots were thoroughly cleaned in water to remove all soil before being placed in the bags. I placed the bags in an oven at about 39°C for at least 2 days. The roots, stems, flowers, and leaves were all weighed separately, including the components from the dead plants. To avoid the plants from rehydrating before they were measured, only a few bags at a time were taken out of the oven.

Starch Content

When measuring the starch content, I focused on the roots because they are a starch storage organ. Also, when looking at the dry weight, the roots had more tissue in most of the plants which made it possible to redo the procedure if necessary, this occurred twice during the experiment. I took the bags of dried roots and selected 5 bags from each species and treatment by choosing every other letter from each treatment group (B, D, F, H, J). If the roots did not have at least the 50mg necessary for the procedure, then I chose the plant directly previous or directly

after in the group. If there were no roots with enough material, then I continued the procedure with what I had. Roots from the 5 plants were ground to pass a 0.5mm mesh so that they were a fine powder and stored in bottles labeled with my initials, the treatment, species, and the letter of the plant. The process used to measure the root starch content in this experiment was the same method that Vanderklein et al. (2015) used when studying the starch content in the Japanese barberry. I extracted any pigments or soluble sugars by heating an 80% ethanol solution to 80°C in a hot water bath. After centrifuging, the liquid was decanted from each sample tube and the process was repeated until the ethanol was clear. The sample tubes were then placed in an oven overnight. The samples were hydrated with a Na-F buffer solution and boiled for 15 minutes before adding amyloglucosidase, then the samples incubated for 24 hours. Also, I set up a series of glucose standards. After obtaining a liquid sample of starch, I added a color reagent and heated the samples and standards to 37°C for 30 minutes. I then read at 450nm using a spectrophotometer and was able to use the glucose standards to build a standard curve. Using the absorbency of the samples and the standard curve, I was able to calculate the starch concentration.

Statistical Analyses

Dead plants were not included in the height analyses, after they died their heights were not recorded. Mortality information was also recorded. Dead plants were included in the biomass and starch analyses, but because some of them died early I can't compare them at week 10 so instead we are comparing their total mass, root/shoot ratio, and starch at the time they were harvested. A 2-way ANOVA was conducted on species, pH/salinity, and the potential interaction between final height/total mass-flowers/root/shoot ratio/starch content with species and pH/salinity being our factors and final height/total mass-flowers/root/shoot ratio/starch content

was the response. If there was an interaction then a one-way ANOVA and Tukey HSD was conducted for each individual species to see how each responded to the treatments.

Results

Growth

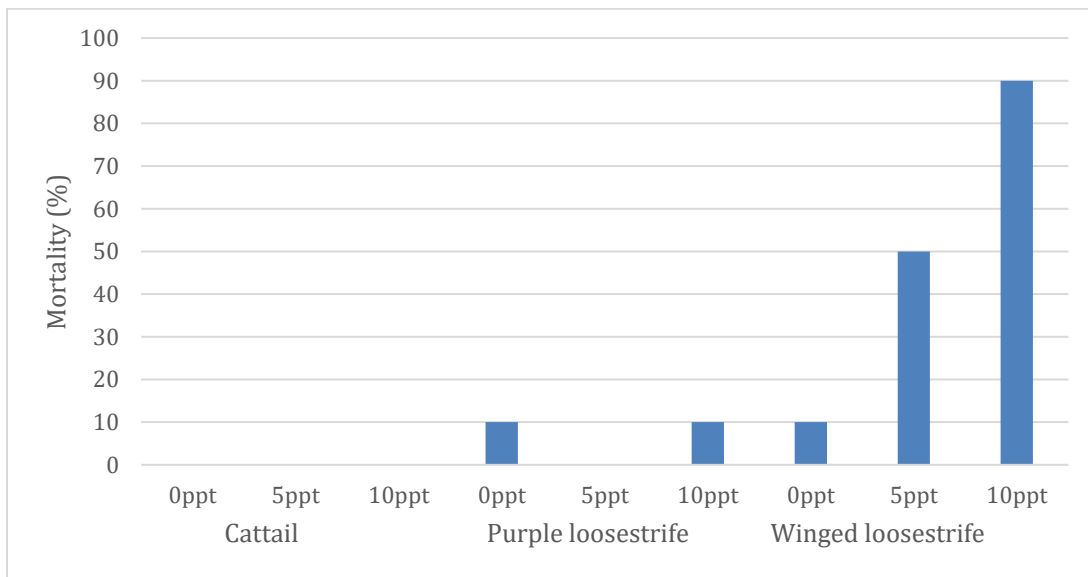
Salinity

In general, the plants grew better in the control group and grew more poorly as the salinity increased. Purple loosestrife had a 10% mortality rate in the 0ppt and 10ppt treatment and a 0% mortality rate in the 5ppt treatment (Fig. 1). According to Fig. 2, the purple loosestrife grew more poorly in 10ppt salinity compared to the 5ppt and 0ppt salinity. The purple loosestrife grew equally well in the 5ppt and the 0ppt groups with nearly the same height. Winged loosestrife had a 10% mortality rate in the 0ppt treatment, a 50% mortality rate in the 5ppt treatment, and a 90% mortality rate in the 10ppt treatment (Fig. 1). Based on Fig. 3 the winged loosestrife grew best in the 0ppt salinity and grew the most poorly in the 10ppt treatment. Cattails had a 0% mortality rate in all of the salinity treatments (Fig. 1). In Fig. 4 the cattails grew the best in the 0ppt salinity and grew the poorest in the 10ppt treatment.

pH

Winged loosestrife and purple loosestrife both suffered high mortality rates in the alkaline conditions, the mortality rate for winged loosestrife was 100% and 90% for purple loosestrife in the 7.5pH treatment (Fig. 1). Purple loosestrife had a 10% mortality rate in the 5.5pH treatment and 0% in the 6.5pH treatment (Fig. 1). In Fig. 5, the purple loosestrife grew the poorest in the 7.5pH treatment and also struggled in the 5.5pH treatment. The purple loosestrife grew best in the 6.5pH treatment and reached a height of over 60cm. Winged loosestrife had a 0% mortality rate in the 5.5pH treatment and 20% in the 6.5pH treatment (Fig. 1). According to Fig. 6, the

winged loosestrife also grew poorest in the 7.5pH treatment and were all dead by week 7. The winged loosestrife in both the 6.5pH and 5.5pH groups reached over 45cm and were nearly equal in average height. The cattail struggled in the more acidic conditions, the mortality rate was 10% in the 5.5pH treatment and 0% in the 6.5pH and 7.5pH treatments (Fig. 1). In Fig. 7, the cattails did fairly well in all the pH conditions, but its growth started to slow down between weeks 4-5 and it more or less leveled off in all three pH groups. Compared to the purple loosestrife and winged loosestrife, the cattails were much taller than the other two species in the 6.5pH and 7.5pH groups.



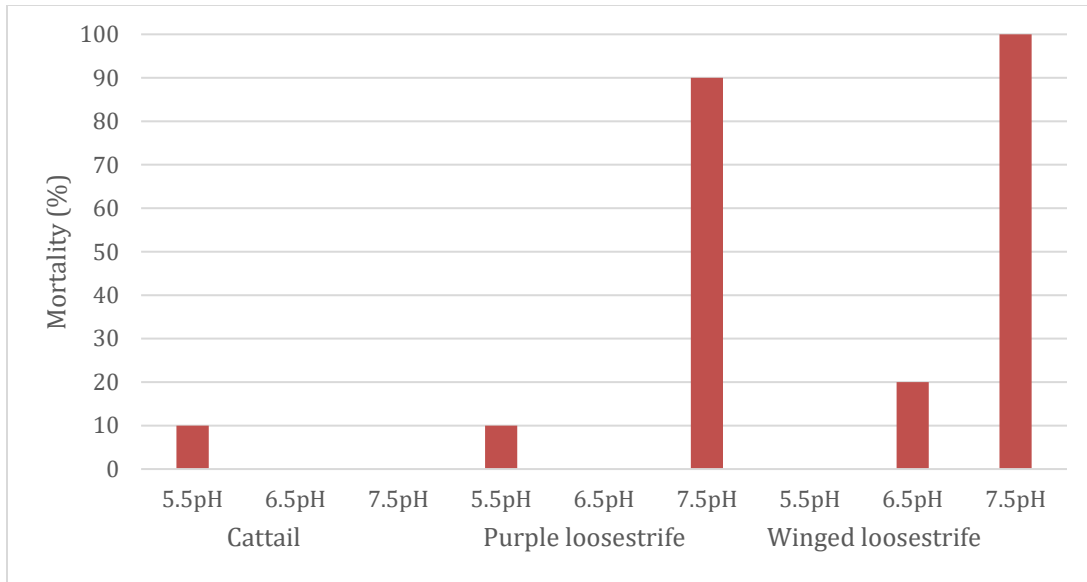


Figure 1 Mortality Rates in all Treatments

The mortality rates of all three species in all treatments over the 10-week growing period.

