The Effects of Low pH Characteristic of Acid Mine Drainage on *Lythrum salicaria*, an Invasive Wetland Species, and *Typha latifolia*, a Native Wetland Species

Triad #37

[redacted]

Section 005

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ABSTRACT

Acid mine drainage, or AMD, affects many wetlands in the Appalachian region by decreasing soil pH. Low soil pH could make these areas vulnerable to non-native, invasive species. To determine the effects of species type and low pH due to AMD on plant growth, seventy-two native *Typha latifolia* plants and seventy-two invasive *Lythrum salicaria* plants were subjected to pH levels of 6.5, 4.0, and 2.5. The interaction of species and pH had a significant effect on biomass (F ratio=3.3289, p=0.0387). *Typha latifolia's* mean biomass decreased steadily with decreasing pH, while pH had a less of an effect on *L. salicaria's* mean biomass, which increased initially, then decreased. The effect of pH on plant height and leaf number does not depend on species. These results indicate that *L. salicaria* may have a competitive advantage over *T. latifolia* in wetlands affected by AMD, that could ultimately result in displacement of native *T. latifolia* and alter species richness and variety in Appalachian wetland ecosystems.

INTRODUCTION

Acid mine drainage is an important environmental issue, especially in the Appalachian region (EMAP 2000, ASMR 2004). Acid mine drainage is water that has drained through mine tailings and contains heavy metals and sulfuric acid (Jambor 1994, Bourg and Loch 1995). Because of its low pH, AMD can have adverse effects on ecosystems that are exposed to it such as wetlands (Stephenson *et al*. 1995, Sibrell and Watten 2002, Stephens *et al*. 2003, ASMR 2004). Wetlands exposed to AMD have markedly reduced plant species richness (Stephenson *et al*. 1995), which may make them susceptible to invasive species.

In wetlands, invasive plant species are known to displace native plants and alter species richness and native community structure because of their ability to produce small seeds that can grow without pretreatment and the ability to form monospecific stands (Goodwin *et al*. 1999, Mack 1996, Thompson *et al*. 1987, Morrison 2002, Farnsworth and Meyerson 2003). Invasion by non-native species can alter habitats for aquatic invertebrates (Gardner *et al*. 2001) and affect nesting and feeding patterns of wetland birds and mammals (Homan *et al*. 2000, Melvin 2002, Connors *et al.* 2000).

AMD makes wetlands susceptible to invasive species because it alters nutrient availability (Wieder and Lang 1984). When soil pH falls below 4.5, heavy metals such as aluminum become more soluble and can block plant up-take of more important nutrients, such as calcium, nitrogen, and phosphorus (Driscoll *et al*. 2001). Also, acid conditions can cause nutrients to be leached from soils (Driscoll *et al*. 2001, Bourg and Loch 1995, Sibrell and Watten 2002), resulting in nutrient depletion that can lead to reduced plant growth and increased susceptibility to stress (Driscoll *et al*. 2002). In wetlands, AMD reduces carbon content in plant tissues and causes a decrease in plant species richness (Stephens *et al*. 2003, Stephenson *et al*. 1995). Because invasive species typically reproduce sexually, yield large numbers of seeds, and seem to have an affinity for disturbed areas (Thompson *et al.* 1987*)*, there is an increased probability that they will be better suited to changed conditions than native species and may be able to eliminate native species through competitive exclusion (Mooney and Cleland 2001). Invasive wetlands species have also been known to tolerate low soil pH and have a superior ability to assimilate nutrients (Thompson *et al*. 1987, Emery and Perry 1995).

Lythrum salicaria is a non-native, invasive wetland plant from Europe with many characteristics that may allow it to spread in areas exposed to AMD (Thompson *et al.*1987, Cox 2002). *Lythrum salicaria* is found all over the world, including areas affected by acid mine drainage (Thompson *et al.* 1987, Goodwin 1975). It typically reproduces via sexual reproduction and has large seed yields (Thompson *et al.* 1987). Its seeds can germinate in a variety of conditions such as low pH and low temperatures (Shamsi and Whitehead 1974, Young and Clements 2001). Also, it has an ability to retain phosphorus that is superior to that of *T. latifolia*, and it has an affinity for disturbed areas (Thompson *et al*. 1987, Morrison 2002, Emery and Perry 1995)

