

Effects of Flooding on Connecticut River Plant Species

by

Hanna W. Atia

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ABSTRACT

Due to dam construction and other human influences, patterns of river water flow, including the timing and duration of flooding, have become increasingly stable in wetlands. Flooding is a natural disturbance event that changes the physical structure and nutrient availability of habitats. The prevention of natural flooding can change species composition in floodplains and assist colonization by non-native species. The presumed mechanism for this pattern is that species differ in their tolerance to flood duration, yet there are few experimental studies that have examined flood tolerances experimentally.

The purpose of this study was to quantify differences in flood tolerance among 25 woody plant species found along the Connecticut River, and to determine the degree to which flood tolerance predicts species distributions along flooding gradients. I additionally analyzed the roles of height, non-native status, growth form, and evolutionary history on flood tolerance. Seedlings were subjected to flood treatments of four different durations, with and without suspended clay sediment. The flood tolerance of each species was calculated by comparing individual species' survival relative to the average survival across all replicates.

The flood and sediment treatments had a significant effect on seedling survival, and the distribution of species on flooding gradients was predicted by the experimentally determined flood tolerance. There was significant variation in height between species, and seedlings submerged more deeply were less healthy than those closer to the surface. Native species had overall better health than non-native species with increasing flood stress. Analysis of growth forms showed that shrubs were significantly healthier than vines with increasing flood stress. There were no significant differences between trees and other growth forms. Higher flood tolerance did not map cleanly onto plant families. The most flood tolerant and least flood tolerant species were both in the genus *Acer* (maples).

INTRODUCTION

What factors affect flooding?

Due to the construction of dams, patterns of water flow, including the timing and duration of flooding, have becoming increasingly stable in floodplain systems (Casanova and Brock 2000). While control of large floods is important for human safety, the prevention of flooding is detrimental to wetland communities. Both the physical and biotic aspects of these habitats are impacted by the absence of natural flood cycles (Junk et al. 1989, Kozłowski 1997, Kozłowski 2002, Jackson and Colmer 2005).

Weather Patterns

Flood intensity in a river naturally varies because it is influenced by a variety of environmental factors such as seasonal change, topographic variation, and regional climate (Junk et al. 1989). Seasonal flood pulses occur in most rivers, and the inundation they cause can last from hours to months, with a range in water depth (Junk et al. 1989). On the Connecticut River mainstem, flooding from snowmelt can last for several weeks (Marks et al. 2014). Other sources of flooding include seasonal hurricanes and tropical storms, and depending on weather conditions, moderate flooding can occur any time of year (Marks et al. 2014). Though these weather patterns would naturally have a large impact on the

fluctuation of water flow, measures put in place to control flooding restrict natural weather-based fluctuations in water level.

Human Impact on Flood Control

The existence of a dam slows the rate at which water can travel downstream. Controlling water flow by allowing only a limited amount of water to pass through a dam reduces flood variation and intensity, substantially inhibiting flood depth and length (Kohri 2008). The resulting decrease in water quantity flowing downstream can lead to water deficiencies, particularly for organisms that depend on extensive seasonal flooding (Kozlowski 2002, Niinemets and Valladares 2006). The presence of water is important for seed germination in general, but it is especially crucial for the survival of plant species with seeds and seedlings that require consistent moisture to germinate and thrive (Kozlowski 2002). In blocking water flow, dams additionally create areas of inundated soil where bodies of stagnant water may form. Plants living upstream of a dam are therefore at high risk of dying, as the physiological effects of long-term submergence in stagnant water can lead to death in even the most flood-tolerant species (Kozlowski 2002).

Flood prevention has significant long-term impacts on physical aspects of the environment. Controlling flood intensity reduces the frequency and severity of disturbance processes that influence floodplain topography (Kozlowski 2002, Kohri 2008). The erosion of riverbanks and deposition of sediments along channels are processes directly caused by river flow (Gurnell et al. 2012). Natural bank scouring is diminished by flow control, and less sediment is carried by the

river and deposited as a result (Kozlowski 2002). The physical boundary a dam creates can trap various substances, and over time sediments, organic matter, and seeds accumulate behind dams (Gurnell et al. 2012). Flood control creates environmental changes and results in physiological dysfunctions that affect growth and development of riparian forest ecosystems (Kozlowski 2002).

What are the impacts of flooding?

Physical Changes to Area

Flooding causes a number of physical changes in topography, sediment, and gas exchange in inundated areas. The processes of erosion in riverbanks and deposition of sediments along channels impacts the topography of an area over time, leading to the formation of oxbows and new bodies of water (Gurnell et al. 2012). Scouring causes changes in riverbanks, and the build up of sediments creates moist, disturbed areas, which are required by some flood-adapted species to germinate and survive as seedlings (Kozlowski 2002). The processes of erosion in riverbanks and deposition of sediments along channels creates new areas for seedling establishment (Gurnell et al. 2012).

Sediments carried by flood waters can also impede plant growth when suspended over submerged plants or deposited on seedlings after a flood. The presence of sediment in flood water reduces the amount of light available to plants, which increases the severity of flood stress by inhibiting photosynthesis (Peterson and Bazzaz 1984).

Riparian areas experience varying levels of disturbance, and can therefore be more susceptible to invasion by opportunistic species when stress levels are altered (Alpert et al. 2000). Though newly disturbed riparian areas are cleared of potential competitors by flood disturbance, they are can be stressful environments for young plants to colonize when floods last for longer durations. During floods, soil inundated with water creates a hypoxic (low-oxygen) or anoxic (no-oxygen) root environment that is especially stressful when flooding is stagnant, as water replaces the air in soil pores. The diffusion of gases through water is significantly slower than in air and soil, leading to plant deficiencies in gases necessary for regular functioning (Kozlowski 2002, Jackson and Colmer 2005, Lambers et al. 2008). This results in a variety of physiological effects on plants, such as stomatal closure, increased use of anaerobic respiration, and increased ethylene production (Kozlowski 2002, Lambers 2008). These effects impact species in different ways, and native floodplain species have developed adaptations to better tolerate submergence (Kozlowski 2002).

Native and non-native species appear to perform differently at different stress levels, with young, colonizing invaders more susceptible to the impacts of anoxic conditions than native flood-adapted species (Alpert et al. 2000). Additionally, floods may clear out young invaders in subsequent years, possibly before they can reproduce, if the colonizers are not flood-adapted. Though disturbance caused by shorter floods can increase resource availability to plant species by clearing out competition, the stresses of more prolonged flooding durations can reduce the

ability of non-native species to colonize riparian areas (Casanova and Brock 2000, Alpert et al. 2000).

Species Distribution

Flood duration, depth, and frequency are important aspects in determining where floodplain forests will occur, and the species compositions within them (Marks et al. 2014). These factors impact which herbaceous and aquatic plant species become established in an area, as wetland diversity results from flooding variation, with different plant community development as an outcome of different flood durations (Casanova and Brock 2000).

There are quantitative estimates of flood duration at transitions between different habitats such as floodplain forests, upland forests, shrub swamps, marshes, and scour shelves. Along the Connecticut River, the shift in dominance from upland to floodplain tree species is at 4.5 days of flooding per year, and the shift from floodplain forest to shrub swamps at over 95 days/year (Marks et al. 2014). The species found along these distributions are determined by the adaptive traits that enable specific species to thrive in a particular area.

Native Species

Physiological responses in plants are the product of historical filters that determine species dispersal and eventual distribution. Plants that persist within stressful conditions respond evolutionarily to these conditions; over generations, the survival of certain phenotypes leads to population level changes in physiology that allow them to better grow and reproduce, resulting in adaptations to the

historical climate (Lambers et al. 2008). Native upland species such as *Prunus serotina* may be less tolerant to flooding, as they generally grow in areas that flood less than 4.5 days/year (Marks et al. 2014). Native species found in floodplains, where flooding occurs more than 4.5 days per year and less than 95 days per year, possess traits that enable them to survive the stresses associated with flooding. Comparing how native and invasive species perform at different stress levels is helpful in understanding the relationship between environmental stresses and invasibility of an area, and how differences in stresses may make an area more or less prone to invasion (Alpert et al. 2000).

Invasive Species

Invasive species are defined as non-native organisms that start successful, spreading populations after initial human introduction to new areas, which have negative ecological impacts on these new environments (Lockwood et al. 2007). Many non-native species are not adapted to the long term abiotic conditions of the environment in which they find themselves, which appears to be the case with flood tolerance. Non-native species seem more sensitive to water changes, with lower abundance and less frequency of invasive shrubs like *Berberis thunbergii* found when flooding is deeper and longer in duration (Casanova and Brock 2000, Marks et al. 2014). A greater number of non-native species can behave invasively during longer dry periods (Casanova and Brock 2000).

Physiological Responses in Plants

Flooded soil leads to suppressed growth in plants, and death and decay in roots, primarily due to the absence of gases required for regular plant functioning (Kozlowski 2002). In completely submerged plants, light availability can be greatly reduced, and stomatal closure occurs to prevent excess water from entering cells. There are a variety of impacts caused by stomatal closure because of decreased gas absorption (Kozlowski 2002).

Respiratory Metabolism

Changes in plant metabolism occur in response to the limitation on aerobic respiration, restricting ATP production (Lambers et al. 2008, Kozlowski 2002). One way plants react to this energetic restriction is the use of fermentation to produce ATP (Lambers et al. 2008, Visser et al. 2003). Though well-adapted species can survive for months under water by using fermentation (Visser et al. 2003), in most plants the use of fermentation is not sustainable for long periods of time, as it requires large amounts of starch reserves, leads to ethanol and lactate accumulation, and is much less efficient than aerobic respiration (Lambers et al. 2008).

Photosynthesis

Excess amounts of water surrounding a plant generally lead to decreased efficiency in photosynthesis due to stomatal closure, limited gas exchange, and decreased light intensity (Lambers et al. 2008, Vriezen et al. 2003).

Stomatal closure limits gas exchange, but the rapid closure seen in floodplain species such as *Populus deltoides* and *Acer saccharinum* is adaptive as it prevents excess water from entering plants through osmosis (Kozlowski 2002). Stomatal closure also results in decreased gas absorption and root respiration, which can lead to decreased leaf turgor, water absorption, and photosynthesis efficiency (Kozlowski 1997). While many plant species die in submerged conditions due to these effects, floodplain species have adaptations that can help them survive periods of prolonged flooding.

In *P. deltoides*, leaves were found to retain their turgor even after stomatal closure, though carbon dioxide absorption was reduced. Other floodplain plants such as *Platanus occidentalis* and *Ulmus americana* also absorb less carbon dioxide due to stomatal closure, but *U. americana* seedlings were able to retain their rigidity (Kozlowski 2002). While early stomatal closure in response to flooding inhibits photosynthesis, it does not reduce leaf turgor in these floodplain species (Kozlowski 2002). Maintained leaf turgor and stem rigidity demonstrates how water absorption in flood-adapted species does not seem to be greatly impacted by stomatal closure, though their physiological functions are altered (Kozlowski 1997, Kozlowski 2002).

Although a plant would be able to photosynthesize longer without the response of stomatal closure, the water surrounding the plant would impact the pressure potential in the plant's cells, which could lead to other negative effects. Stomata play an important role in photosynthesis, and over longer periods of time

photosynthesis declines in flooded plants because of alterations in the photosynthetic process and a decrease in leaf chlorophyll (Kozłowski 2002).

The presence of sediment in flood water also impacts photosynthetic processes by reducing the amount of light available to plants (Peterson and Bazzaz 1984). Though plants submerged in water containing sediments photosynthesize at lower rates than plants submerged in clear water, the presence of sediment within flood water has less of an impact on photosynthetic rates than flood duration (Peterson and Bazzaz 1984).