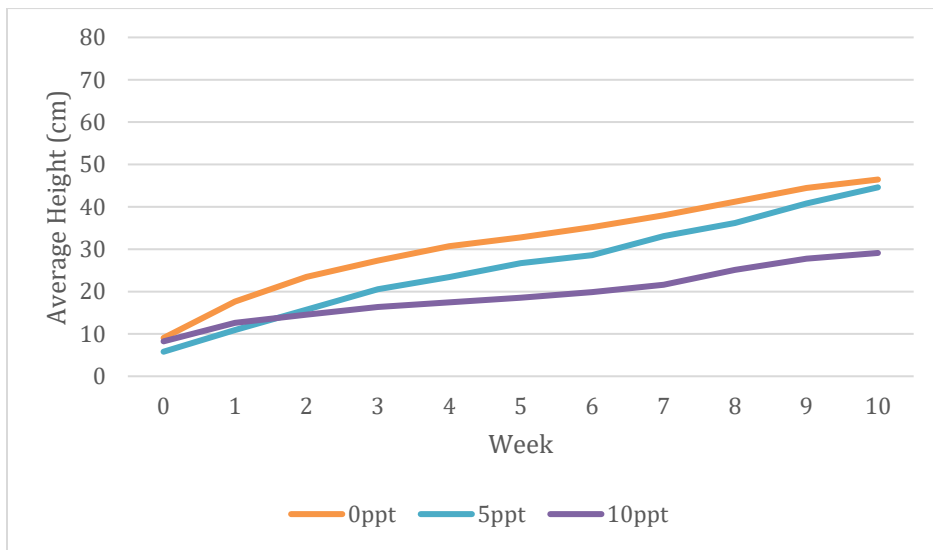


Figure 2 Average Height of Purple Loosestrife-Salinity Group

The average height of purple loosestrife in the salinity group for 10 weeks. If the plant died, their height was removed for the remaining weeks. The mortality rate was 10% in the 0ppt and 10ppt groups and 0% in the 5ppt group.

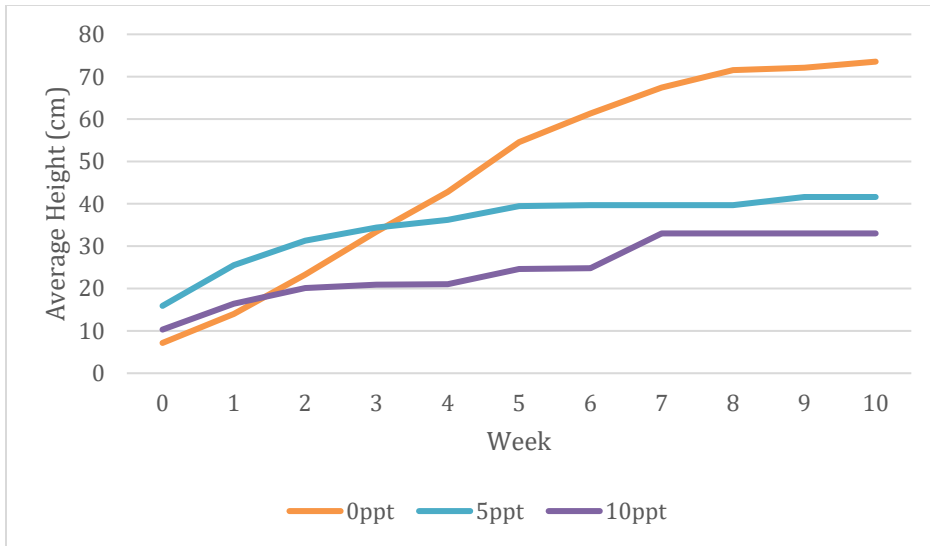


Figure 3 Average Height of Winged Loosestrife-Salinity Group

The average height of winged loosestrife in the salinity group for 10 weeks. If the plant died, their height was removed for the remaining weeks. The mortality rate was 10% in the 0ppt treatment, 50% in the 5ppt treatment, and 90% in the 10ppt treatment.

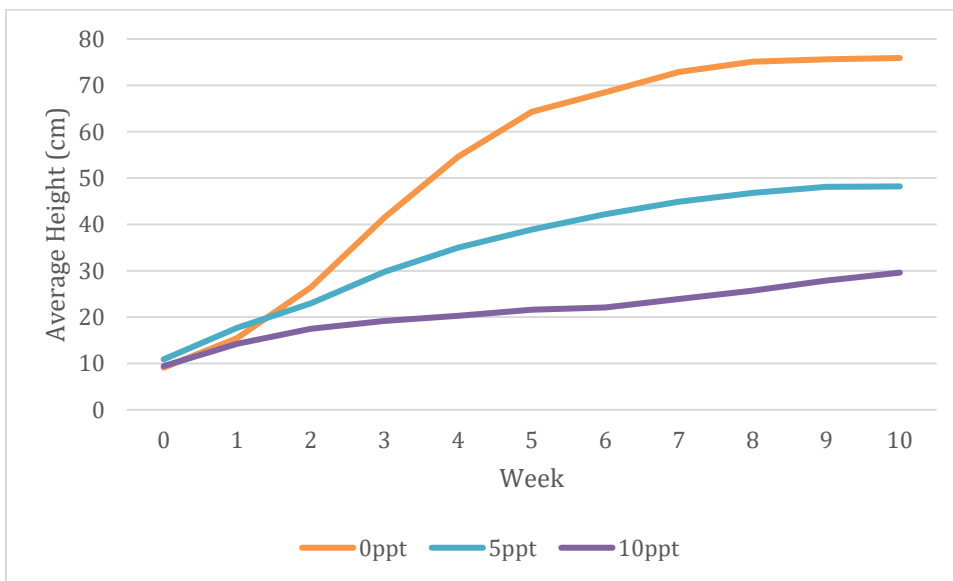


Figure 4 Average Height of Cattail-Salinity Group

The average height of cattail in the salinity group for 10 weeks. The mortality rate was 0% for all treatments.

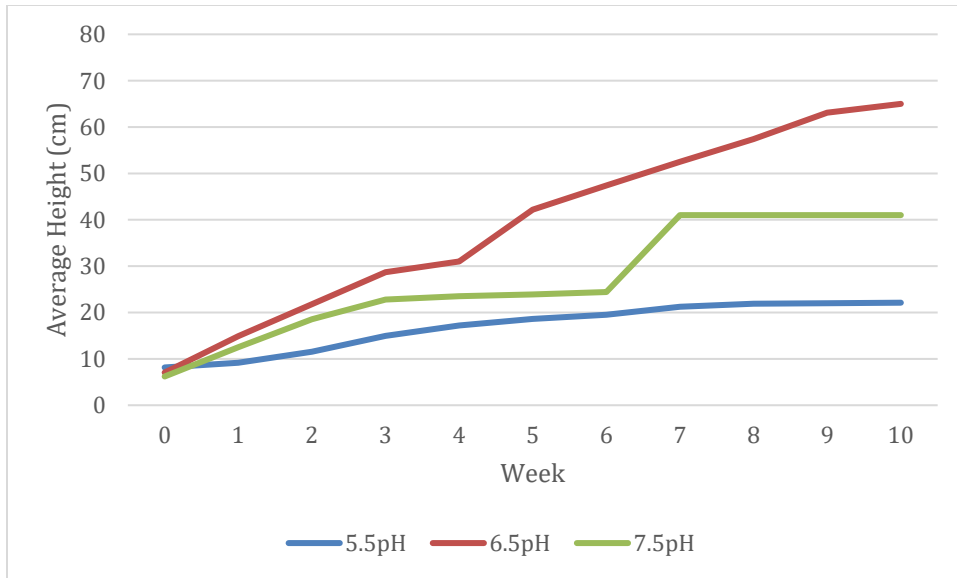


Figure 5 Average Height of Purple Loosestrife-pH Group

The average height of purple loosestrife in the pH group for 10 weeks. If the plant died, their height was removed for the remaining weeks. The mortality rate was 10% for the 5.5pH, 0% for the 6.5pH, and 90% for the 7.5pH.