One native wetland species that coexists with *L. salicaria* is the cattail, *Typha latifolia* (Thompson *et al.* 1987). *Typha latifolia* is found all over the United States and southern Canada (Cox 2002) including areas that are subject to AMD, and it is commonly used in AMD treatment wetlands (Weider and Lang 1985, Stephens *et al.* 2003, Goodwin 1975). *Typha latifolia* is an integral component of wetland ecosystems because wetland birds and mammals such as cranes and muskrats rely heavily on it for food, nesting grounds, and lodging material (Fassett 1940, Conners *et al.* 2000, Homan *et al.* 2000, Melvin 2002).

Lythrum salicaria has already been shown to displace *T. latifolia* in wetland areas that are not affected by AMD (Morrison 2002, Thompson *et al*. 1987). Due to its affinity for establishing disturbed areas (Thompson *et al.* 1987, Wilcox 1995) and ability to survive in a variety of pH ranges, with seed germination recorded in soil pH as low as 4.0 (Sashimi and Whitehead 1974, Thompson *et al*. 1987), *L. salicaria* may have competitive advantage over *T. latifolia* in AMD-exposed wetlands. This may ultimately result in the displacement of cattail in these areas as well, leading to a decrease in suitable habitat for wildlife (Rawinski and Malecki 1984, Gardner *et al.* 2001, Thompson *et al*. 1987) and changes in hydrology and nutrient cycling (Emery and Perry 1995, Dixon and Johnson 1999).

Because they are located in areas that receive AMD, it is important to understand how *L. salicaria* and *T. latifolia* respond to low pH (Thompson *et al.* 1987, ASMR 2004). Although extensive research has been done on *L. salicaria's* ability to displace *T. latifolia* in environments unaffected by AMD (Thompson *et al*. 1987, Morrison 2002, Farnsworth and Ellis 2001), this experiment compares how each species responds to low soil pH levels that simulate the pH values of AMD. Also, the effects of pH on mature plants of these species has already been studied to some extent (Kittle 1993, Thompson *et al*. 1987), but we will be examining the effects of low pH on juvenile plants. This will help to determine how low pH affects the establishment of new plants, rather than those that are already established.

The main objective of this study was to determine whether native *T. latifolia* and invasive *L. salicaria* exhibit a differential growth response to decreased soil pH.

METHODS

To determine whether the effect of pH depended on species, we used a two-way factorial design. This first factor was species and had two levels, invasive, or *Lythrum salicaria,* and native, or *Typha latifolia*. The second factor was pH and had three levels, 6.5, 4.0, and 2.5. A pH of 6.5 was chose because it is the optimal level for nutrient uptake in *T. latifolia* (Brix *et al.* 2002). The pH levels 4.0 and 2.5 represent pH levels in soils that are subject to AMD (Bourg and Loch 1995) (Table 1). There were twenty-four replicate plants per treatment combination for a total of 144 plants.

Because we wanted to determine the effects of AMD on young plants, we decided to start our plants from seed. Approximately 200 *L. salicaria* and 200 *T. latifolia* seeds were spread on top of water-saturated soil in two different trays and placed in growth carts with constant light. Within 3-4 days, the *L. salicaria* seeds had germinated, but after two weeks there was no germination of *T. latifolia*. *Typha latifolia* seeds were spread between water-saturated paper towels in plastic trays covered with plastic wrap. These seeds germinated within two days.

Seventy-two seedlings of each species were then transferred into individual 5cm pots filled with sand. Sand was used instead of soil because it has little to no buffering capability. There was one seedling per pot, for a total of 144 potted seedlings.

The pots were then arranged in six large, clear, plastic bins according to treatment combinations. There were two bins for each pH level, and each species was randomly placed in each bin. We placed the bins back in the growth cart, and then added distilled water to each bin. The bin design was used to keep the pots in 1-3cm of standing water so the sand could stay saturated at all times. The plants were kept in standing distilled water for approximately six days to allow time for recuperation after transplantation.