Ethylene

Anaerobic conditions can stimulate ethylene synthesis due to decreased efficiency of photosynthesis (Kozłowski 2002, Vriezen et al. 2003). Increased ethylene concentrations are associated with responses such as leaf epinasty and abscission, tissue hypertrophy, and horizontal cell growth (Burg 1973, Kozłowski 2002). However, in flood-adapted species, the build-up of ethylene in response to submergence can lead to accelerated shoot elongation, a strategy plants may use to quickly grow tall enough to reach air and survive total submergence (Voeselek et al. 2003, Vriezen et al. 2003). The seedlings of two floodplain species, *Ulmus americana* and *Platanus occidentalis*, were found to contain higher ethylene concentrations when flooded, which suggests that they may use elevated ethylene concentrations to grow quickly (Kozłowski 2002).

The Project

Exploring the mechanisms that affect species distribution and composition in floodplain forests is significant to conservation efforts. The alteration of natural river flows through dam construction has been linked to native species declines and the invasion of non-native and upland species in the Connecticut River floodplain, among other areas (Casanova and Brock 2000, Marks et. al 2014). Designing ecological flow prescriptions that mimic natural flooding is a way to facilitate the preservation of unique ecosystems created and supported by this important ecological process (Kozlowski 2002).

Flood duration affects species distributions in floodplains, wetlands and river banks by acting as a physiological filter on plant life (Casanova and Brock 2000, Lambers 2008, Marks et. al 2014). Differences in species' flood tolerance is presumed to be the mechanism enabling distinct patterns of species distribution and plant communities to occur in flooding areas, yet few experimental studies have compared a broad range of species flood tolerances experimentally. The purpose of this study was to quantify differences in flood tolerance among woody plant species that grow in different habitats along the Connecticut River. Specifically, we seek to determine the degree to which flood tolerance predicts species distribution along flooding gradients in floodplain forests by comparing species' experimentally calculated flood tolerance to their natural distribution in these areas.

In this experiment, 25 species of woody plants both native and exotic to the Connecticut River were flooded for various durations of time. In order to mimic the effects of real floods with water and soil movement, we flooded some plants and added clay, and compared these to plants flooded with only water.

I hypothesize that species would have different experimental flood tolerances that reflect where they are found on the flood gradient. I additionally predict that species found in areas that flood more frequently will have lower mortality with increasing flood duration.

Native species develop within an area, adapting to the environmental pressures within a habitat. I hypothesize that native species will have higher rates of survival and higher flood tolerances than non-native species because non-native species tend to be more sensitive to water submergence (Casanova and Brock 2000).

I hypothesize that taller seedlings will survive flooding treatments better than short seedlings because by being closer to the surface, they have better access to light and diffused gases than shorter seedlings. Because trees grow to be taller than shrubs or vines, and many dominant floodplain species are trees, I predict that trees will survive best of these growth forms.

I predict that species that are more closely related will respond to flooding similarly because they all shared a common ancestor at some point, and as a result they may react to the same stresses in similar ways (Niinemets and Valladares, 2006).

METHODS

Experimental Design

We tested 25 different species of woody plants from the Connecticut River watershed for flood tolerance. In this experiment, seedlings were subjected to flood treatments of four different durations with and without suspended sediment (Table 1). Trays containing 25 potted seedlings (one from each species) were placed in bins, which were filled with water to completely submerge the seedlings. All bins were filled to the same point, so the water level between treatments was uniform at the start of the experiment. The location of species within each tray was randomized. Likewise, the locations of bins with different treatments were randomly assigned within the area where the experiment took place. Each bin and tray was labeled with a code describing the treatment and marking which direction the tray was to be facing within the bin. Flood durations were 1 day, 1 week, 3 weeks, and 6 weeks. In half of the bins we stirred in red powdered potter's clay to create suspended sediment in the flood water. The experiment included 8 replicates of each treatment.

Table 1. Flood and suspended sediment treatment combinations in the flood tolerance testing experiment. For each treatment there were 8 trays with all 25 species in each tray.

1 day, no clay	1 week, no clay	3 weeks, no clay	6 weeks, no clay
1 day + clay	1 week + clay	3 weeks + clay	6 weeks + clay

The experiment was conducted over 10 weeks, from July 22, 2015 until September 30, 2015, at the Richard Cronin National Salmon Station in Sunderland, MA. On the first day of the experiment, trays of seedlings for all treatments were placed into their respective bins where they were completely submerged. The standard depth to which bins were filled meant that trays were completely submerged and even the tops of the tallest seedlings were below the surface of the water. The source of the water for filling the bins was a series of natural groundwater springs at the Salmon station. After the bins were filled with water, the trays were left alone until their treatment was complete. Water in the bins remained stagnant for the duration of the flood treatment. When the end of the flood duration was reached, all of the trays from that treatment were lifted out of the bins and the water was drained. Seedling trays were replaced into the now empty bins for the remainder of the experiment, where they continued to be monitored.

After each flood duration was complete, the treated seedlings were given enough water to keep the bottom of the pots submerged about an inch. Water was periodically added or removed from the post-treatment bins based on temperature and precipitation in order to keep the seedlings sufficiently moist, but prevent soil from becoming saturated with water. The water level within bins still undergoing flooding treatments was unaltered after the onset of the experiment, aside from naturally occurring evaporation or precipitation. None of the seedlings grew tall

enough to emerge past the flooded water level during the course of the experiment.

The pots used were D27 lightweight cylindrical pots (diameter=6.4 cm, depth=17.8 cm, volume=444 ml) from Stuewe & Sons Inc, in Tangent, Oregon. The pots were filled with a commercial potting soil (Earthgro brand) that contained mostly compost and peat with a small amount of perlite and sand. The trays had dimensions of 35.6 cm by 35.6 cm and held 25 pots in rows of five. The top lip of the pots was 17.8 cm from the bottom of the bins. The plastic storage bins that we used were 68 liters, with dimensions of 61 cm x47 cm, with a depth of 40 cm. The lip of each pot was 20 cm from the top of the bins. See Figure 1 for a side view of a bin.

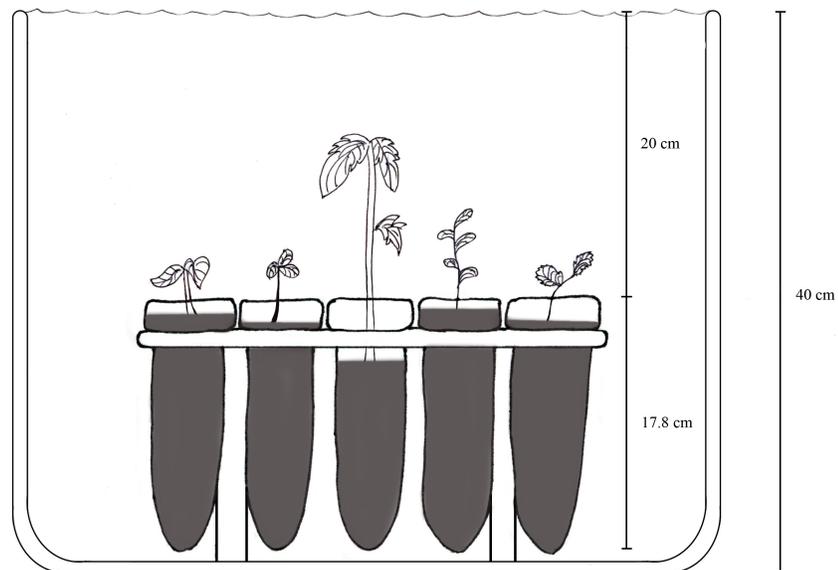


Figure 1. An illustration of the side view of a bin containing a submerged tray holding plants. Plants were measured from the lip of each pot to account for inconsistencies in soil depth.

We stirred 222.5 ml of red potter's clay into the water in the bins immediately after submersion to create a suspension (Fig. 2A). While suspended, the sediment obscured light reaching submerged seedlings. Even after about two weeks, when most of the clay had settled out of suspension, the clay reduced light by coating leaf surfaces (as well as all the other surfaces in the bins and pots), as can also be observed on floodplain plants after natural riverine floods (Fig. 2B).



Figure 2A.



Figure 2B.

Figure 2. Images of the experiment. 2A shows how the clay treatment obscured light compared to the clear treatment. In 2B, a tray from the clear treatment is next to a clay-treated tray to show the effects of the clay suspension after it settled onto the surfaces of the bin.

At the completion of each experimental flooding period, we measured the height of each plant in that flooding treatment. Because of differences in soil height between pots, we measured seedlings from the lip of each pot to the top of the plant. Doing so kept a consistent reference with respect to the water surface, and quantified the depth to which the top of each seedling was submerged.

Health and height data were collected from all the seedlings in each group immediately after the duration of flooding treatment was over. Additional seedling health data was periodically collected following initial assessment (Table 2). Each plant was assessed as vigorous (0), stressed (1), or dead (2) after the completion of its flooding treatment and after completion of each additional

flooding treatment. In addition, all plants were assessed a final time, four weeks after the end of the six week flooding treatment (Table 2). This means that the plants treated for one day were assessed the most, five times over the course of the experiment, while the plants treated for six weeks were assessed twice.

Multiple assessments were done to give seedlings initially marked dead a chance to resprout, decreasing the chance of incorrectly counting plants as dead.

Table 2. The assessment dates for each treatment. Dates where seedlings were assessed are marked by “X” and “height” indicates when height was measured.

	7/23/2015	7/30/2015	8/12/2015	9/2/2015	9/30/2015
1 day	X height	X	X	X	X
1 week		X height	X	X	X
3 weeks			X height	X	X
6 weeks				X height	X

Using the collective data for each seedling from the original three-point assessment scale, I assessed each plant as healthy, stressed, or dead (Table 3). Seedlings were ultimately scored based on the overall data trend. Individuals consistently marked as vigorous were recorded as healthy (0), those with a combination of the three levels were marked as stressed, and those mainly marked as stressed and dead were counted as dead (2).

I additionally assessed each seedling as dead (0) or alive (1) based on their final stress assessment (Table 3). Seedlings considered alive or stressed were marked as alive, while dead seedlings were marked dead.

I additionally created a five-point health scale of vigorous (5), healthy (4), stressed (3), unhealthy (2), or dead (1) with the data from the three-point stress scale. Because we conducted multiple assessments, I was able to create this new scale by looking at the trend of each seedling over the course of the experiment. Seedlings that consistently scored healthy (0) on the three-point stress scale were marked as vigorous (5), while seedlings that were consistently scored as stressed (1) were marked as stressed (3). I created specific guidelines for each treatment duration so plant health would be assessed consistently. See Table 3 for examples.

For all plant assessments, seedlings marked as “dead” on the final day of assessments (9/30/15) were not ultimately considered dead unless also marked dead in prior assessments. This was done because the leaves on other plants in the area were starting to abscise with the increasingly cooler weather, and we did not want to incorrectly mark seedlings as dead when their leaf loss was due to autumn abscission. Additionally, the main purpose for assessing all seedlings 4 weeks after the conclusion of the 6 week treatment was to check for resprouting in seedlings that may have been marked dead immediately after flood treatments.

Table 3. Examples of how seedlings from each duration treatment were scored for each assessment scale. Survival was measured as alive (1) or dead (0). Stress was measured as healthy (0), stressed (1) or dead (2). Health was measured as vigorous (5), healthy (4), stressed (3), unhealthy (2), or dead (1).