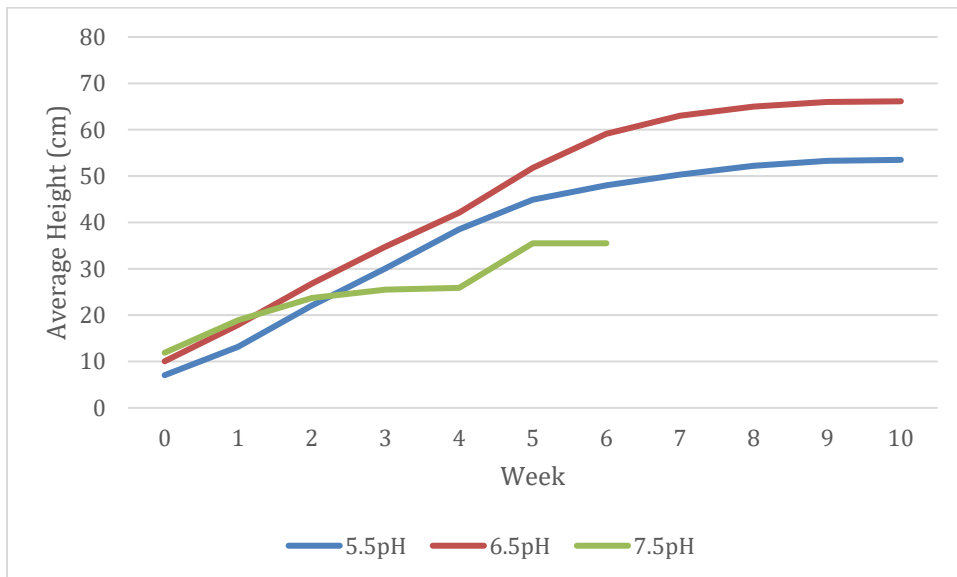


Figure 6 Average Height of Winged Loosestrife-pH Group

The average height of winged loosestrife in the pH group for 10 weeks. If the plant died, their height was removed for the remaining weeks. The mortality rate was 0% in the 5.5pH treatment, 20% in the 6.5pH treatment, and 100% in the 7.5pH treatment.

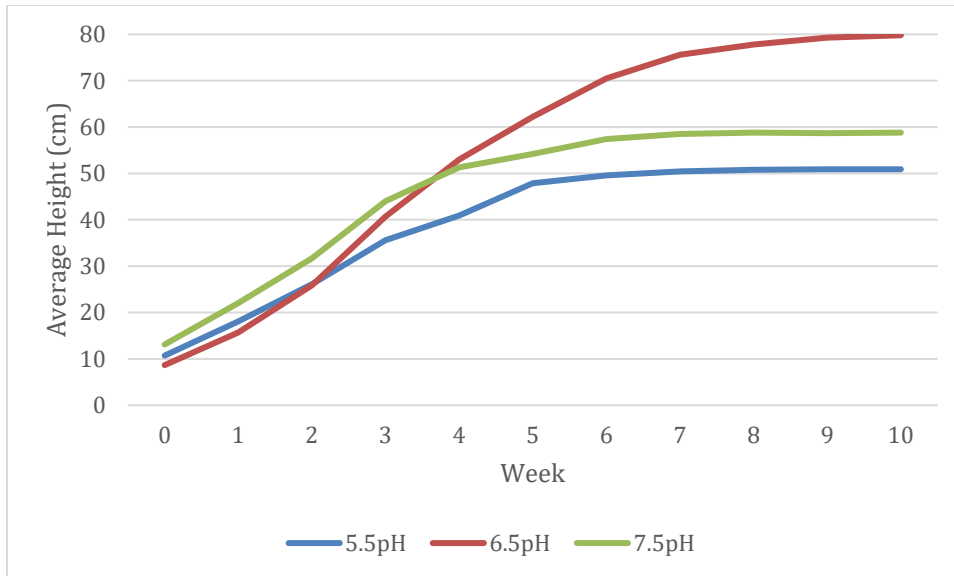


Figure 7 Average Height of Cattail-pH Group

The average height of cattail in the pH group for 10 weeks. If the plant died, their height was removed for the remaining weeks. The mortality rate was 10% in the 5.5pH treatment and 0% in the 6.5pH and 7.5pH treatment.

Dry weight

Salinity

Generally, the plants had more biomass in all of their components in the control group and decreased in biomass as the salinity increased. In Fig. 8, the purple loosestrife in the 0ppt group had the most dry weight in its roots, but only about half as much dry weight in the leaves and stem. The purple loosestrife in the 10ppt salinity had the least amount of dry weight and had slightly less biomass in the roots compared to the other components. For winged loosestrife, according to Fig. 9, the 0ppt group had the most dry weight in its roots, but only half as much in the leaves and stems and practically no flowers. In the 10ppt group, the winged loosestrife had the least amount of dry weight in all of its components, except for the flowers. The three components in the 5ppt group had nearly equal amounts of biomass but had slightly less in the roots. According to Fig. 10, the cattails in the 0ppt had the highest average biomass compared to

the other treatments. Most of the cattails' average biomass was focused on the roots in each salinity group. Cattails have no stems or flowers so there were no results for those components.

pH

In general, all three plant species had the most biomass in the control group. The purple loosestrife and winged loosestrife had the least amount of biomass in the alkaline groups, but the purple loosestrife also had very low biomass in the most acidic conditions. In Fig. 11, the purple loosestrife had the highest amount of dry weight in the 6.5pH soil. The 7.5pH had the least amount of dry weight and most of its dry weight was concentrated in the stem, while the roots and leaves were nearly equal and there were no flowers (Fig. 11). In Fig. 12, the winged loosestrife in the 5.5pH group had the most dry weight in its roots compared to the other pH groups, but the leaves and stems had less than half as much. The 6.5pH group had the most dry weight in its stem and leaves compared to the other groups, but it had less dry weight in its roots. The 7.5pH group had the least amount of dry weight in all its components, especially the roots. According to Fig. 13, the cattails in the 6.5pH group had the most dry weight in all its components compared to the other groups and the amount in its roots was nearly twice as much as the leaves. The cattail had the least amount of dry weight in the 5.5pH group in both the roots and leaves. The 7.5pH group had only slightly more dry weight in both components compared to the 5.5pH group.