Treatments were initiated six days after the bins were organized. Water was drained from all the bins and replaced with treatment solutions that had been adjusted to the appropriate pH level. Two bins received water with a pH of 6.5, two received water with a pH of 4.0, and the final two bins received water with a pH of 2.5. The treatment solutions were created using 1M sulfuric acid (H_2SO_4). On average, one drop H_2SO_4 was added to 3.5L of distilled water to create the 6.5 pH solution, ten drops were added to 3.5L for the 4.0 solution, and 20mL were added to 3.5L to create the 2.5 pH solution. The pH of each solution was tested using a Model 10, Accumet pH meter before the solution was applied to the plants. Treatments continued for twenty days. The water level of each bin was checked on a daily basis. The appropriate pH solution was mixed and added to each bin whenever the standing solution in the bin fell below 1cm which was approximately once per week.

Measurements of plant height and leaf count began five days after treatments were initiated. Measurements continued to be taken biweekly for three weeks. *Lythrum salicaria* plant height was measured from the base of the stem to the tip of the stem in centimeters. For *T. latifolia*, which is a grass species, the length of the longest blade was measured as plant height. The number of blades for each *T. latifolia* plant was considered leaf count, while the number of true leaves present on *L. salicaria* was considered leaf count.

Treatments were ended after three weeks, when both root and shoot sections of each plant were harvested and rinsed. Each plant was put into an individual paper bag and placed in a drying oven at 65 degrees Celsius for forty-eight hours, after which the biomass of all plants was measured in grams using a Model A-160, Denver Instrument Digital Scale.

Data was entered into SAS-JMP software. The effect of species type, pH level, and an interaction of the two was determined by comparing plant height, leaf number and biomass means of each treatment group using a 2-way analysis of variance. A p-value less than 0.05 indicated a statistically significant effect of the treatment.

RESULTS

The effect of pH on biomass depended on species (F ratio=77.3717, $p=<0.0001$). The mean biomass of *T. latifolia* at pH of 6.5 was eleven times greater than the mean biomass at a pH of 4.0, and fifty times greater than at a pH of 2.5 (Table 2; Figure 1). Conversely, *L. salicaria* plants in 4.0 pH had a mean biomass that was larger than that of plants in 6.5 or 2.5 pH (Figure 1). There was also a significant effect of pH on biomass (F ratio=3.7887, p=0.0387). Low soil pH resulted in an overall decrease in biomass of both species (Figure 1). The effect of species type on biomass was statistically significant (F ratio=2.3713, p=0.0972). The biomass of *Lythrum salicaria* plants in all three treatment groups was at least two times greater than the biomass of *T. latifolia* (Table 2; Figure 1).

Species type had a statistically significant effect on plant height (F ratio=115.4780, $p<0.0001$). *Typha latifolia* was on average three times shorter than *L. salicaria* (Table 3; Figure 2). The effect of pH on plant height did not depend on species type (F ratio=2.3713, p=0.0972). Also, the effect of pH on plant height was not significant (F=0.5255, p=0.5924). Decreased pH did not seem to have a large negative on either species, but it did seemed to increase *Lythrum salicaria* height means slightly (Figure 2; Table 3).

As expected, the effect of species on leaf number was statistically significant (F ratio=221.0181, p<0.0001). *Lythrum salicaria* generally had three times as many leaves as *T. latifolia* (Table 4; Figure 3). On average, *Lythrum salicaria* had between eight and ten leaves, while *T. latifolia* plants had on average three leaves with little variation between groups of the same species (Table 4; Figure 3). The effect of pH on leaf number was not statistically significant (F ratio=1.0641, p=.3479), and the effect of pH on leaf number does not depend on species (F ratio=2.2813, p=0.1060). Both species' leaf number appeared to be unaffected by pH (Table 4; Figure 3).

DISCUSSION

The interaction of species and pH had a significant effect on biomass, leading to the rejection of the null hypothesis that there was no difference between means of the treatment groups. It appears that at this stage of development invasive *L. salicaria* is better suited to a low pH environment, possibly due to several of its characteristics. *Lythrum salicaria* has a history of thriving in disturbed environments such as those with low soil pH and other human caused perturbations (Thompson *et al*. 1987). Also, *L. salicaria* relies heavily on sexual reproduction and has large seed yields (Thompson *et al*. 1987) which may result in a large amount of genotypic variation within the *L. salicaria* population. Therefore, it may have an increased probability of surviving in altered environmental conditions such as AMD affected wetlands. Finally, *L. salicaria* exhibits a better ability to retain phosphorus than *T. latifolia* (Emery and Perry). This characteristic is advantageous in a low pH environment, because low pH can leach phosphorus from soils and solubilize heavy metals that block phosphorus up-take by plants (Driscoll *et al*. 2001). *Lythrum salicaria*'*s* response to low pH in this study corresponds to the results of Shamsi and Whitehead (1974) and Thompson *et al.* (1987), which indicate that *L. salicaria* seeds can germinate in low pH, and mature plants can thrive in a variety of pH levels.