	7/23/15	7/30/15	8/12/15	9/2/15	9/30/15	Survival Score	Stress Score	Health Score
1 day	0	0	0	0	0	1	0	5
	0	0	0	0	2	1	0	4
	0	0	1	2	1	1	1	3
	0	0	2	2	1	1	1	2
	0	0	0	2	2	0	2	1
1 week		0	0	0	0	1	0	5
		0	0	0	1	1	0	4
		1	0	1	1	1	1	3
		1	2	2	1	1	1	2
		1	2	2	2	0	2	1
3 weeks			0	0	0	1	0	5
			1	0	0	1	0	4
			1	1	1	1	1	3
			2	0	2	1	1	2
			2	2	2	0	2	1
6 weeks				0	0	1	0	5
				1	0	1	0	4
				0	2	1	1	3
				1	2	1	1	2
				2	2	0	2	1

Seedlings

Through the months of June and July 2015, up until about a week before the experiment began, we collected seedlings and seeds of the 25 different plant species. We planted each seedling in an individual pot. After transplanting seedlings into pots, they were well-watered and observed for between one to six weeks to ensure that they were healthy at the start of the experiment.

The species used were both native and non-native to New England. For the purposes of this experiment, species originating from the United States that are not native to the northeast are classified as non-native species.

The way seedlings were collected varied between species depending on the source. We wanted the seedlings of all species to be at the developmental stage and size that they would naturally be in the field if they encountered a summer flood. Thus we collected seedlings that had germinated in the spring of 2015 at nearby field sites. We looked for cotyledons on the seedlings to insure that they did indeed germinate in spring 2015. For some species that produce seed in the spring, we planted the seed a few days after it ripened in May. Finding sufficient (i.e., 64) seedlings that had germinated naturally in the field in the same spring for 25 species proved difficult. As a result we sometimes had to collect all of the seedlings for some species at the same site. In the case of a few species, we did not find 64 individuals. Specifically, *Catalpa speciosa* was missing one replicate, *Toxicodendron radicans* was missing three, and *Ulmus rubra* was missing 17 (out of 64). Most of the seedlings were collected from sites in Massachusetts, such as the floodplain forest on the Green River in Greenfield, (see Table 4). For *Ulmus americana*, *Ulmus rubra*, *Acer rubrum*, and *Acer saccharinum* we collected seeds and planted them directly in the pots between June 1 and 4.

The seedlings of *Prunus virginiana* were purchased from a native plant nursery in Brunswick, Vermont, as were the majority of *Acer negundo* seedlings. These plants came from seeds collected in Brunswick, Vermont along the

Connecticut River floodplain. The seeds were planted outside at the nursery in the preceding fall. Due to the more northerly location of Brunswick, the seedlings of these two species were perhaps a bit more behind in their growth than they would have been had they germinated in Massachusetts.

Table 4. The species used in the experiment. All species were collected as seedlings, except where noted as from seed. Species codes and nomenclature follow the USA NRCS plants online database: <http://plants.usda.gov/java>

Species Code	Common Name	Scientific Name	Family Name	Order	Location Collected
ACNE2	Boxelder	<i>Acer negundo</i> L.	Aceraceae	Sapindales	Brunswick, VT (nursery)
ACPL	Norway maple	<i>Acer platanoides</i> L.*	Aceraceae	Sapindales	Northampton, MA
ACRU	Red Maple	<i>Acer rubrum</i> L.	Aceraceae	Sapindales	Northampton, MA (from seed)
ACSA2	Silver maple	<i>Acer saccharinum</i> L.	Aceraceae	Sapindales	Turner's Falls, MA (from seed)
BETH	Japanese barberry	<i>Berberis thunbergii</i> DC.*	Berberidaceae	Ranunculales	East Haddam, CT
BELE	Black birch	<i>Betula lenta</i> L.	Betulaceae	Fagales	Northampton, MA
CACA18	Musclewood	<i>Carpinus caroliniana</i> Walter	Betulaceae	Fagales	Greenfield, MA
CASP8	Northern catalpa	<i>Catalpa speciosa</i> (Warder) Warder ex Engelm.*	Bignoniaceae	Lamiales	Northampton, MA
CEOR7	Oriental bittersweet	<i>Celastrus orbiculatus</i> (Thunb.) Siebold*	Celastraceae	Celastraceae	Greenfield, MA
ELUM	Autumn olive	<i>Elaeagnus umbellata</i> Thunb.*	Elaeagnaceae	Rosales	Greenfield, MA
EUAL13	Burning bush	<i>Euonymus alatus</i> Thunb.*	Celastraceae	Celastraceae	Greenfield, MA
FRAL4	Glossy buckthorn	<i>Frangula alnus</i> Mill.*	Rhamnaceae	Rosales	Easthampton, MA
PIST	White pine	<i>Pinus strobus</i> L.	Pinaceae	Pinales	Amherst, MA Keene, NH Swanzey, NH
PLOC	American Sycamore	<i>Platanus occidentalis</i> L.	Platanaceae	Proteales	Greenfield, MA
PODE3	Eastern Cottonwood	<i>Populus deltoides</i> Bertram ex Marsh.	Salicaceae	Malpighiales	Greenfield, MA
PRSE2	Black cherry	<i>Prunus serotina</i> Ehrh.	Rosaceae	Rosales	Northampton, MA
PRVI	Choke cherry	<i>Prunus virginiana</i> L.	Rosaceae	Rosales	Brunswick, VT (nursery)
QRUR	Red oak	<i>Quercus rubra</i> L.	Fagaceae	Fagales	Northampton, MA
ROPS	Black locust	<i>Robinia pseudoacacia</i> L.*	Fabaceae	Fabales	Northampton, MA
ROMU	Multiflora rose	<i>Rosa multiflora</i> Thunb.*	Rosaceae	Rosales	Greenfield, MA
TIAM	American basswood	<i>Tilia americana</i> L.	Tiliaceae	Malvales	Greenfield, MA
TORA2	Poison-ivy	<i>Toxicodendron radicans</i> (L.) Kuntze	Anacardiaceae	Sapindales	Easthampton MA
ULAM	American elm	<i>Ulmus americana</i> L.	Ulmaceae	Rosales	Greenfield, MA (from seed)
ULRU	Slippery elm	<i>Ulmus rubra</i> Muhl.	Ulmaceae	Rosales	Greenfield, MA (from seed)
VIRI	River grape	<i>Vitis riparia</i> Michx.	Vitaceae	Vitales	Greenfield, MA

* Non-native species in Northeastern North America

Of the seedlings that we collected, we chose the 64 healthiest seedlings of each species to use in the experiment and only used seedlings that were short enough to be fully submerged in the water-filled bins. The heights of seedlings ranged from less than 1 cm, for species such as *Betula lenta* and *Platanus occidentalis*, to 14 cm for species, such as *Acer negundo* and *Acer saccharinum*.

Additionally, conducting the experiment outside provides additional realism, as the seedlings are subject to the natural weather patterns that they would have experienced had we not uprooted them. While a greenhouse would have provided a more controlled environment in terms of temperature, precipitation, and light, keeping the seedlings outside throughout the experiment makes it more applicable to the field. Air temperature fluctuated diurnally between 15.5 and 27 degrees C, while water temperature fluctuated between 16 and 25 degrees C. There was no difference in water temperature between bins with clay and without clay.

It should be noted that species were selected to have relatively equal representation of all parts of the flood gradient in terms of species distribution. The design was not balanced with respect to other species differences, such as native status or growth form. Of the 25 species used, 16 are native. There were three liana and six shrub species used in the experiment, with the majority of species growing as trees.

STATISTICAL ANALYSES

I compiled all data in Microsoft Excel. To ensure all data was input correctly, I used Excel to count the number of species within each bin and the number of seedlings of each species on the datasheet, and compared the original paper data for individual seedling rack and bin number to the electronic data. I performed all statistical analyses with the statistical software package R version 3.2.2 (R Core Team, 2013). I used a 0.05 cut off level to test significance.

Treatment Effects

I conducted a two-way analysis of variance (ANOVA) (two-tailed; $\alpha=0.05$) using the ANOVA command in the R “stats” package to test for effects of flooding and suspended sediment treatments on seedling survival as well as the presence of an interaction between these factors. I used the formula ANOVA(formula=Mortality ~ Duration * Clay) to analyze differences between treatments. To run these analyses, I first calculated the mean mortality of all seedlings in their respective tray for all trays using the two-point survival scale, and used this mean tray mortality as my response variable. The overall N for this analysis was, therefore, 64, with 8 replicates in each duration by clay treatment combination.

Flood Tolerance

To quantify the effects of both treatment duration and presence or absence of clay on each individual bin, I used the calculated mean mortality of all seedlings in a given tray (includes 1 from each of the 25 species) as a biological indicator of the flood stress in that bin. Each bin, therefore, had its own quantified value for flood stress, calculated by the collective seedling response to all the stresses in an individual bin. I will refer to this bioindicator of flood stress in the trays as the flood stress index (FSI). The advantage of using this index is that the effects of flood duration and suspended sediment treatments on flood stress can be combined into a single quantitative measure. Moreover, actual flood stress varies within treatments because of differences in temperature among bin locations, algal growth in the water, visitation by frogs respiring in the water, etc. Unlike the nominal treatment, the FSI can capture this variation by shifting the focus towards stress as experienced by the seedlings collectively.

For each species, I ran a linear regression of seedling survival versus the flood stress index (so 64 total replicates of each species vs. the flood stress index of the bin each seedling was in). The formula used for each species' regression was $\text{lm}(\text{Survival} \sim \text{FSI})$. As flood stress increases, fewer seedlings survive. Species differences in seedling survival result in differences between the regression line slopes for each species. The slope of each regression line is therefore used as a measure of species' flood tolerance, with a slope closer to zero indicative of higher flood tolerance.

I used the slopes of these regression lines to compare the flood tolerance of different species. Using a general linear model I tested for differences in species survival with the formula $\text{lm}(\text{formula} = \text{Survival} \sim \text{FSI} * \text{Species})$. To visualize the data, I plotted a separate regression line for each species, and colored the lines differently based on slope value to illustrate differences in flood tolerance.

Distribution Data

To test the relationship between species distribution in the field and experimental flood tolerance, I used distribution data from Marks et al. (2014). The data was collected by establishing transects at various sites along the Connecticut river, and then recording the species of every living tree over 10 cm in diameter on those transects. The ground elevation at the base of each tree was measured, and used to calculate the amount of flooding experienced by that tree. The projected flood exposure was computed based on hydraulic models of flood regime and USGS stream gage flow data, recorded and calculated for each site (see Marks et al. 2014 for method details). In the 1-meter radius around the base of every tree, all woody species present were recorded, including seedlings, shrubs, and woody vines. The species included in the data were those with 50 or more occurrences in the field.

The distribution data was divided into data for the tree layer and the small woody species (shrub/seedling) layer. Neither of these data sets alone had all 25 of the species used in this experiment, though some species were represented in

both layers. Using both the tree and shrub/seedling layer data, all 25 species from this experiment were represented.

The data from Marks et al. (2014) quantified species distribution with respect to flooding by calculating the percentage of days per year that each individual in a species experienced flooding. The thresholds associated with species distribution limits were the percentage of days per year that 90th percentile individuals of a species experienced flooded conditions, or the 10% of individuals that experienced the greatest amount of flooding per year. For each species, I plotted the experimental flood tolerance (calculated from the slope of each species-specific regression line) against the percentage of days per year that 90th percentile individuals in that species were flooded in the field. To test to what degree these distributional limits are related to species ability to survive flooding as seedlings, I analyzed the relationship between experimental flood tolerance and distribution in the field with a linear regression using the formula $\ln(\text{formula} = X_{90} \sim \text{Flood Tolerance Slope})$. Both the tree and shrub/seedling layer data was plotted and analyzed in this way.