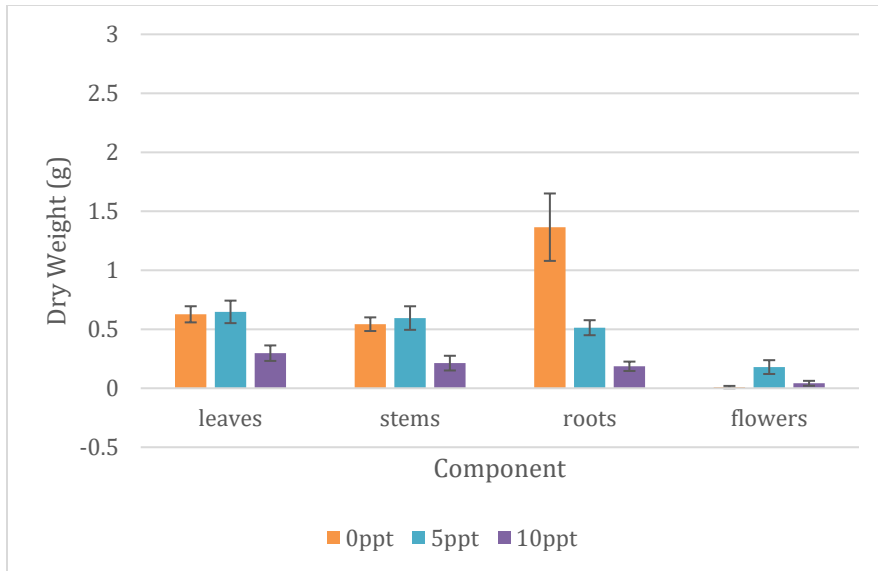


Figure 8 *Purple Loosestrife Average Dry Weight at Harvest-Salinity Group*

The average dry weight of purple loosestrife components for the salinity group. Any dead plants harvested throughout the experiment were included. The error bars represent standard error.

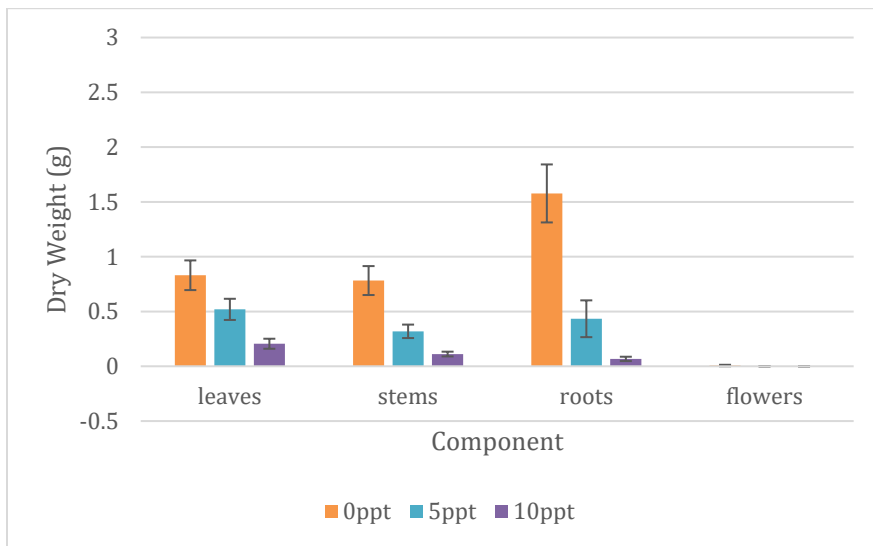


Figure 9 *Winged Loosestrife Average Dry Weight at Harvest-Salinity Group*

The average dry weight of winged loosestrife components for the salinity group. Any dead plants harvested throughout the experiment were included. The error bars represent standard error.

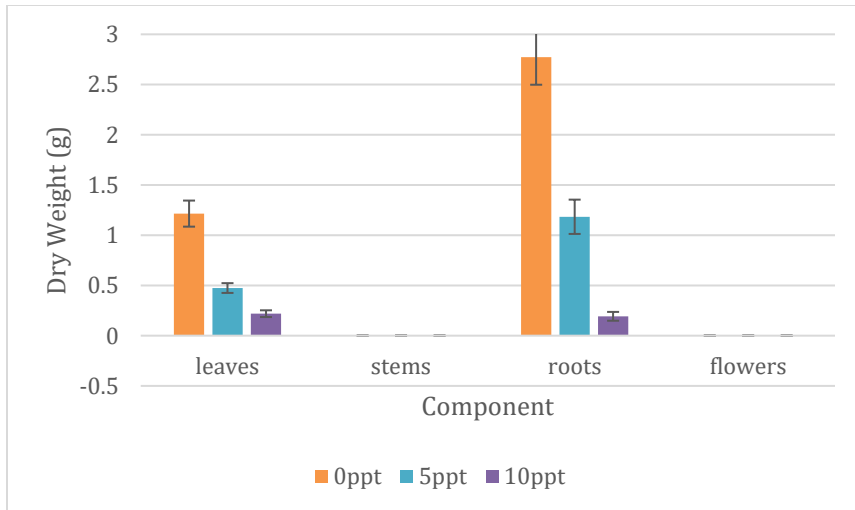


Figure 10 *Cattail Average Dry Weight at Harvest-Salinity Group*

The average dry weight of cattail components for the salinity group. Cattails do not have stems or flowers; these were marked as 0. Any dead plants harvested throughout the experiment were included. The error bars represent standard error.

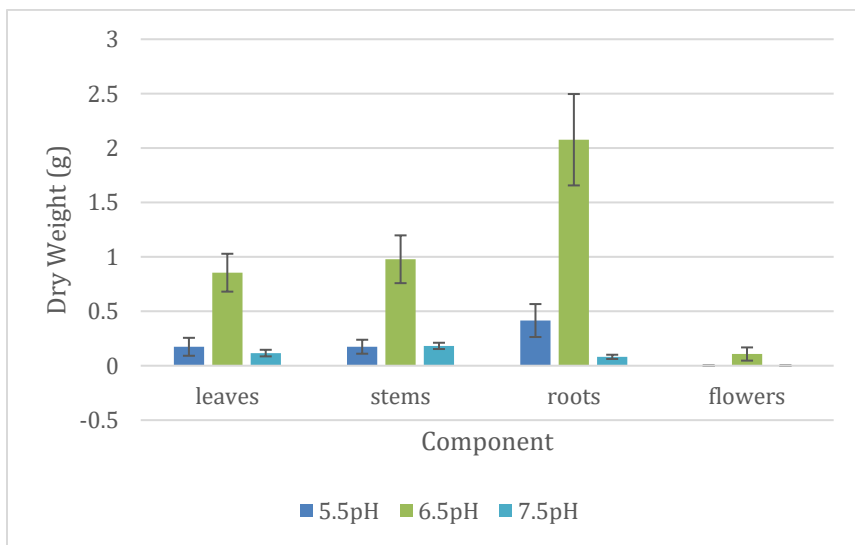


Figure 11 *Purple Loosestrife Average Dry Weight at Harvest-pH Group*

The average dry weight of purple loosestrife components for the pH group. Any dead plants harvested throughout the experiment were included. The error bars represent standard error.

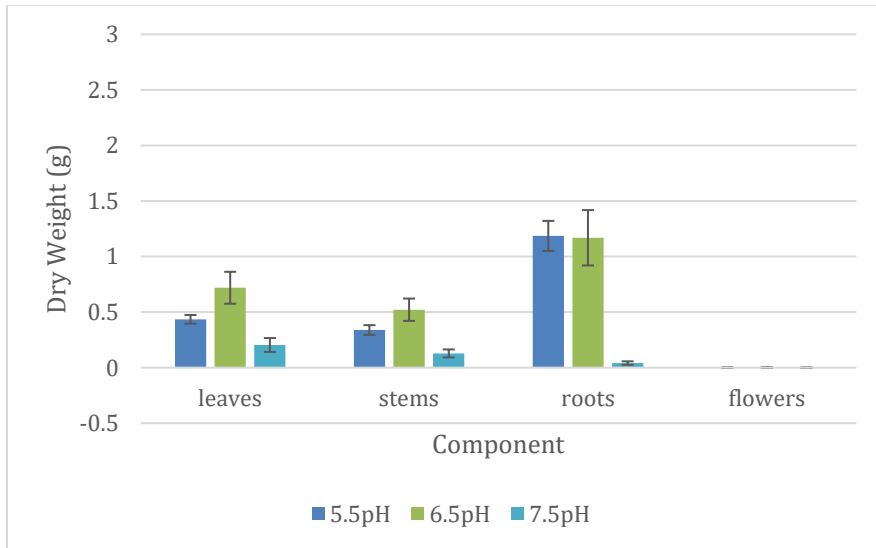


Figure 12 *Winged Loosestrife Average Dry Weight at Harvest-pH Group*
 The average dry weight of winged loosestrife components for the pH group. Any dead plants harvested throughout the experiment were included. The error bars represent standard error.