The greater negative response of *T. latifolia* to low pH is similar to the results of a study by Kittle (1993), which also found that low pH produces decreased biomass in this species. Because *L. salicaria* had less of a negative response to decreased pH, it could have a competitive advantage in wetlands subjected to AMD. This could ultimately result in the displacement of *T. latifolia* by *L. salicaria* and lead to further changes in species richness and variety, which would alter wetland wildlife habitats.

Decreased pH had a significant effect on biomass, but not on plant height or leaf number. The plants were young, so they may have put more energy into increasing their height and leaf number to increase photosynthetic area rather than accumulating biomass. Further research could be done in this area to see if, in fact, this is the case. Also, the insignificant effect of pH on plant height and leaf number may be explained by Kittle's (1993) research concerning the effects of AMD on several wetland species, including *T. latifolia*. Kittle found that wetland species have a greater intolerance to the metals present in AMD rather than pH alone.

Species type had a significant effect on all parameters of growth measured. The larger leaf number per plant for *L. salicaria* was expected. Young *L. salicaria* plants typically have more leaves than young *T. latifolia* plants have blades due to differences in the species (Thompson *et al.*1987). An unexpected result was that *L. salicaria* had greater plant height means than *T. latifolia*. Research by Thompson *et al*. (1987) indicated these species had similar height when found together; however, our research indicates that *T. latifolia* had a larger percentage increase in height. This may indicate that *L. salicaria* has greater increases in height at this stage of development, which may give it an advantage if competing with *T. latifolia* for light and further contribute to the displacement of *T. latifolia.* Species type appeared to have the largest effect on biomass. Biomass of *L. salicaria* was at least two times greater than the biomass of *T. latifolia*. This was interesting because the two species generally have similar biomass means in the field (Emery and Perry 1995, Thompson *et al.* 1987). *Typha latifolia* began germination some time after *L. salicaria*, so this could have had some effect on biomass, height, and leaf number means.

Low soil pH is only one aspect of how AMD can alter soil chemistry. Increases in heavy metal concentration are also typically associated with AMD. Further research focusing on how *T. latifolia* and *L. salicaria* interact with AMD and each other should progress to experiments that examine the effects of low soil pH combined with increased heavy metal concentrations. It has already been found that *T. latifolia* forms a protective iron plaque on its roots (Taylor *et al.* 1997). It would be interesting to see if *L. salicaria* has a similar evolutionary defense.

The results of this experiment lead to many implications about the establishment of young plants for both species in AMD affected areas. These results indicate that juvenile invasive *L. salicaria* plants are more tolerant of low pH than *T. latifolia*. This research contributes to the hypothesis of previous experiments by Thompson *et al.* (1987) in that L. *salicaria's* success as an invasive species is in part due to ability to colonize areas affected by human-caused disturbances and its amazing ability to thrive in low pH. Our results also indicate that *L. salicaria* exhibits more growth than *T. latifolia* at this stage of development. In summation, *L. salicaria's* invasive efficiency may allow it to out-compete *T. latifolia* and possibly other native wetland speices in areas disturbed by AMD.

Acid mine drainage is changing soil chemistry of many wetlands in the Appalachian region. Because *Lythrum salicaria* exhibits more growth than *T. latifolia's* at this stage, and *L. salicaria* is more tolerant to low pH, it may be able to decrease richness of *T. latifolia* by competitive exclusion during establishment of new stands by juvenile plants. A variety of ecological consequences could follow. Invasion by *L. salicaria* would not only reduce richness of *T. latifolia*, but species richness and diversity of the entire area could be affected (Thompson *et al.* 1987). This could have a detrimental effect on wetland wildlife species that rely heavily on *T. latifolia* for food and nesting material (Connors *et al.* 2000, Melvin 2002) and possibly produce changes in hydrology and nutrient cycling with in AMD affected wetlands(Dixon and Johnson 1999, Emery and Perry 1995).