Seedling Depth and Height

I was interested in analyzing the effects of height both within a species and across different species. However, due to inconsistencies in soil depth between pots, we measured plant height from the same point on each pot. This gave us a consistent measure of depth across all bins. The measure of depth is highly correlated, but not identical to a height measurement. When creating figures

analyzing the effects of depth, I used the values measured for height from the lip of each pot.

To see if depth had an effect on health across all species, and to test for an interaction between flood stress and depth, I ran a general linear model with the formula $\text{glm}(\text{formula}=\text{Health} \sim \text{FSI} * \text{Depth})$ using the five-point health scale. To analyze species effects of depth, I ran a general linear model with the formula $\text{lm}(\text{formula}=\text{Survival} \sim \text{FSI} + \text{Depth} + \text{Species})$.

To analyze the effects of depth between species of different flood tolerance, I grouped species based on their experimental flood tolerance level levels. I plotted these values, and looked for an interaction between depth and flood tolerance with the formula $\text{lm}(\text{Depth} \sim \text{Duration} * \text{Flood Tolerance Level})$. I additionally ran a two-way ANOVA (two-tailed; $\alpha=0.05$) comparing the mean depths of different flood tolerance levels grouped by treatment duration to test for the effects of depth on seedling survival and interaction between these factors.

To visualize species differences in survival as a function of depth, I plotted the regression slopes of depth and survival for each species. Each species' regression line was calculated with the formula $\text{lm}(\text{Survival} * 100 \sim \text{Depth})$.

Native Status

Using all seedling data points, I ran a general linear model using the formula $\text{glm}(\text{formula}=\text{Health} \sim \text{FSI} * \text{Native Status})$ to analyze the effect of flood stress and native status on seedling health, and to test for the presence of an interaction between flood stress and native status. This analysis used seedling data from the

five-point health scale as a function of flood stress. The health of native and non-native species was compared to test for the effects of native status independent of flood stress.

Growth Form

Each species was categorized as a tree, shrub, or liana. With the five-point health scale, I ran a general linear model using all seedlings to test the effects of growth form on seedling health as a function of flood stress index with the formula $\text{glm}(\text{formula}=\text{Health}\sim\text{FSI}*\text{Form})$. I also tested for the presence of an interaction between growth form and flood stress. I then ran a multiple comparisons of means Tukey contrast to compare the mean health between all growth forms.

Phylogenetic Relationships

Using the website PhyloT, I constructed a phylogenetic tree of the 25 species used in this experiment based on nucleotide sequencing retrieved from the NCBI taxonomy database (Letunic 2015). I then edited the tree, mapping on the calculated flood tolerance of each species using the Interactive Tree of Life website (Letunic and Bork 2007). This was done to see if flood tolerant species were clustered on a few branches, or if they were dispersed throughout the tree.

RESULTS

Flood Duration and Suspended Sediment Treatment Effects

The different treatments had significant effects on seedling survival. Flood duration significantly affected seedling mortality in all treatments, with longer flood duration resulting in decreased seedling survival ($F = 498.075$; $df = 3, 56$; $p < 2e-16$, Table 5). Figure 3 demonstrates the negative relationship between the percent of seedling survival in each tray and the duration of treatment. The six week treatment affected the seedlings most, with 39.59% survival in the treatment without clay, and 35.2% percent survival in the treatment with clay added as a suspension to the flood water (Table 6).

Table 5: Coefficients from ANOVA of seedling mortality as a function of flood treatments. Data compares mortality between all trays.

ANOVA(formula=Mortality ~ Duration*Clay)

Factor	Df	Sum Sq	Mean Sq	F value	Pr(>F)	Signif.
Duration	3	31770	10590	252.127	<2e-16	***
Clay	1	354	354	8.422	0.00529	**
Duration X Clay	3	119	40	0.947	0.42442	
Residuals	56	2352	42			

Significance codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

The presence of clay also had a significant impact on seedling survival, with the addition of clay resulting in significantly increased mortality overall (Fig. 3),

but there was no significant interaction between clay and flooding duration (Table 5).

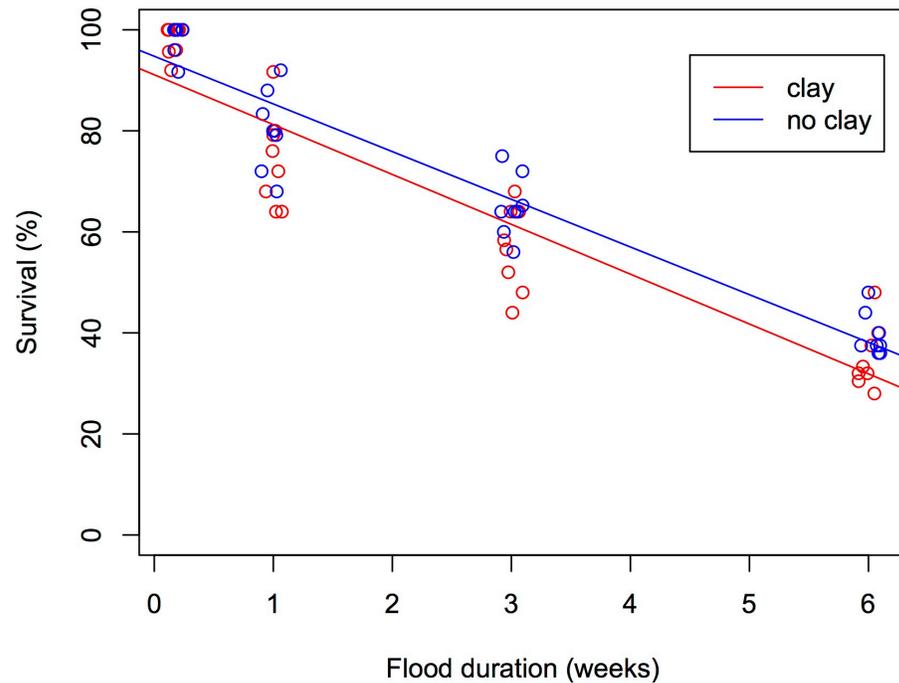


Figure 3. The relationship between seedling survival and treatment. Each point represents the mean percent survival of all the seedlings in a given tray. The slopes were generated from two linear models, for treatments with clear water and treatments with clay, with survival as a function of duration. Note that the points were jittered on the x-axis to show overlapping points.

$\text{lm}(\text{formula}=\text{Survival} \sim \text{Duration})$

There was much variation in survival among replicates within the same treatments (Table 6, Fig. 3). The most variation was within the one and three week treatments, more so when clay was present (Table 6).

Table 6. Mean percent survival of seedlings in each treatment and standard deviation of survival in each treatment.

		1 Day	1 Week	3 Weeks	6 Weeks
No Clay	Survival	98.4848	80.3030	64.9746	39.5939
	SD	3.0805	7.8104	6.0714	4.2964
Clay	Survival	97.9695	74.2424	57.0707	35.2040
	SD	3.0588	9.3945	8.4303	6.4439

Flood Stress Index Effects

With the slopes of the regression lines of seedling survival and flood stress index within each bin, each species has an experimentally determined measure of flood tolerance, given in Table 7. This measure of flood tolerance divided species into three main groups, of high, medium, and low flood tolerance (Fig. 4).

The most flood tolerant species were all native floodplain trees, such as *A. saccharinum*, which had the highest tolerance (Table 7). Some invasive non-native shrubs showed substantial flood tolerance, such as *E. umbellata*, which had the highest flood tolerance of the non-native species. The species with the highest and lowest flood tolerances were both from the *Acer* genus, with *A. platanoides* as the least flood tolerant species in the experiment.

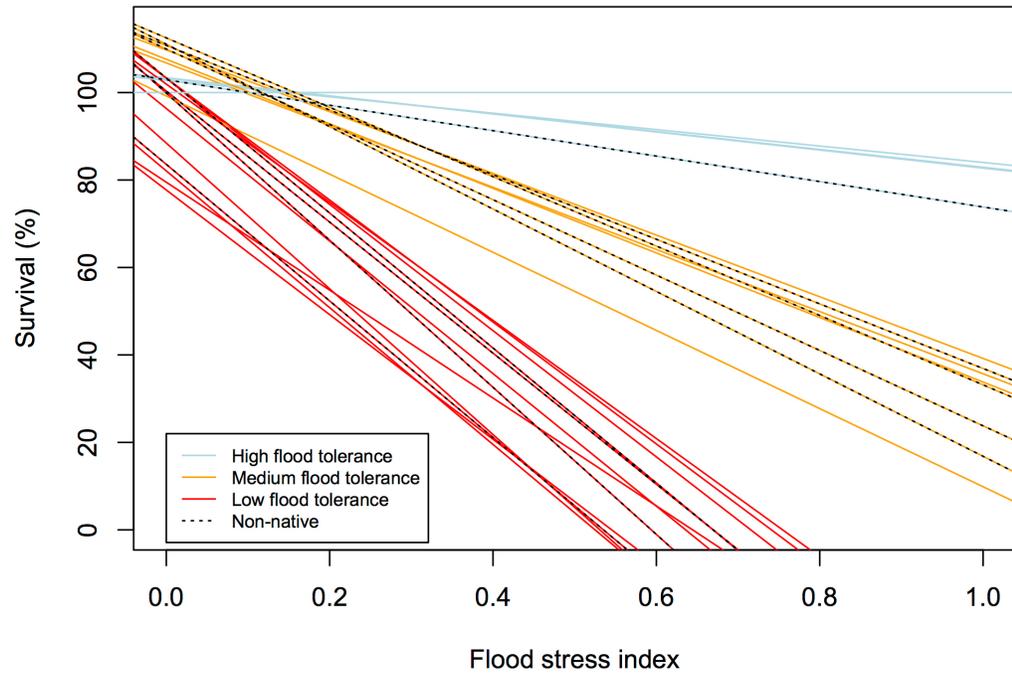


Figure 4. Regression lines showing seedling survival as a function of flood stress index for all species used in the experiment. Each line represents a different species. Species flood tolerance was calculated based on the slope of each species' regression lines, and colored according to flood tolerance. Shallower, less negative slopes indicate higher flood tolerance. High flood tolerance species range between a slope of 0 and -30. Medium flood tolerance species range between -70 and -95. Low flood tolerance species range between -123 and -169.

Table 7. Flood tolerance of each species calculated through the slope of the regression comparing individual species' survival and the calculated flood stress index in each bin. Steeper, more negative slopes indicate lower flood stress tolerance. Colors of each species reflect the lines in Figure 4, with black dots indicating invasive species. Species are arranged by their taxonomic orders to compare species' relatedness to their experimental flood tolerance.