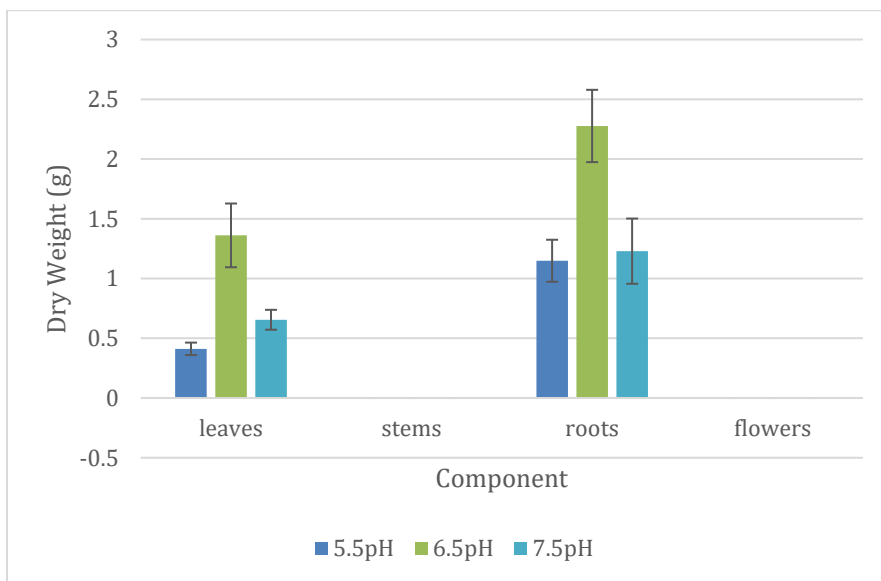


Figure 13 *Cattail Average Dry Weight at Harvest-pH Group*
 The average dry weight of cattail components for the pH group. Cattails do not have stems or flowers; these were marked as 0. Any dead plants harvested throughout the experiment were included. The error bars represent standard error.

Final Height

Salinity

It was found that there were significant difference among species ($F_{2,64}=8.7215$, $P<0.001$), salinity treatments ($F_{2,64}=43.8340$, $P<0.001$), and species*salinity interaction ($F_{4,64}=7.1376$, $P<0.001$), indicating a difference in the species' responses to salinity. A one-way ANOVA was conducted on each species and the means were compared using Tukey HSD. In general, all three species did significantly more poorly in the high salinity conditions. It was found that the cattails had a significant difference in the final heights among all three different levels of salinity ($F_{2,27}=109.9575$, $P<0.001$; Fig. 14). The purple loosestrife also showed a significant difference in final height based on salinity level ($F_{2,25}=7.8613$, $P=0.002$), with growth significantly reduced at 10ppt (Fig. 14). Finally, the result of the winged loosestrife analysis showed a significant difference in heights among the salinity levels ($F_{2,12}=8.2617$, $P=0.005$), with control treatment (0ppt) being significantly taller than the 5ppt salinity treatment (Fig. 14).

pH

The 2-way ANOVA showed that there were significant differences among species ($F_{1,60}=9.4309$, $P=0.003$), pH treatments ($F_{1,60}=35.7465$, $P<0.001$), and the interaction between species*pH ($F_{3,60}=4.3631$, $P=0.008$). As a result of the significant interaction term, 1-Way ANOVAs were completed for each species to assess their response to pH. Results from the analysis on cattails showed there was a significant difference among pH treatments and final heights ($F_{2,26}=14.2315$, $P<0.001$), with final height significantly greater at pH 6.5 than the other two treatments (Fig. 14). In the purple loosestrife, the differences in the final heights were also statistically significant depending on pH level ($F_{2,17}=11.3328$, $P=0.001$). The mean final heights of the 5.5pH and 7.5pH were not significantly different compared to each other, but the 5.5pH

group was significantly lower than the 6.5pH group (Fig. 14). Finally, the winged loosestrife had a mortality rate of 100% in the 7.5pH group so there was no final height data to analyze (Fig. 1). In the 6.5pH and 5.5pH groups there was no significant difference between the final heights and pH levels ($F_{1,17}=0.4261$, $P=0.523$; Fig. 14).

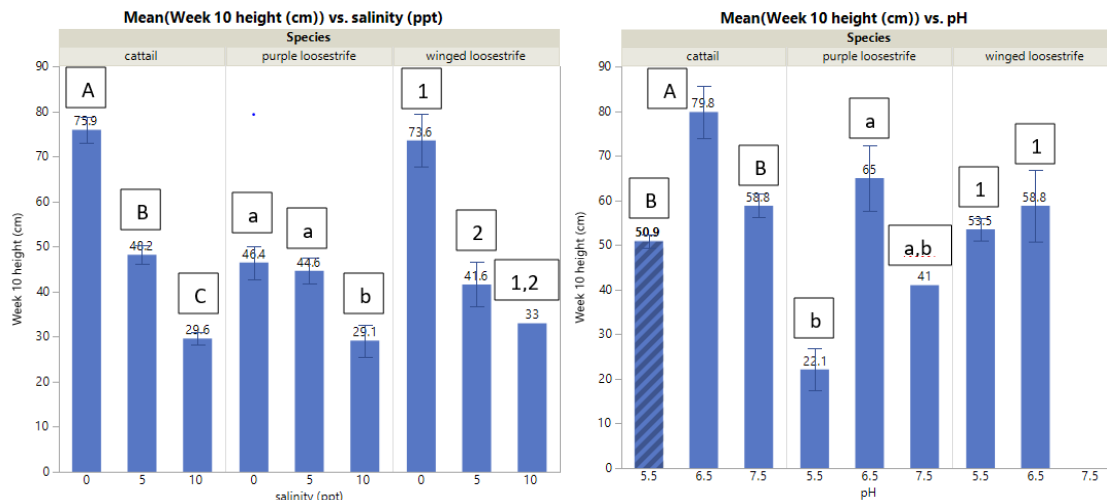


Figure 14 Mean (Week 10 height (cm)) vs. salinity (ppt) and Mean (Week 10 height (cm)) vs. pH Effect of salinity/pH on average Final height for all individuals of all species, not including dead plants; Different letters and numbers indicate significant differences within species. (Capital letters for Cattails, lowercase letters for Purple loosestrife and numbers for Winged loosestrife)

Total mass not including flowers

Salinity

Since not every species produced flowers, the mass of flowers was subtracted from the total mass of the species that did produce flowers. The 2-way ANOVA for species, salinity, and total mass-flowers showed there was no significance in the amount of total mass across species ($F_{2,81}=1.6644$, $P=0.196$), but there was significance between salinity ($F_{2,81}=65.2513$, $P=0.001$) and the interaction between species*salinity ($F_{4,81}=2.7266$, $P=0.035$). Therefore, a one-way ANOVA was conducted on the species separately as well as a Tukey HSD. In general, all the species had less mass in the high salinity treatment, but the winged loosestrife and cattail also had less biomass in the medium salinity treatment. In the cattails it was found that there was

significance between total mass and salinity level ($F_{2,27}=49.9346$, $P=0.001$), the Tukey HSD showed that the average means differed significantly between all three salinity levels. In Fig. 15, it is clear that the 0ppt group had a much higher mean total mass than the 10ppt group and the 5ppt group is somewhere between the two of them. The purple loosestrife also showed a significance between the different salinity levels and the amounts of total mass in each group ($F_{2,27}=11.8452$, $P<0.001$). However, the mean total masses of the 0ppt and 5ppt group did not differ significantly between each other and 10ppt differed from both (Fig. 15). Finally, the winged loosestrife also had a significant difference between the total masses and the salinity levels ($F_{2,27}=16.4114$, $P<0.001$). The Tukey HSD showed that there was a significant difference between the mean total mass of the 0ppt salinity group and the other two salinity groups but the 5ppt and 10ppt were not significantly different from each other. In Fig. 15, the mean for 0ppt salinity was much higher than the other two means.