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Table 1 – Experimental Design Showing Species vs. pH Level and Number of Replicates per Combination

		pH Level		
		6.5	4.0	2.5
Species	*Typha latifolia*	N=24	N=24	N=24
	Lythrum salicaria	N=24	N=24	N=24

Table 2 – Replicate N and Biomass Means (+/- standard errors) for *Typha latifolia* and *Lythrum salicaria* in 6.5, 4.0, and 2.5 pH.			
Species	pH	N	Mean Biomass (+/- s.e.) *in* grams
Lythrum salicaria	6.5	24	0.02695g (+/- 0.003222g)
	4.0	24	0.033412g (+/- 0.00459875g)
	2.5	24	0.02044583g (+/- 0.00224593g)
Typha latifolia	6.5	24	0.01030417g (+/- 0.00504906g)
	4.0	24	0.00091913g (+/- 0.0003711g)
	2.5	24	0.0020537g (+/- 0.00009216g)

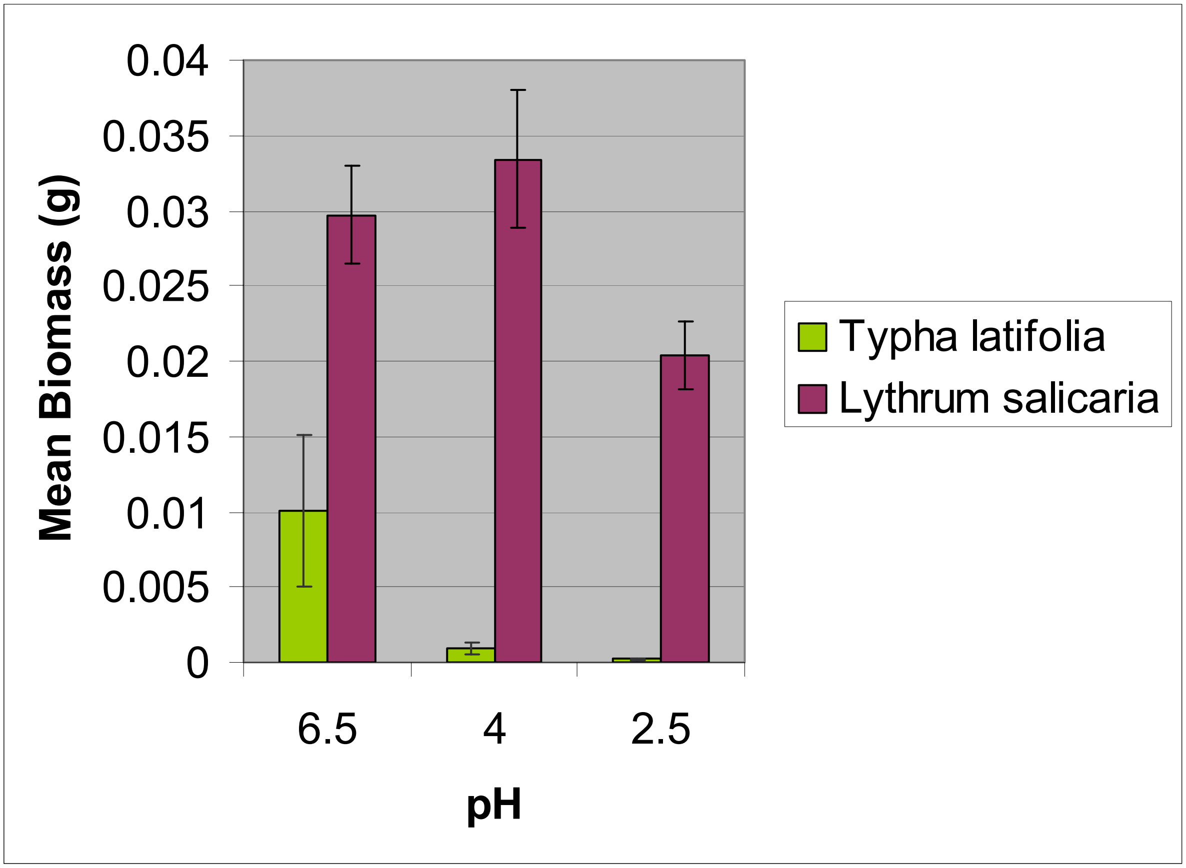
Table 3 – Replicate N and Plant Height Means (+/- standard errors) in centimeters for *Typha latifolia* and *Lythrum salicaria* in 6.5, 4.0, and 2.5 pH.			
Species	pH	N	Plant Height in cm (+/- s.e.)
Lythrum salicaria	6.5	24	3.7875 (+/- 0.42958155)
	4.0	24	4.42083333 (+/- 0.38097792)
	2.5	24	4.60833333 (+/- 0.46195539)
Typha latifolia	6.5	24	1.54583333 (+/- 0.2206692)
	4.0	24	1.575 (+/- 0.18932087)
	2.5	24	0.87916667 (+/- 0.17764351)