Species Code	Common Name	Scientific Name	Order	Slope	Color
CEOR7	Oriental bittersweet	<i>Celastrus orbiculatus</i> (Thunb.) Siebold*	Celastraceae	-149.8472	Red with black dots
EUAL13	Burning bush	<i>Euonymus alatus</i> Thun*	Celastraceae	-154.3454	Red with black dots
ROPS	Black locust	<i>Robinia pseudoacacia</i> L.*	Fabales	-156.1541	Red with black dots
BELE	Black birch	<i>Betula lenta</i> L.	Fagales	-123.373	Red
CACA18	Musclewood	<i>Carpinus caroliniana</i> Walter	Fagales	-139.4539	Red
QURU	Red oak	<i>Quercus rubra</i> L.	Fagales	-135.1034	Red
CASP8	Northern catalpa	<i>Catalpa speciosa</i> (Warder) Warder ex Engelm.*	Lamiales	-94.2288	Red with black dots
PODE3	Eastern Cottonwood	<i>Populus deltoides</i> Bertram ex Marsh.	Malpighiales	-18.7822	Blue
TIAM	American basswood	<i>Tilia americana</i> L.	Malvales	-144.3500	Red
PIST	White pine	<i>Pinus strobus</i> L.	Pinales	-142.5214	Red
PLOC	American Sycamore	<i>Platanus occidentalis</i> L.	Proteales	-20.6610	Blue
BETH	Japanese barberry	<i>Berberis thunbergii</i> DC.*	Ranunculales	-86.1705	Red with black dots
ELUM	Autumn olive	<i>Elaeagnus umbellata</i> Thunb.*	Rosales	-29.0855	Red with black dots
FRAL4	Glossy buckthorn	<i>Frangula alnus</i> Mill.*	Rosales	-79.3390	Red with black dots
PRSE2	Black cherry	<i>Prunus serotina</i> Ehrh.	Rosales	-166.6113	Red
PRVI	Choke cherry	<i>Prunus virginiana</i> L.	Rosales	-156.3918	Red
ROMU	Multiflora rose	<i>Rosa multiflora</i> Thunb.*	Rosales	-73.8763	Red with black dots
ULAM	American elm	<i>Ulmus americana</i> L.	Rosales	-73.8407	Red
ULRU	Slippery elm	<i>Ulmus rubra</i> Muhl.	Rosales	-151.5377	Red
ACNE2	Boxelder	<i>Acer negundo</i> L.	Sapindales	-20.3908	Blue
ACPL	Norway maple	<i>Acer platanoides</i> L. *	Sapindales	-168.1749	Red with black dots
ACRU	Red Maple	<i>Acer rubrum</i> L.	Sapindales	-70.522	Red
ACSA2	Silver maple	<i>Acer saccharinum</i> L.	Sapindales	0	Blue
TORA2	Poison-ivy	<i>Toxicodendron radicans</i> (L.) Kuntze	Sapindales	-89.3722	Red
VIRI	River grape	<i>Vitis riparia</i> Michx.	Vitales	-71.0758	Red

*Non-native species in Northeastern North America

The experimentally determined flood tolerances were plotted against the quantified species distribution found naturally in flooding areas. The

resulting plot shows species with low, medium, and high flood tolerances clustered together respectively (Fig. 5). Flood tolerance predicts the distribution of species on flooding gradients, with the experimental flood tolerance explaining half of within-species variation in the amount of flooding experienced by the most flood exposed trees (90th percentile) in a given species.

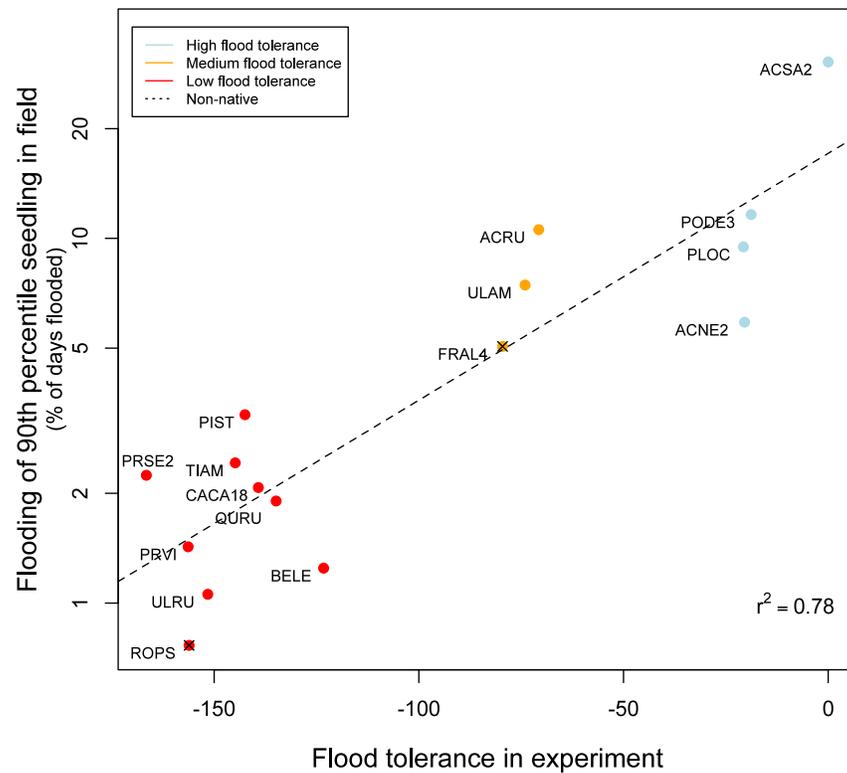


Figure 5A. Tree layer distribution.

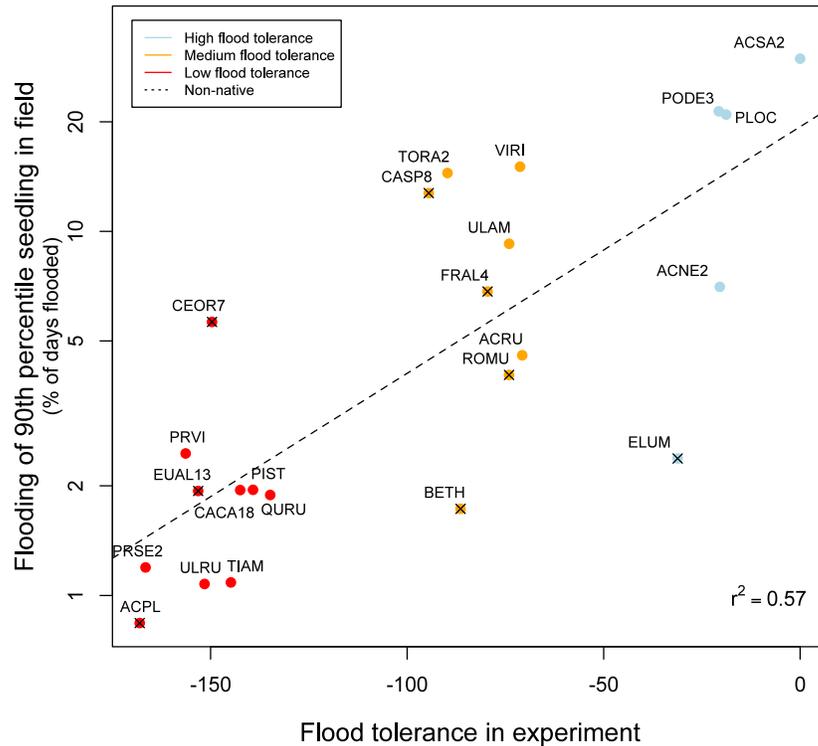


Figure 5B. Shrub layer distribution.

Figure 5. The relationship between species experimental flood tolerance and distribution in the field as trees (5A) and in the shrub/seedling layer (5B). The adjusted r^2 value for the linear regressions are 0.78 and 0.57, respectively, represented by the dashed lines. Both regressions were highly significant ($p=4.10e-06$ and $p=1.68e-05$, respectively). Species flood tolerance was estimated by slope of the regression of seedling survival, given in Table 6. Species distribution limits were quantified by the amount of flooding that 90th percentile individuals in that species experience in nature (data from Marks et al. 2014). Refer to Table 6 for species codes and colors.

Seedling Depth and Height Effects

Differences in seedling depth beneath the water surface appeared to affect seedling survival. Between different plant species there was significant variation in seedling height. Depending on the species, some seedlings were beneath more or less water than others due to their height differences. To see if depth had an

effect on seedling health, I ran a general linear model with seedling health as a function of Flood Stress Index (FSI) and depth. Seedling depth has a significant effect on health (Table 8). More deeply submerged seedlings were less healthy than taller seedlings whose tops were closer to the surface of the water.

Table 8. Coefficients from general linear model of seedling health as a function of FSI and seedling depth beneath water surface.

lm(formula= Health ~ FSI * Depth)

	Df	Sum Sq	Mean Sq	F value	Pr(>F)	Signif.
FSI	1	1787.06	1787.06	1126.3514	<2.2e-16	***
Depth	1	118.78	118.78	74.8638	<2.2e-16	***
FSI:Depth	1	11.26	11.26	7.0943	0.0078	**
Residuals	1575	2498.89	1.59			

Significance codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1'

Figure 6 shows the differences in height between high, medium, and low flood tolerant species, depicted by plant heights measured from the same point on each pot. The highly flood tolerant species are the tallest, and are therefore closest to the surface of the water, while the less tolerant species were beneath significantly more water (Table 9).

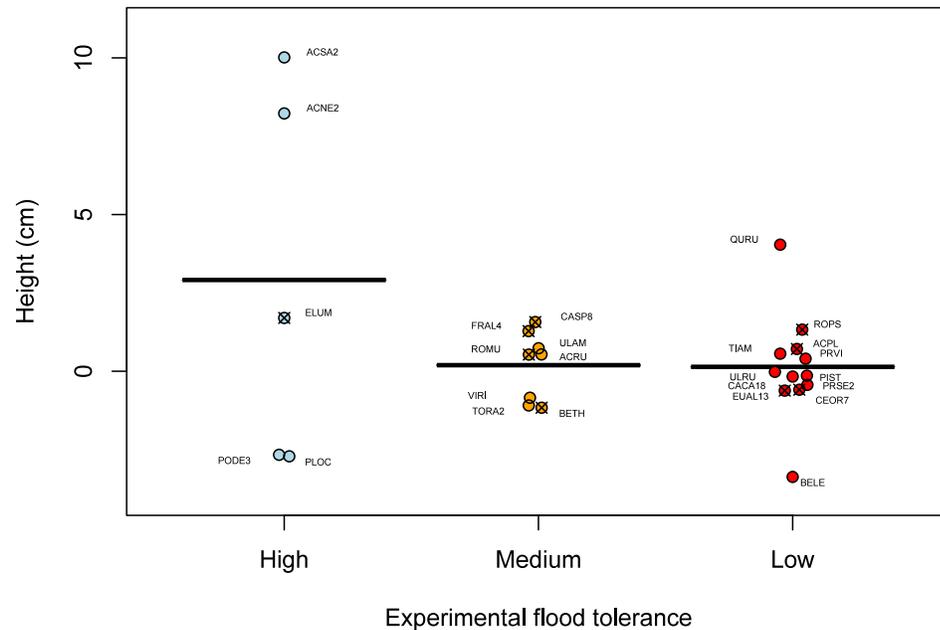


Figure 6. Height of species grouped by experimental flood tolerance. The mean height of each group is represented by solid black lines. Species are colored by level of flood tolerance, given in Figure 4 and Table 7. Note that the points were jittered on the x-axis to show overlapping points.

There is a significant, positive interaction between flood stress and height, which suggests that the negative effect of increasing flood stress on seedling health is smaller in taller seedlings whose tops are closer to the water surface (Fig. 8, Table 8). There is a significant difference in height between the highly flood tolerant group and the medium and low flood tolerance groups, though there is more variation in the high flood tolerance group (Fig. 7, Table 9).