pH

The 2-way ANOVA showed that there was a significance between species ($F_{2,81}=3.2518$, $P=0.044$), pH ($F_{2,81}=29.7332$, $P<0.001$), and the interaction between species*pH ($F_{4,81}=3.7155$, $P=0.008$). This shows that there was an interaction and thus a one-way ANOVA and Tukey HSD was conducted. In general, the cattails and purple loosestrife both had less mass in the 7.5pH and 5.5pH treatments (Fig. 15). The winged loosestrife also had less biomass in the 7.5pH group compared to its control group but was less affected by acidity compared to the purple loosestrife and cattails (Fig. 15). The cattails showed significance between the amounts of total mass and pH levels ($F_{2,27}=8.0706$, $P=0.002$). According to the Tukey HSD, the 6.5pH group had a significantly higher mean total mass than the 5.5pH or 7.5pH (Fig. 15). The purple loosestrife also had a significant difference in total mass depending on the pH level ($F_{2,27}=15.4374$,

P=0.001). There was a significant difference between the total mass of the 6.5pH group and the other pH groups (Fig. 15). Finally, the total masses of the winged loosestrifes were also significantly affected by the level of pH ($F_{2,27}=11.6505$, $P<0.001$). The Tukey HSD showed that the mean for the 7.5pH group was significantly lower than the other two groups and the 5.5pH and 6.5pH groups were not significantly different from each other (Fig. 15).

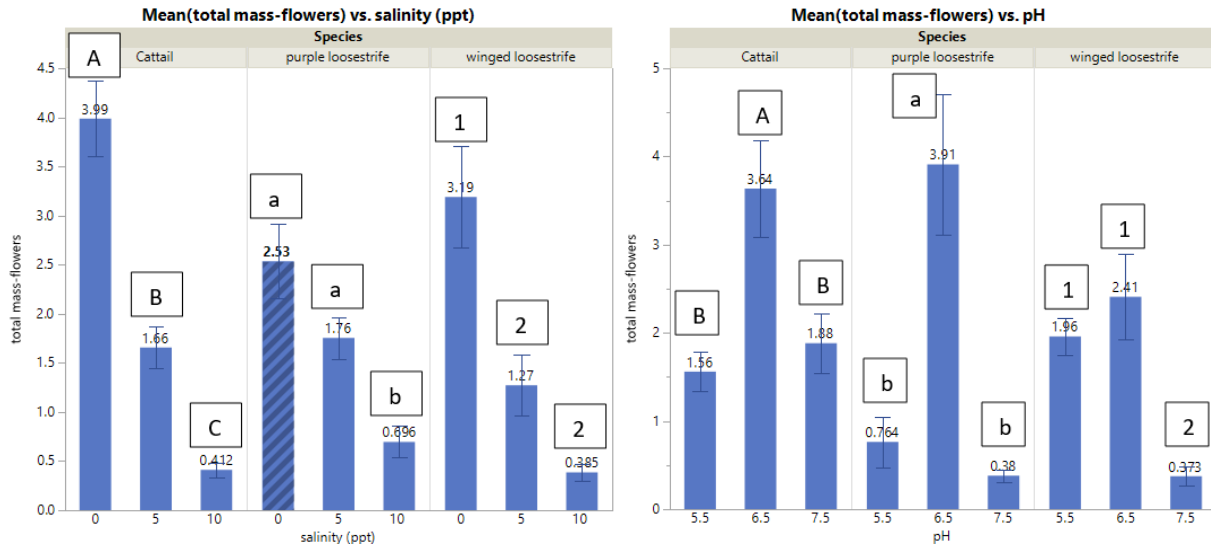


Figure 15 Mean(total mass-flowers (g)) vs. salinity (ppt) and Mean (total mass-flowers (g)) vs. pH

Effect of salinity/pH on the average Total mass (g dry weight) of all components (leaves, roots, stems) – flowers for all species, including dead individuals; Different letters and numbers indicate significant differences within species. (Capital letters for Cattails, lowercase letters for Purple loosestrife and numbers for Winged loosestrife)

Root/shoot

Salinity

The 2-way ANOVA showed that there was a significant effect between the root/shoot ratio and species ($F_{2,81}=75.0788$, $P<0.001$) and salinity ($F_{2,81}=31.9383$, $P<0.001$) as well as a significant interaction between species and salinity ($F_{4,81}=8.2864$, $P<0.001$). Therefore, a one-way ANOVA and Tukey HSD were conducted for each species. In general, both the purple loosestrife and winged loosestrife had a lower root/shoot ratio with increasing salinity, but the

cattails had significantly higher root/shoot ratio in the control group and 5ppt salinity group compared to the 10ppt group (Fig. 16). It was found that the cattails had a significant difference between the root/shoot ratios and the levels of salinity ($F_{2,27}=16.7418$, $P<0.001$). The Tukey HSD showed that there was no significant difference between the mean root/shoot ratios of the 0ppt group and 5ppt group but both were significantly different from the 10ppt group (Fig. 16). The root/shoot ratios of the purple loosestrife were also found to be significantly different ($F_{2,27}=11.8001$, $P=0.001$). In this case, the Tukey HSD showed that there was no significant difference between the mean ratios of the 5ppt group and 10ppt group and both were different from the 0ppt group (Fig. 16). Finally, the winged loosestrife also showed a significant difference across salinity levels ($F_{2,27}=19.9884$, $P<0.001$). According to the Tukey HSD, the winged loosestrife showed a similar pattern to the purple loosestrife. The mean of the 0ppt group was significantly higher than the 5ppt or 10ppt group (Fig. 16).

pH

The 2-way ANOVA showed that there was a significance between the root/shoot ratio and species ($F_{2,81}=49.7472$, $P<0.001$) and pH ($F_{2,81}=22.7325$, $P<0.001$) as well as a significant interaction between species and pH ($F_{4,81}=2.7697$, $P=0.033$). Therefore, a one-way ANOVA and Tukey HSD were conducted for each species. In general, the purple loosestrife and winged loosestrife had a low root/shoot ratio in the alkaline conditions and winged loosestrife had a significantly higher ratio in the 5.5pH group compared to the control group whereas the purple loosestrife did not. Also, the cattail did not have a significant difference in root/shoot ratio based on the pH level ($F_{2,27}=2.6409$, $P=0.090$). The purple loosestrife did have significantly different root/shoot ratios depending on the pH level ($F_{2,27}=15.8841$, $P=0.001$). According to the Tukey HSD, the means of the 6.5pH and 5.5pH groups were not significantly different from each other

but both were different from the 7.5pH group (Fig. 16). Finally, the winged loosestrife also showed a significant difference between the root/shoot ratios and the levels of pH ($F_{2,27}=81.7897$, $P<0.001$). According to the Tukey HSD, the mean root/shoot ratios for all three pH levels were significantly different from each other (Fig. 16).

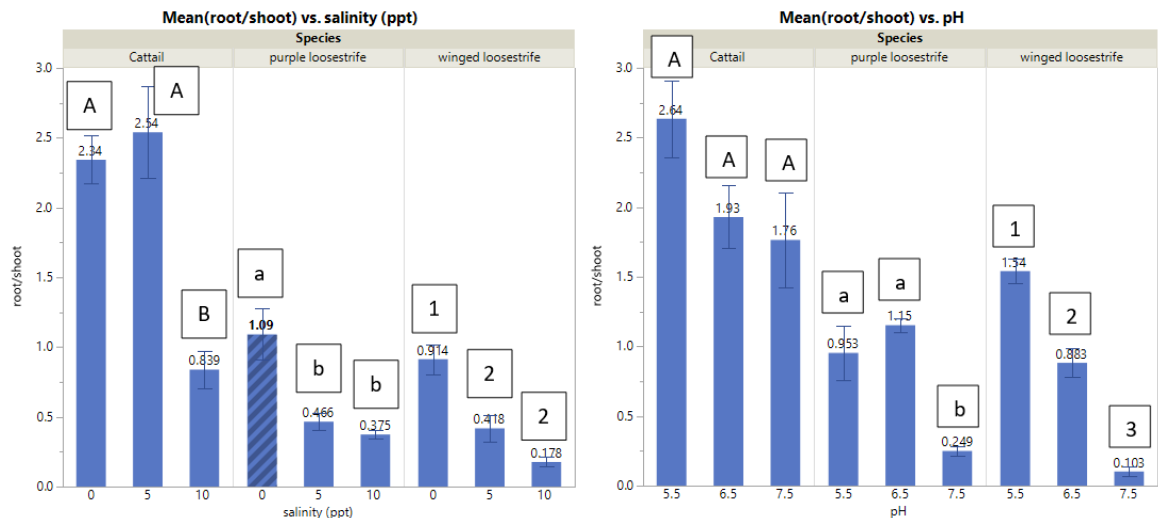


Figure 16 Mean(root/shoot) vs. salinity (ppt) and Mean(root/shoot) vs. pH Effect of salinity/pH on the root to shoot (leaves+stem) ratio for all species, including dead individuals; Different letters and numbers indicate significant differences within species. (Capital letters for Cattails, lowercase letters for Purple loosestrife and numbers for Winged loosestrife)