Table 4 – Replicate N and Leaf Number Means per Plant (+/- standard errors) for *Typha latifolia* and *Lythrum salicaria* in 6.5, 4.0, and 2.5 pH.			
Species	pH	N	Mean Leaf Number per Plant (+/- s.e.)
Lythrum salicaria	6.5	24	9.666667 (+/- 0.50957025)
	4.0	24	10.2916667 (+/- 0.55324853)
	2.5	24	8.375 (+/- 0.82929274)
Typha latifolia	6.5	24	2.75 (+/- 0.32554302)
	4.0	24	2.70833333 (+/- 0.35344663)
	2.5	24	3.05416667 (+/- 0.32554302)

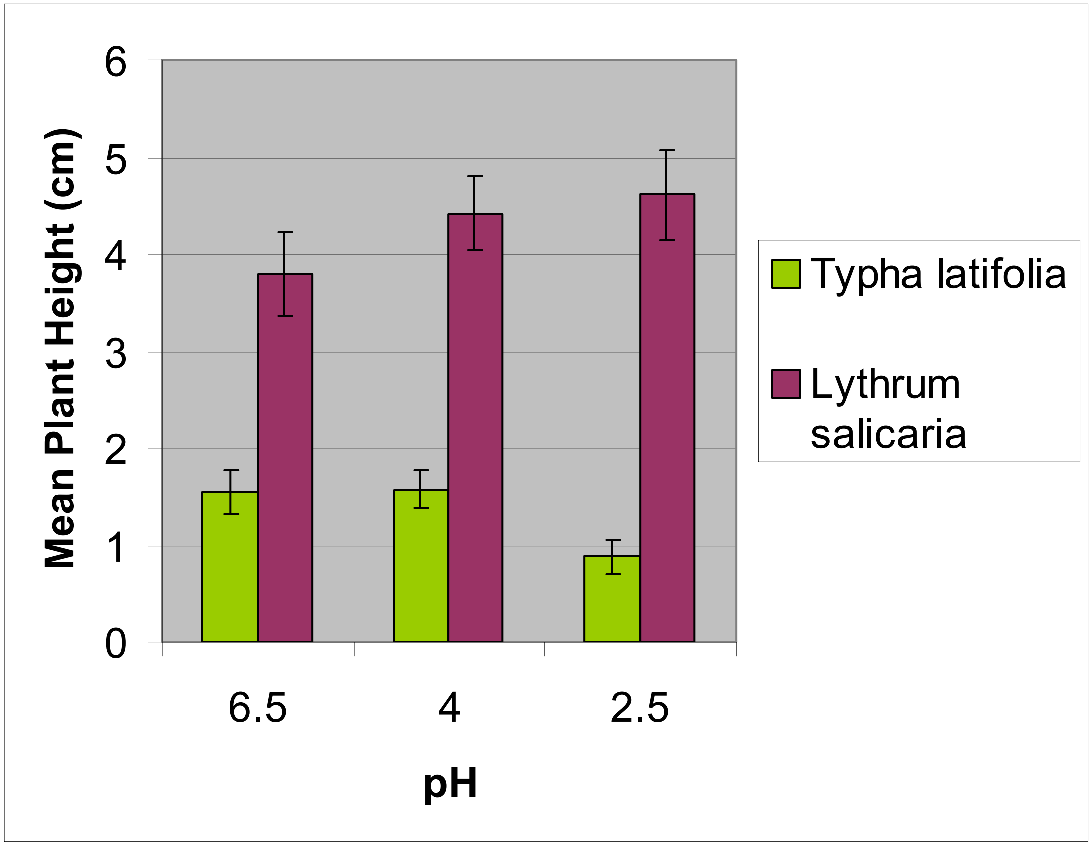
Figure 1. Mean biomass (+/- standard errors) for T*ypha latifolia* and *Lythrum salicaria* in 6.5, 4.0, and 2.5 pH.