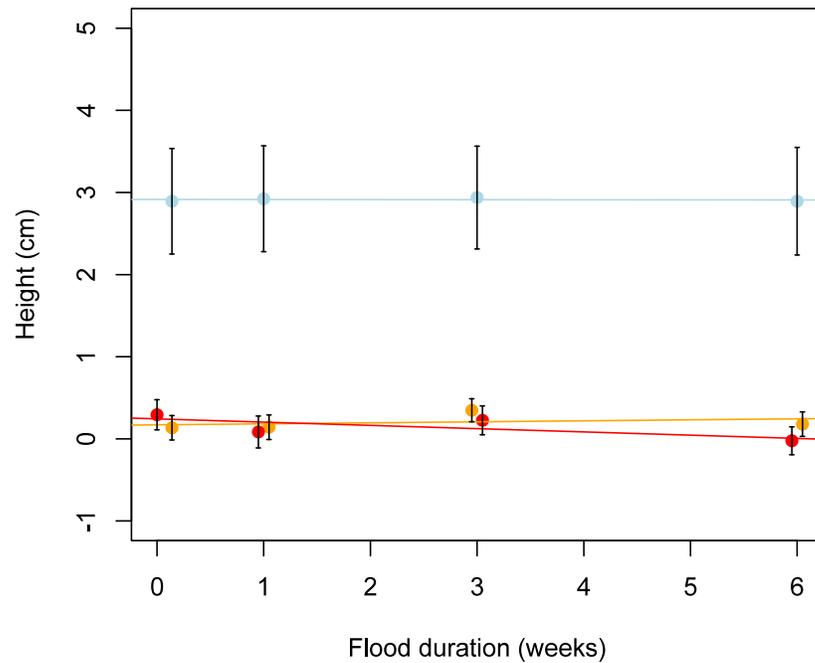


Figure 7. The mean height of species, grouped by flood tolerance level, for each treatment duration. Points are colored by level of flood tolerance, given in Figure 4 and Table 7. Standard error bars for each group are included. Note that the points were jittered on the x-axis to show overlapping points.

$\text{lm}(\text{Height} \sim \text{Duration} * \text{Flood Tolerance Level})$

Table 9. ANOVA table comparing the mean heights of different flood tolerance levels, grouped by treatment duration.

$\text{ANOVA}(\text{Height} \sim \text{Duration} * \text{Flood Tolerance Level})$

	Df	Sum Sq	Mean Sq	F value	Pr(>F)	Signif.
Duration	1	0.006	0.006	0.603	0.467	
Flood Tolerance Level	2	20.025	10.012	1064.133	2.2e-8	***
Duration X Flood Tolerance Level	2	0.030	0.015	1.611	0.275	
Residuals	6	0.056	0.009			

Significance codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Taking into account the effect of species difference on survival, height was still highly significant (Table 10). This means that within a particular species,

seedlings that were shorter in height, or beneath more water, were less likely to survive than taller seedlings.

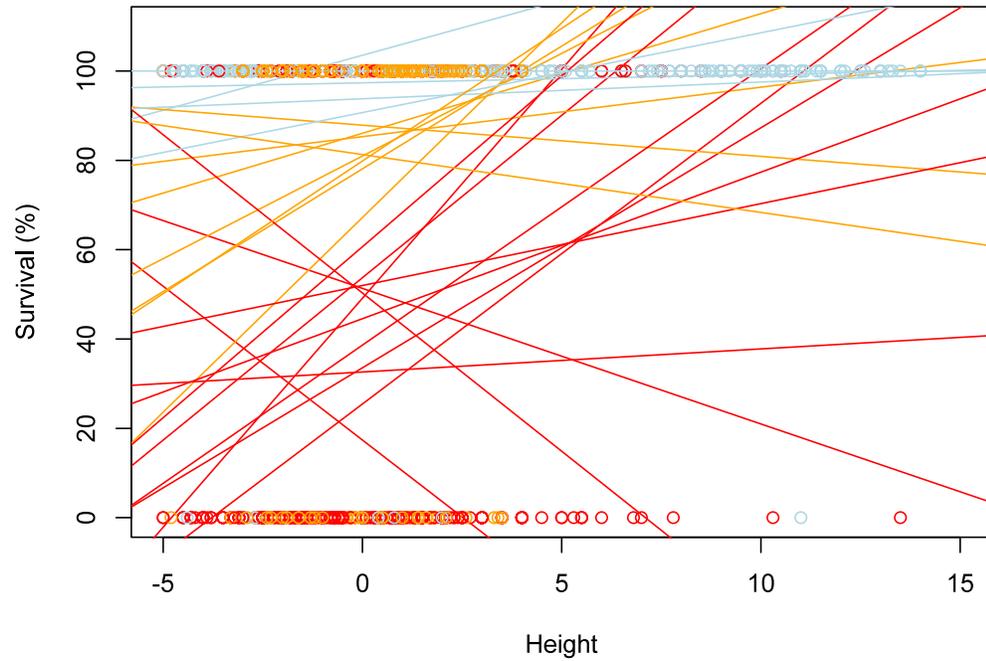


Figure 8. Regression slopes of height and survival for each species. Lines are colored by species' flood tolerance level, given in Figure 4 and Table 7. Each point represents an individual seedling. Each species' regression line was calculated by $\text{lm}(\text{Survival} * 100 \sim \text{Height})$.

Table 10. Coefficients from general linear model of seedling survival as a function of FSI, depth of submergence, and species. Note that species effects are compared to *A. saccharinum*, the most flood tolerant species in the experiment.

lm(formula=Survival ~ FSI + Depth + Species)

	Estimate	Std. Error	t value	Pr(> t)	Sig.
(Intercept)	1.4926	0.0641	23.2859	4.13E-103	***
FSI	-0.0100	0.0004	-27.7111	1.18E-137	***
Depth	0.0179	0.0048	3.7274	0.0002	***
SpeciesACNE2	0.0007	0.0592	0.0115	0.9908	
SpeciesACPL	-0.3648	0.0737	-4.9518	8.16E-07	***
SpeciesACRU	0.0445	0.0742	0.6005	0.5482	
SpeciesBELE	-0.3542	0.0870	-4.0728	4.88E-05	***
SpeciesBETH	0.0281	0.0795	0.3539	0.7234	
SpeciesCACAI8	-0.2240	0.0763	-2.9357	0.0034	**
SpeciesCASP8	-0.0351	0.0714	-0.4920	0.6228	
SpeciesCEOR7	-0.2790	0.0776	-3.5942	0.0003	***
SpeciesELUM	0.0862	0.0709	1.2163	0.2241	
SpeciesEUAL13	-0.2629	0.0777	-3.3832	0.0007	***
SpeciesFRAL4	0.0312	0.0720	0.4334	0.6648	
SpeciesPIST	-0.4902	0.0762	-6.4325	1.67E-10	***
SpeciesPLOC	0.1965	0.0847	2.3209	0.0204	*
SpeciesPODE3	0.1956	0.0845	2.3153	0.0207	*
SpeciesPRSE2	-0.4536	0.0771	-5.8807	4.99E-09	***
SpeciesPRVI	-0.4999	0.0746	-6.7022	2.86E-11	***
SpeciesQURU	-0.2994	0.0652	-4.5884	4.83E-06	***
SpeciesROMU	0.0446	0.0742	0.6014	0.5477	
SpeciesROPS	-0.5008	0.0719	-6.9632	4.89E-12	***
SpeciesTIAM	-0.2527	0.0741	-3.4096	0.0007	***
SpeciesTORA2	-0.0918	0.0798	-1.1515	0.2497	
SpeciesULAM	0.0098	0.0736	0.1328	0.8944	
SpeciesULRU	-0.3333	0.0798	-4.1743	3.15E-05	***
SpeciesVIRI	0.0380	0.0784	0.4846	0.6280	

Significance codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Effect of Native Status on Flood Tolerance

Native status did not have a statistically significant effect on seedling health independent of flooding at the 0.05 cut off level (Table 11). However, there was an overall trend of higher flood tolerance and seedling health in native seedlings (Fig. 9, Fig. 10). Native species were significantly healthier than invasive species

with increasing flood stress (Table 11, Fig. 9). There is a significant interaction between flood stress index and native status such that native species are more healthy than non-natives with increasing flood stress (Fig. 9)

Table 11. Coefficients from general linear model of seedling health as a function of flood stress index and seedling native or non-native status. Note that status effects are compared to non-native species.

lm(formula=Health ~ FSI * Native Status)

	Df	Sum Sq	Mean Sq	F value	Pr(>F)	Sig.
FSI	1	1787.06	1787.06	1080.1467	<2.2e-16	***
Native Species	1	5.05	5.05	3.0502	0.0809	.
FSI: Native Species	1	18.09	18.09	10.9369	0.0009639	***
Residuals	15754	2605.78	1.65			

Significance codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1'

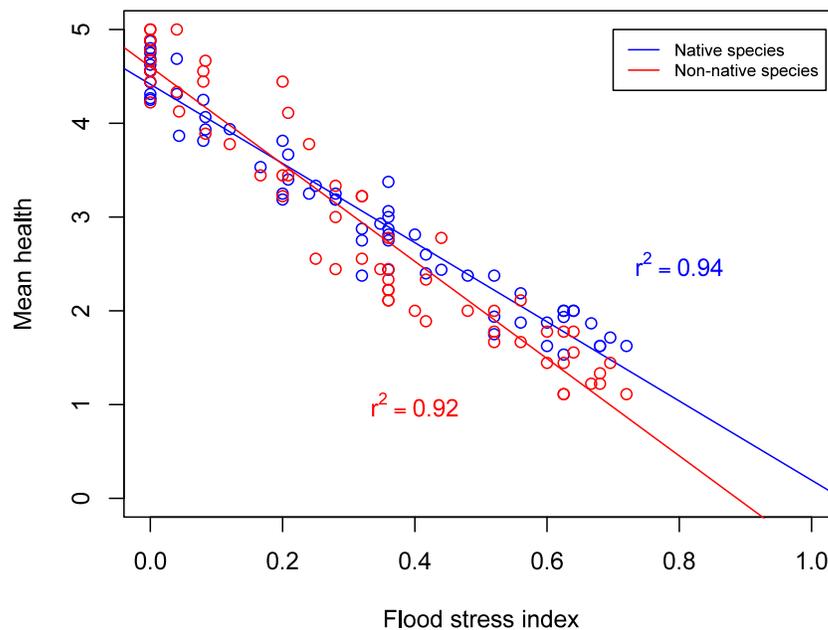


Figure 9. A plot displaying the interaction between flood stress and seedling health. For each of the 64 bins, the mean health of all native species and all non-native species in a given bin is plotted.

In Figure 10, the health of native species is higher within each level of flood tolerance. The effect of native status is significant independent of flood tolerance level (Table 12).

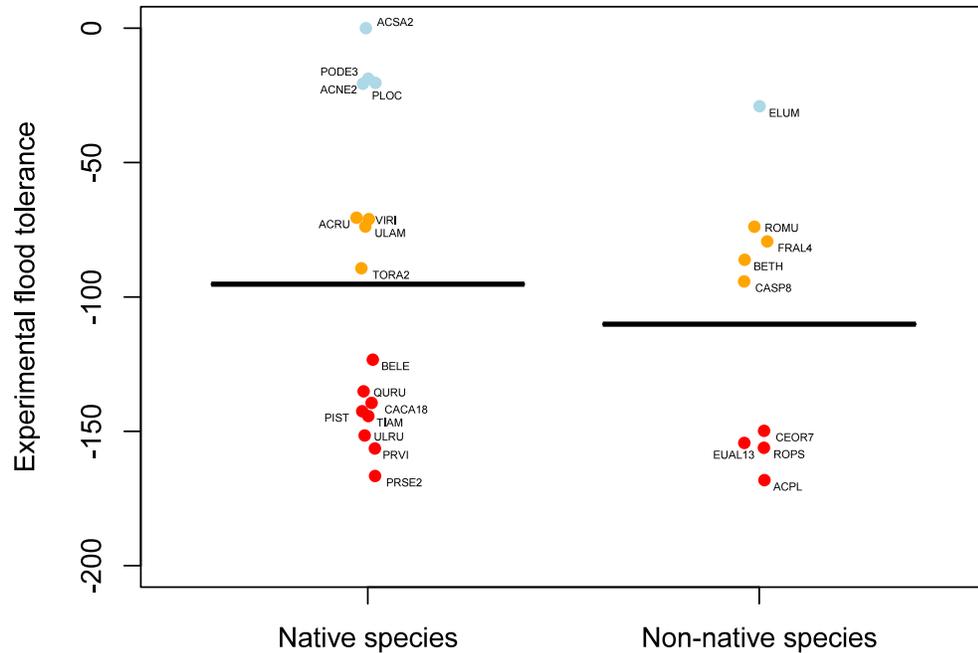


Figure 10. Experimental flood tolerance divided by native and non-native species. The solid black lines represent the mean experimental flood tolerance of each group. Note that the points were jittered on the x-axis to show overlapping points.