Starch content

Salinity

According to the 2-way ANOVA, there is no significant difference in the starch content across species ($F_{2,62}=2.7552$, $P=0.071$), but there is a significant difference across salinity levels ($F_{2,62}=5.3518$, $P=0.007$) and the interaction between species*salinity ($F_{4,62}=2.7220$, $P=0.037$). Once the one-way ANOVA was conducted, the amount of starch in purple loosestrife did not show significance between the starch content and the salinity level ($F_{2,18}=2.5599$, $P=0.105$). The winged loosestrife had a significant difference in the starch contents among salinity levels ($F_{2,26}=5.4960$, $P=0.010$), but the only significant difference was between control (0ppt) and the

highest salinity treatment (10ppt, Fig. 17). The cattails were found to have a significant difference in the starch concentration across different salinity levels ($F_{2,18}=4.2036$, $P=0.032$). The Tukey HSD showed that there was a significant difference between the mean starch content of the 5ppt group and the 10ppt group but the 0ppt group was in the middle and was not significantly different from either one (Fig. 17).

pH

The 2-way ANOVA showed a significant difference in the starch concentrations across both species ($F_{2,55}=5.4022$, $P=0.007$) and pH levels ($F_{2,55}=4.7523$, $P=0.012$) as well as a difference in the species' responses to the pH levels ($F_{4,55}=6.3775$, $P<0.001$). Generally, the purple loosestrife and winged loosestrife had less starch in the alkaline conditions, but the amount of starch in the cattails was not affected by pH. The one-way ANOVA showed that the cattails ($F_{2,22}=3.2652$, $P=0.057$) did not have a statistically significant difference, but the means appear to be noticeably different (Fig. 17). The starch concentrations in purple loosestrife were significantly different between pH levels ($F_{2,14}=6.5619$, $P=0.010$). The Tukey HSD showed that the mean starch content of the 6.5pH group was significantly different from the 7.5pH group and the 5.5pH group was not significantly different from either one (Fig. 17). Finally, the winged loosestrife showed a significant difference between the starch concentrations across the pH levels ($F_{2,19}=7.2951$, $P=0.004$). According to the Tukey HSD, the mean starch content of the 6.5pH and 5.5pH groups were not significantly different but both were different from the 7.5pH group (Fig. 17).

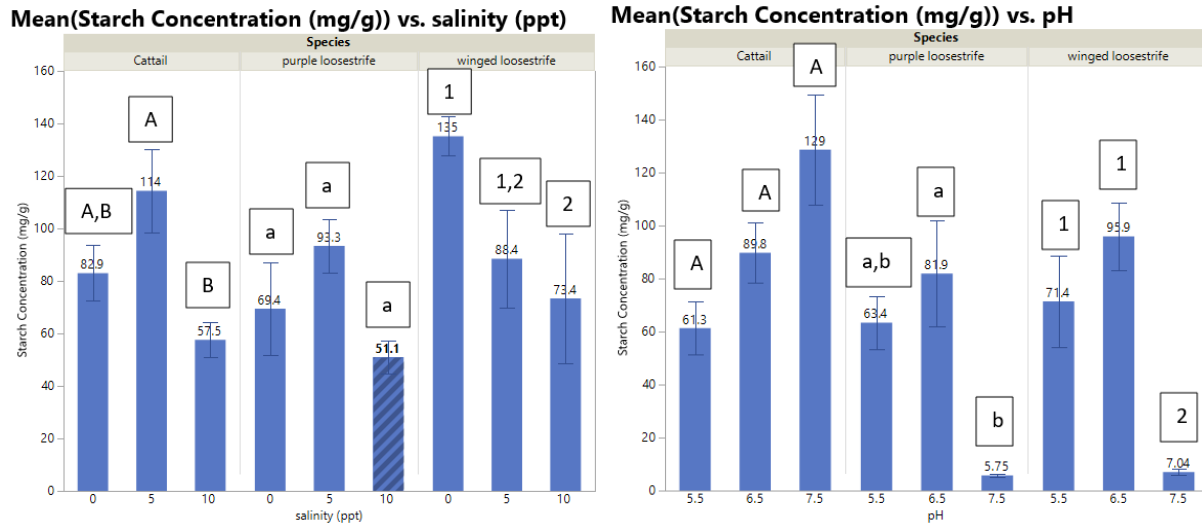


Figure 17 Mean(Starch Concentration (mg/g)) vs. salinity (ppt) and Mean(Starch Concentration (mg/g)) vs. pH

Effect of salinity/pH on the average Starch content for all species, including dead individuals; Different letters and numbers indicate significant differences within species. (Capital letters for Cattails, lowercase letters for Purple loosestrife and numbers for Winged loosestrife)

Discussion

The hypothesis was that if the purple loosestrife could tolerate and thrive in the different soil conditions then it would have higher growth, more dry weight, and higher mean starch content values in all the different treatments compared to the winged loosestrife and cattail. Purple loosestrife are related to winged loosestrife and purple loosestrife often grow in cattail marshes, meaning they often have to outcompete cattails (Rogers, 2019). However, these hypotheses were not supported. The purple loosestrife struggled in the 10ppt and 7.5pH soil conditions and did not perform significantly better compared to the other species

Salinity

In general, based on all of the data from the salinity treatments, all of the plants did not grow well in the 10ppt salinity conditions. This suggests that not only was growth stunted (Fig. 14), but starch reserves were reduced as well (Fig. 17). This supports the findings of Ribeiro et

al. (2022) who found starch reserves are depleted during times of abiotic stress. Perhaps surprisingly, the 5ppt group for cattails had slightly more starch and a higher root/shoot ratio than the 0ppt group, despite the fact that it had a significantly lower total mass and it had a lower average final height. However, the differences in starch and root/shoot ratio were not statistically significant, which suggests that it may simply be by chance that the cattail was able to store that much starch. Most species of *Typha* generally have lower salt tolerance compared to other halophytes which restricts it from most saline environments (Bansal, et al., 2019). It could be that the 5ppt group just happened to allocate more resources to storage rather than growth because it wasn't as stressed out in the saline soils as one may predict. The common cattails, *Typha latifolia*, are known to be able to tolerate moderate salinity as well as reduced soil conditions (plants.ces.ncsu.edu). This particular species of cattail may simply be naturally tolerant towards the 5ppt salinity conditions compared to other species.

The purple loosestrife also had a low tolerance for the 10ppt salinity soil (Fig. 2). The 0ppt and 5ppt groups grew to almost the same height, whereas the 10ppt group grew steadily but to a shorter height. However, it appears that purple loosestrife may have struggled to store starch in the 5ppt and 10ppt salinity conditions (Fig. 8). In the saline groups, the amount of dry weight in the roots is almost equal to the other components, except for the flowers, but the 0ppt control group had significantly more biomass in the roots compared to the rest of the components. This suggests that the purple loosestrife struggles in high saline environments and may have dedicated more effort to above ground growth in the 5ppt group as suggested by Li, et al. (2020) and their work on invasive Chinese tallow trees and Al Hassan et al. (2016) and their work with invasive salt marsh plants. In Figures 14, 15, and 16, the total mass in the 5ppt group was higher than the 10ppt group, but the root/shoot ratios and starch contents were not significantly different. This

further supports the theory that the purple loosestrife in the 5ppt group allocated its resources towards above ground growth rather than storing starch and the purple loosestrife was stressed in the 10ppt salinity. It is possible, perhaps, that the starch was being stored in preparation for winter given that purple loosestrife is a perennial plant, as stated by Vriet (2014) in their paper on the importance of starch for regrowth, storing starch is important for perennial plants in order to grow back in spring.