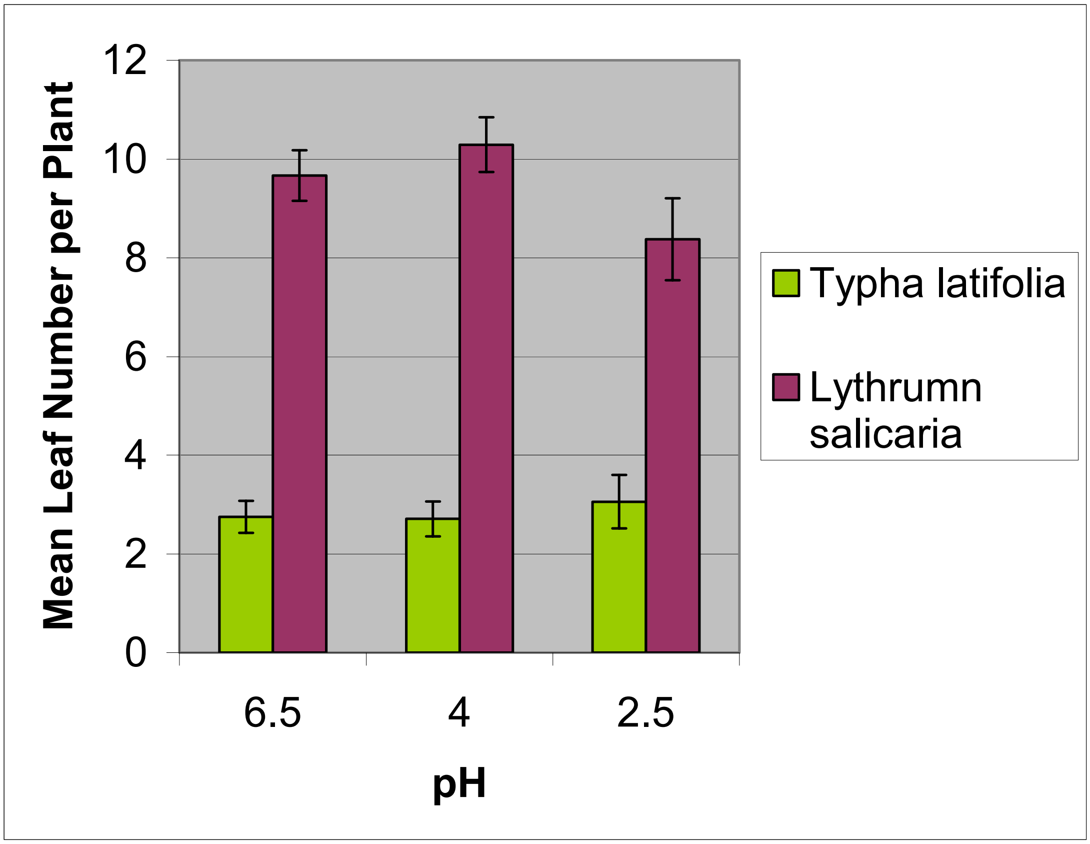
Figure 2. Mean plant height (+/- standard errors) for T*ypha latifolia* and *Lythrum salicaria* in 6.5, 4.0, and 2.5 pH.

Figure 3. Mean leaf number per plant (+/- standard errors) for T*ypha latifolia* and *Lythrum salicaria* in 6.5, 4.0, and 2.5 pH.



Mean Biomass (g)
0.04
0.035
0.03
0.025
0.02
0.015
0.01
0.005
0
6.5
4
2.5
pH
Typha latifolia
Lythrum salicaria


Mean Plant Height (cm)
6
5
4
3
2
1
0
6.5
4
2.5
pH
Typha latifolia
Lythrum salicaria



Mean Leaf Number per Plant
12
10
8
6
4
2
0
6.5
4
2.5
pH
Typha latifolia
Lythrumn salicaria