Table 12. ANOVA table comparing experimental flood tolerance between native and non-native species for the different levels of flood tolerance.

ANOVA(Flood Tolerance ~ Native Status * Flood Tolerance Level)

	Df	Sum Sq	Mean Sq	F value	Pr(>F)	Sig.
Native Status	1	1276	1276	11.042	0.00358	**
Flood Tolerance Level	2	65195	32597	281.996	7.49e-15	***
Native Status X Flood Tolerance	2	40	20	0.173	0.84252	
Residuals	19	2196	116			

Significance codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Effect of Growth Form on Flood Tolerance

Flood stress index had a significant negative effect on seedling health independent of growth form (Table 13). There was a significant difference in health between shrub and liana species (Table 13), but between shrub and tree species and between liana and tree species the p value was not significant (Table 13, Table 14).

Table 13. Coefficients from general linear model of seedling health as a function of flood stress index and seedling growth form. Note, form effects are compared to trees, the most common plant form used in the experiment.

lm(formula = Health ~ FSI * Form)

	Df	Sum Sq	Mean Sq	F value	Pr(>F)	Sig.
Mortality	1	1787	1787.06	1077.2716	<2e-16	***
Form	2	10.16	5.08	3.0619	0.04708	*
Mortality x Form	2	9.35	4.67	2.8169	0.06009	.
Residuals	1573	2609.42	1.66			

Significance codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1'

Table 14. Coefficients from Tukey contrasts comparing the mean health between each form.

	Estimate	Std. Error	z value	Pr(> z)	Signif.
liana - tree == 0	-0.1988	0.1712	-1.161	0.4699	
shrub - tree == 0	0.2856	0.1302	2.194	0.0696	.
shrub - liana == 0	0.4844	0.1921	2.522	0.0304	*

Significance codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1'

Phylogenetic Relationships with Flood Tolerance

I created a phylogenetic tree of the 25 species, and colored each species based on whether it had high, medium, or low flood tolerance (Fig. 4, Table 7). Highly flood tolerant species were spread throughout the tree, suggesting that flood tolerance evolved repeatedly. The species in the Fagales order, *B. lenta*, *C.*

caroliniana, *Q. rubra* all have similar flood tolerance (Table 7). Overall, however, differences in flood tolerance are spread throughout orders, with groups of species that greatly vary in flood tolerance, like the Aceraceae family, with both the most and least flood tolerant species in the experiment (Table 7, Fig. 11).

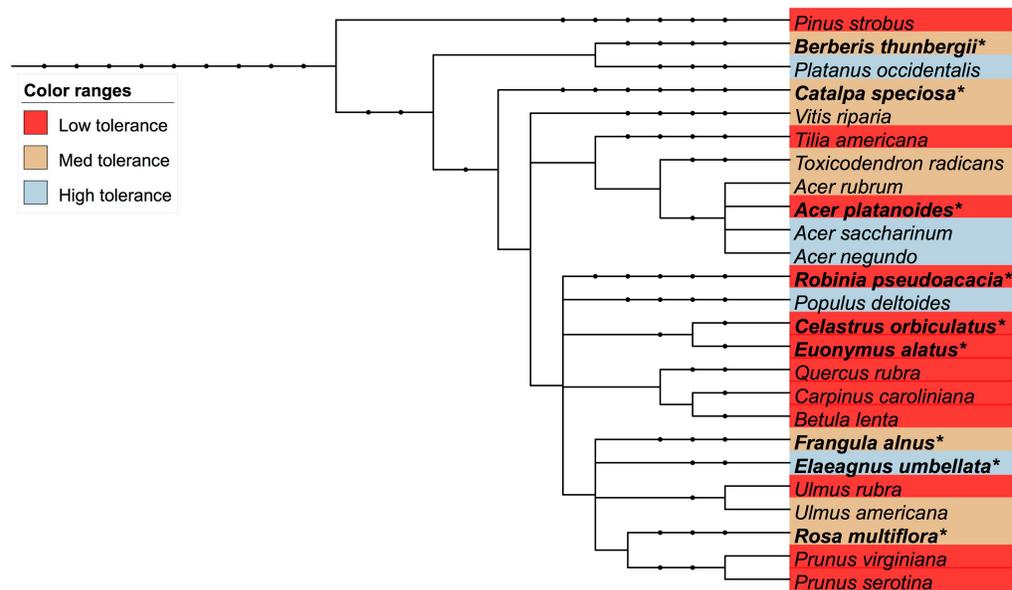


Figure 11. Phylogenetic tree showing relationships between species. Species are colored based on the results shown in Figure 3 and Table 7. Nodes represent various levels of taxonomic classification (in a way, the number of the individual segments indirectly indicates the evolutionary distance between taxa.) Species in bold font are non-native. Edited with itol.embl.de (Letunic and Bork 2007). Created with phylot.biobyte.de, which creates trees based on the NCBI taxonomy database (Letunic 2015).

Table 15. Non-native species sorted by date of introduction to New England. Species origin, flood tolerance, and native habitat are given (Alien Plant Working Group 2010, Danoff-Berg 2003, USDA and NRCS 2016).

Species	Origin	Flood Tolerance	Date Introduced	Natural Habitat
<i>Robinia pseudoacacia</i>	Southeastern US, lower slopes of Appalachian Mountains	-156.1541	N/A	Appalachian Mountains, Ozark Mountains. Deep, well-drained soil.
<i>Catalpa speciosa</i>	Midwestern US	-94.2288	N/A	Moist, deep, well-drained soil, but adapts to wet or dry soils
<i>Fragula alnus</i>	Eurasia	-79.3390	Prior to 1900's	Marshes, bog, shorelines, ditches, fields, disturbed areas
<i>Berberis thunbergii</i>	Japan	-86.1705	1875	Mountains
<i>Rosa multiflora</i>	Japan, Korea, Eastern China	-73.8763	1866	Wide range of conditions
<i>Euonymus alatus</i>	Northeastern Asia, Japan, Central China	-154.3454	1860	Well-drained soils, open or disturbed areas, forests
<i>Celastrus orbiculatus</i>	Eastern Asia, Korea, China, Japan	-149.8472	1860	Lowland slopes, or thickets at higher altitudes
<i>Elaeagnus umbellata</i>	East Asia	-29.0855	1830	Riverbanks, streams, roadsides, disturbed areas, grasslands, fields
<i>Acer platanoides</i>	Europe and Western Asia	-168.1749	1756	Fields, mature forests, disrupted habitats

DISCUSSION

Flood duration and the presence of sediment significantly impacted plant survival. In treatments with longer flood duration and with sediment present, higher flood stress levels were generated, leading to greater seedling mortality. Species flood tolerance clustered into three main groups, of high, medium, and low flood tolerance, and predicted natural species distribution in a floodplain. There is a trend of higher flood tolerance and seedling health in native seedlings. Based on the phylogenetic tree, flood tolerance appears to have evolved independently at the individual species level, rather than at a higher taxonomic level.

Duration and Clay

With longer flooding duration, there was greater seedling mortality. When clay was added to treatments, seedling mortality also was greater, though clay suspension had less of an effect than increasing flood duration (Table 5, Fig. 3).

This implies that in nature, flood duration is more influential than suspended sediments on restricting flood intolerant species from colonizing lower floodplain areas. The clay treatment had a consistent effect on plant survival, regardless of duration (Fig. 3). However, we cannot say that suspended sediments are not important, as the nutrients these sediments bring into floodplains play a role in the survival of floodplain plants (Kozlowski 2002, Gurnell et al. 2012). The

sediments deposited along river banks in floods additionally create specific habitats with qualities that some flood-adapted seeds require to germinate, including consistent moisture and low competition (Kozlowski 2002).

The decrease in survival with prolonged submergence is attributed to a number of factors. As a result of flooding, seedlings have less access to oxygen, carbon dioxide, and light, especially in treatments that include a clay suspension (Jackson and Colmer 2005). Additionally, longer durations of flooding may permanently damage stomata, particularly in plants that are not flood-adapted (Peterson and Bazzaz 1984). Stomata damage leads to water loss and a decline in photosynthesis, both of which reduce plant survival, and could help explain why plant mortality increases with longer flood duration (Peterson and Bazzaz 1984). Additionally, changes take place when soil is inundated for longer periods of time. Aerobic bacteria is replaced by anaerobic bacteria as soil pores containing oxygen are filled with water, and oxygen is depleted within that water (Kozlowski 2002). These micro-organisms can change soil through the products that accumulate due to their anaerobic metabolism, which in turn impacts plant growth and development (Jackson and Colmer 2005).

As there is a broad range of species used in this experiment, differences in flood tolerance can be at least partially attributed to a number of different potential adaptations. Flood-tolerant species appear to close stomata quickly in response to flooding, and recover regular stomatal functioning quickly once flooding subsides (Peterson and Bazzaz 1984, Kozlowski 2002). Other potential

physiological adaptations to flooding include internal aeration to avoid oxygen deficiency (Jackson and Armstrong 1999), anoxia tolerance (Gibbs and Greenway 2003), and the capacity to prevent or repair damages caused by anoxia during re-aeration (Blokhina et al. 2003).

Though sediment had less of an impact on plant survival than duration, the presence of sediment leads to increased plant mortality. The effect of the clay was fairly consistent, even with increasing duration (Fig. 3). In the experiment we used a standard amount of sediment for all treated bins, but how might differing amounts of sediment impact plant survival? Specifically, is there a maximum amount of sediment where, beyond that amount, plant survival would be unaffected? Additionally, how would different species be impacted by varying amounts of sediment?

Flood Tolerance and Species Distribution

Species flood tolerance, calculated by plotting seedling responses to the flood stress index within each treatment bin, generated three main clusters of high, medium, and low flood tolerance species (Fig. 4). Based on the research done by Marks et al. (2014), I expected that species naturally found in areas with a higher percentage of days flooded per year would also have a higher experimental flood tolerance. Generally this was true, and species found in areas with infrequent floods had lower flood tolerance, such as dominant upland species *Prunus serotina*, *Tilia americana*, and *Ulmus rubra* (Marks et al. 2014). In contrast,

dominant floodplain species *Acer saccharinum* and *Populus deltoides* were among the most flood tolerant species.

The calculated flood tolerance reflected where species were naturally found on flood gradients. The ability of seedlings to survive complete submergence can help explain variation in distributions of tree and shrub species with respect to flooding in the field, and be used to predict where seedlings will most likely survive (Fig. 5). Higher variation in the seedling/shrub layer is to be expected, as it includes younger seedlings that will not necessarily survive to maturity. Species in the tree layer have successfully survived long enough to reach a substantial size, suggesting these individuals are in adequate locations to suit their needs.

While the experimental flood tolerance of dominant upland and floodplain species were consistent with our predictions, other species tolerated flooding much more readily than was predicted based on their distributions. *Elaeagnus umbellata* was the most striking example of this, with 90% of *E. umbellata* dominated locations experiencing flooding less than 2.4% of the year but a high experimental flood tolerance (Marks et al. 2014).