The winged loosestrife did particularly poorly in the saline soil (Fig. 4), with growth for both the 10ppt and 5ppt treatments showing impacts somewhere around week 4 and they never recovered for the rest of the experiment. Around week 4 of the experiment, the temperatures in the greenhouse reached 90-100F. The winged loosestrife is not as competitive or hardy as the purple loosestrife and struggles to survive under hot and/or dry conditions (illinoiswildflowers.info). Also, winged loosestrife tends to be found in marshes, along rivers and streams, in fens, and other places where freshwater is available ([illionoiswildflowers.info](http://illinoiswildflowers.info)). The arid conditions combined with the salt stress may have been too overwhelming for the winged loosestrife. In Figures 9, 15, and 16, the control group had more biomass in its roots and a higher total mass and root/shoot ratio compared to the salinity groups. This suggests that the winged loosestrife struggled to grow and store starch in saline conditions, the values are also very similar to the purple loosestrife so it is possible that the two related species similarly struggled. However, looking at Figures 17, the winged loosestrife managed to obtain more starch than the purple loosestrife, despite the fact that the purple loosestrife endured the high temperatures. According to the New Hampshire Department of Environmental Services (des.nh.gov), the purple loosestrife can survive in fluctuating water levels and full sunlight which would stress native plants. It is also likely that this happened because the purple loosestrife focused more on

aboveground growth compared to the winged loosestrife. As stated in Li, et al., (2020), invasive species tend to focus more on aboveground growth rather than starch storage.

pH

In general, the winged loosestrife and purple loosestrife had a low tolerance for the 7.5pH soil the most and the cattails had a low tolerance for the 5.5pH soil. In Figures 7, 13, 14, 15, 16 and 17, the cattails in the 5.5pH group did not grow as tall as the 7.5pH group or have as much biomass in its roots, but there was no significant difference between the total mass or root/shoot ratios. This could suggest that the 5.5pH group allocated its resources to repair and handle the stress. It's possible that the 7.5pH group stored more starch than the control group because it couldn't allocate resources towards growth, this may be due to a lack of specific nutrients or ions that were blocked due to the alkalinity. As stated by Zhang and Mu (2009), high pH caused by alkaline stress may inhibit the uptake of inorganic anions and cause the plants to become more reliant on organic anions which may prevent them from regaining ionic balance. Without a stable tissue pH, plants cannot maintain their normal metabolism and thus their growth may be stunted as we are likely seeing here with the cattails.

The purple loosestrife in the 7.5pH group started dying off around week 6, which was around the time the temperatures in the greenhouse reached 90-100F. The winged loosestrife also died off around this time even though Figure 6 shows the die off occurring two weeks earlier. The purple loosestrife grew more quickly than the winged loosestrife and cattails, so the treatments started two weeks earlier despite them being planted at the same time. While for the first few weeks it seemed like the 7.5pH group was growing better than the 5.5pH group (Fig. 5), the fact that only plants in the 7.5pH group died likely means that the plants in that group were already struggling. In Figure 11, the average final height for the purple loosestrife in the alkaline

soil was 41cm because only one plant survived in the 7.5pH group. In Figures 14, 15, 16, and 17, the 7.5pH group had less biomass and a lower root/shoot ratio than the other groups but there is no significant difference between the total mass and starch content of the 5.5pH group and 7.5pH group. In this case, the alkalinity likely caused sufficient stress to cause the purple loosestrife to deplete their starch reserves. As for why the 5.5pH group had so little aboveground growth despite the amount of starch it had reserved, hydrogen ion toxicity likely prevented the purple loosestrife from absorbing necessary cations, as suggested by Kidd and Proctor (2001), thus preventing the plants from growing.

The winged loosestrife also struggled in the 7.5pH soil, Figures 1 and 6 show all of the winged loosestrife in the 7.5pH group were dead by week 7. On the other hand, the plants in the other pH soils grew to be nearly the same height at almost the same rate. In Figure 14, while the average for the 7.5pH group was 0, the average values for the 5.5pH and 6.5pH were almost the same. However, in Figure 11 the allocation of biomass appears to be very different between the 5.5pH and control groups. While the 7.5pH had very little biomass in all its components, likely because they died mid-experiment, the 6.5pH group had most of its biomass in its stems and the 5.5pH group had most of its biomass in its roots. This would suggest that the 6.5pH group focused on growth, not necessarily upward growth but perhaps branch growth, and the 5.5pH group stored starch in its roots. This is supported by Figures 15 and 16, while the mean total masses for the 5.5pH and 6.5pH groups were not significantly different, the root/shoot ratio for the 5.5pH was significantly higher than the control group. This suggests that the 5.5pH group had more biomass in its roots than in the leaves or stem and despite the fact that they were nearly the same average height, the 6.5pH group had less mass in its roots and/or more mass in its leaves and stems. However, in Figure 17 the mean starch content for the 6.5pH group was

reportedly higher than the 5.5pH group, even though the difference was not statistically significant. This suggests that the difference in starch content was merely by chance, however, the fact that the 6.5pH group has almost equal amounts of starch could mean that the 5.5pH group did not have as much starch as was previously thought despite the amount of roots it had.

Conclusion

Invasive species are a threat to global biodiversity and can cost countries millions of dollars in their attempts to remove them (Allendorf and Lundquist, 2003). Invasive plants can be particularly difficult to remove given their reproductive abilities and the lack of suppressing herbivores and pathogens (Allendorf and Lundquist, 2003). Habitat compatibility is one important factor as to whether or not a species can establish in a new area. Purple loosestrife has spread through North America over the past 200 years and continues to damage wetland environments. Purple loosestrife can grow faster than many native species and quickly spreads over vast areas due to its high seed count. Further, purple loosestrife can hybridize with the native winged loosestrife, which may lead to it becoming more compatible in the United States compared to its native range (Houghton-Thompson, et al., 2005). Most of the control and removal methods for purple loosestrife have failed. Due to these failures, researchers are investigating biocontrol methods which may harm the native species as well (Blossey, et al., 2001). In order to effectively limit the spread and manage the current populations of purple loosestrife, it is important to learn about what sort of environment they are able to live in. It is necessary to study if the purple loosestrife is capable of outcompeting native species in less-than-optimal growing conditions. If purple loosestrife can survive in areas where native species struggle, then purple loosestrife could potentially spread to areas that had not been previously considered at risk. Testing their tolerance to environmental conditions can help predict where

they may thrive and it can help us find ways to prevent them from spreading any further in areas where they can establish themselves.

The results of these experiments suggest that the purple loosestrife struggles in high salinity as well as in high pH environments. This may suggest that areas with a high number of roads may not be suitable for purple loosestrife because of the presence of road salts. Also, purple loosestrife may not grow in areas where humans have dumped or added chemicals that raise pH such as near factories, farms that use chemical pesticides, or gardens that use lime. Areas of naturally occurring alkaline or high saline conditions will likely also be protected from the spread of purple loosestrife. However, winged loosestrife and cattails, as well as other native species, may not be able to grow in some of these areas either and may also suffer from being sprayed by solutions meant to control purple loosestrife. Winged loosestrife especially had a similar reaction to the different treatments compared to purple loosestrife and therefore may struggle in similar environments in a natural setting. The results also suggest that it may be possible to control the populations of purple loosestrife using alkaline or highly saline solutions to kill the invasive plant. In any case, it is up to other researchers to find ways to control purple loosestrife populations without seriously damaging the native plant populations.

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