One reason for the discrepancy between experimental flood tolerance and natural distribution in *E. umbellata* could be the dispersal techniques employed by the species, which uses animal and water dispersal, as well vegetative reproduction (Kohri 2008). Differences in dispersal mechanisms used by plant species may help explain some of the variation between experimental flood tolerance and shrub/seedling distribution, as the species used in the experiment

have a variety of different techniques. *P. occidentalis*, *P. deltoides*, and *A. platanoides* all use wind dispersal, while many of the others species used, like *R. multiflora* and *P. virginiana*, produce fruits that animals and birds ingest and disperse (USDA and NRCS 2016). With these different methods, some species are able to disperse their seeds farther than others. As a result, there some species will have more variation in where their seedlings are located, which would impact their distribution along the flood gradient.

Though this experiment imitates flooding in terms of the presence of water surrounding a plant and additional sediment, it does not account for aspects of natural flooding such as strong currents, which would test the durability of a plant's root system. Some species may be able to survive in still water, but along a river bank they may not be adapted to surviving intense currents and erosion that occur due to flooding, even as mature plant. The effects of still water compared to flowing water on seedling survival could help explain some of the variability in the shrub layer distribution (Fig. 5B). *Berberis thunbergii* and *E. umbellata* are examples of species with relatively high experimental flood tolerance, considering their distribution in the field.

The results of this experiment demonstrate the importance of flooding as a physiological filter. The presence of flooding creates distinct communities of species, and can help explain the distribution of organisms found along flood gradients (Fig. 5). Different plant species evolved different adaptations to best survive and reproduce within particular habitats, giving them advantages over

other species (Lambers et al. 2008). Floodplain species have evolved to tolerate flooding through adaptations such as enhanced shoot growth in response to ethylene, the formation of aerenchyma, and the prevention of phytotoxin build-up in hypoxic conditions (Kozłowski 2002, Visser et al. 2003, Jackson and Colmer 2005, Seago et al. 2005, Lambers et al. 2008). In other species, elevated concentrations of ethylene elicited by the anaerobic flooded environment leads to different responses, such as leaf abscission (which we observed in many non-native species) and tissue hypertrophy, both of which can impede plant survival and recovery after flooding subsides (Vriezen et al. 2003). Floodplain species have a variety of adaptations that can help explain why they were able to better survive conditions of higher flood stress (Fig. 4).

Native and Invasive Species

Native floodplain trees had the highest flood tolerance of all species, though native status did not have a statistically significant effect on seedling health independent of flooding (Table 11, Table 12). However native species had higher flood tolerance overall and were more healthy with increasing flood stress (Fig. 9, Fig. 10, Table 11). Looking at species grouped by low, medium, and high flood tolerance, we can see that within each level, native species are more resistant to flood stress than non-native species (Fig. 10).

The differences in how non-native, native upland, and native floodplain species respond to flooding have implications for how species diversity and community is impacted by changes to natural flood patterns. Apart from *E.*

umbellata, all of the high flood tolerance species were native floodplain trees. Species with seedlings that are adapted to survive the stress that comes with weeks of complete submergence have an advantage in succession within these areas (Kozlowski 2002). When floods are available to eradicate the potential competition floodplain seedlings face from upland and non-native species, these stress-adapted seedlings are able to colonize newly disturbed areas.

Floods provide an environmental stress that can influence the invasibility of an area, as many non-native and upland species are unable to withstand the effects of prolonged submergence (Alpert et al. 2000). Many upland and non-native seedlings are good competitors, but cannot survive in conditions of prolonged flood stress (Casanova and Brock 2000). For example, *Acer platanoides* seedlings grow quickly to develop huge leaves that shade out smaller plants growing around them, but when faced with increasing levels of flood stress, they drop their leaves and rapidly die (Danoff-Burg 2003, Table 7). The occurrence of invasive species in locations with regular yearly flooding, such as *Celastrus orbiculatus*, *Fragula alnus*, and *Rosa multiflora*, may seem to suggest tolerance to flood stress, yet their abundances also decline with increased flooding (Marks et al. 2014). All of the non-native species introduced from outside north America were brought from different continents for ornamental reasons, and many were chosen to be cultivated due to their hardiness, which is why many can survive in a variety of different conditions (Alien Plant Working Group 2010, Danoff-Berg 2003, USDA and NRCS 2016).

Not all native species are flood tolerant, and not all non-native species are intolerant to flooding. Looking at non-native species requires taking into account the effect of historical filters on plant development, since species that evolve in different regions can have similar adaptations (Lambers et al. 2008). Even if a species has not developed within a particular area, with the appropriate adaptations in its native range it may still be able to survive in that area, and may even survive better than native species for a variety of reasons. Flood-adapted non-native species like *E. umbellata* have advantages over native floodplain species due to lack of predators, dispersal techniques utilizing both animals and flood cycles, and nitrogen-fixing roots (APWG 2010, Kohri 2008). The speed with which this species has spread along riparian areas in North America as well as its own native habitat has implications for how it could continue to spread over time to threaten biodiversity and impact river flows if it continues to grow unmanaged (Church et al. 2004, Kohri 2008).

When considering where a species will successfully survive, there are many factors to consider. Historical filters determine the geographical location a species is dispersed within. The historical filters on a species seem to have a less important role than the physiological adaptations developed based on the environmental conditions a species is exposed to (Lambers et al. 2008). This is exemplified when considering the wide range of flood tolerance for the native species within this experiment, all of which developed in North America, and the

reasons non-native species such as *E. umbellata* can survive so successfully outside their native range.

Depth and Height

Depth had a significant effect on seedling health. Shorter seedlings submerged deeper under the surface were less healthy than taller seedlings (Table 10).

There was significant variation in height between species, with some seedlings beneath more or less water than others (Table 11). For individual seedlings within a species, shorter seedlings were less likely to survive (Table 11).

Though seedlings submerged beneath more water were less healthy, it is difficult to attribute this relationship to depth alone, as greater initial plant heights could correspond to more initially vigorous individuals. Because we did not measure seedlings until after each treatment, we don't know how height changed over the course of flooding, and our measurements more accurately depict seedling depth. Yet in Figure 7 the low tolerance group shows the greatest decrease in height, while the better adapted species stay the same or increase in height throughout the experiment. This suggests that the least flood-adapted species are especially vulnerable to oxygen deficiency caused by complete submergence in water, which leads to reduced structural integrity due to decreased turgor pressure (Kozlowski 1997). A decrease in turgor results in wilting, which ultimately can cause a decrease in height (Lambers et al. 2008).

Taller species may survive flooded environments better because they are able to receive more light and gases diffusing through the water surface, as they are

beneath less water. Diffusion create a gradient in the amount of dissolved oxygen, which decreases with greater depths, and could help account for differences in survival between shorter and taller seedlings (Fig. 8).

Increased seedling height is one adaptation floodplain species use to survive flooding. When faced with total submergence, the decreased gas exchange and light intensity promote the biosynthesis of ethylene in plants (Vriezen et al. 2003). Floodplain species can utilize this ethylene to promote accelerated shoot elongation, which can help them grow above the water line to access the ingredients necessary for photosynthesis (Visser et al. 2003, Vriezen et al. 2003, Jackson and Colmer 2005). The species within the high flood tolerance group have the greatest mean height (Fig. 6, Fig. 7).

Typically, the biosynthesis of ethylene has negative effects on plants, including leaf abscission and tissue hypertrophy (Kozlowski 2002). Differences in response to ethylene synthesis can help explain some of the results of this experiment, particularly why the highly flood tolerant species were able to maintain their height or grow, while the least tolerant species decreased in height over a longer flood duration (Fig. 7). Additionally, while the most flood-adapted species, such as *A. saccharinum*, *P. deltoides*, and *Platanus occidentalis* retained their leaves and remained green over the experiment, we observed the abscission and browning of leaves from many of the non-native and upland species, particularly *A. platanoides*, *Acer negundo*, *Robinia pseudoaccacia*, and *R.*

multiflora. These observations support the theory that differences in response to ethylene synthesis play a role in how plants survive flooding conditions.

The experiment measured plant depth beneath the water surface, but plant height is highly correlated with depth. Due to this high correlation, we cannot say for certain that the effects on plant health and survival is caused by either height or depth alone. Further experimentation, such as flooding plants under varying water depths, would be required to disentangle these factors.

Phylogenetic Tree, Evolutionary Implications

Though I predicted that more related species would have similar responses to the same stresses, this did not appear to be the case. The way flood tolerance mapped onto a phylogenetic tree of the 25 species used in this experiment suggests that the development of flood tolerance arises independently within individual lineages. While the species from the order Fagales all have similar flood tolerance, the majority of plant species in this experiment do not have similar responses to flooding within their families. For example, the *Acer* genus has both the most and least flood tolerant species (Table 7, Fig. 11).

The phylogenetic tree additionally suggests that flood tolerance develops within a much larger timeframe than the time since non-natives were introduced to the area. The dates when invasive species were introduced to the United States has no relation to how flood-tolerant they are (Table 15). The least flood-tolerant invasive, *A. platanoides*, was introduced in 1756, the earliest of all the non-native species used in this experiment (APWG 2010). This implies that resistance to

flood stress depends primarily on whether a species' ancestral habitat had flooding.

Differences in flood tolerance are spread throughout orders, and groups of species within a same family greatly vary in flood tolerance.

Management Implications

Flooding is an important physiological filter on plant growth that creates the distinct communities of species found along flood gradients. The low experimental flood tolerance of upland species compared with the incredible flood tolerance of native floodplain species demonstrates how great of an impact flooding can have on species composition, and implications for areas where natural floods are restricted. Floodplain species rely on seasonal floods to reduce competition from upland and non-native species, as well as for dispersal and nutrients, and unfortunately declines in the abundance of floodplain species is a real consequence of restricting water flow through dams (Kozlowski 2002, Kohri 2008). For a plant to survive long enough to grow and reproduce on the Connecticut River flood gradient, it must have appropriate physiological traits to withstand periods of flooding, unless it is located on higher ground. Showing how species variation in flood tolerance can account for species distribution along a flood gradient demonstrates the significance of flooding as a physiological filter on plant life. These results have implications for how the prevention of natural flooding will continue to influence species composition in the future.

While the experiment imitates flooding in terms of the presence of water surrounding a plant and additional sediment, it does not account for other aspects of natural flooding such as strong currents, which would test the durability of a plant's root system. That being said, stagnant water can be more detrimental to plants than moving water, since it contains less diffused gasses critical to plant survival (Lambers 2008). One issue created by the presence of a dam is the build up of water which would be otherwise be flowing through the area, and flooding river banks as water level rise. The soil inundation resulting from water build-up behind dams creates an environment that even the most flood-adapted plant species have difficulty surviving in for longer periods of time, due to inhibition of branch and root growth, and root decay (Kozlowski 1997). Permitting water to flow more freely through dams would help to maintain natural species composition by preventing the build-up of stagnant water and allowing some degree of flooding to occur.

This experiment shows that even short durations of flooding, so long as the water is deep enough to completely submerge seedlings, has a great impact on which seedlings will continue to survive in an area. There is a natural shift from upland forest species to floodplain species with increased flooding, suggesting that many native floodplain species require flooding, and that stimulating natural flooding regimes may help to exclude non-native species. Non-native species in these habitats do not currently show signs of strong adaptations to flood plain

habitats, but this lack of evolutionary response may reflect current reduced flood patterns.

Based on this experiment, allowing natural spring freshets to flow, rather than holding back the water behind dams, would be greatly beneficial to the flora of floodplain ecosystems. Prescriptive flood durations, ideally between three to six weeks, would prevent upland and non-native species from invading dammed areas that can no longer flood as frequently.

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