

**Assessing the Allelopathic Effect of Invasive
Phragmites australis on *Sida hermaphrodita* and
Ammannia robusta; Two Species at Risk in Southern
Ontario**

by
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Abstract

In Ontario, invasive *Phragmites australis* threatens to displace many species including the endangered species *Sida hermaphrodita* and *Ammannia robusta*. Germination and growth assays measured the effect of *P. australis* aqueous extracts from the leaves, rhizomes, and roots on *S. hermaphrodita* and *A. robusta*. Germination was inhibited by some of the treatments, but growth was not. The tissues inhibited germination differently for *S. hermaphrodita* (leaf > rhizome > root) compared to *A. robusta* (root > rhizome > leaf) indicating that the allelopathic effect was species-specific. However, the laboratory results show that allelopathic effects are weak. This result is consistent to the field study results showing an increase in *S. hermaphrodita* area and density over time. Results from this project inform management options by indicating which part of the plant needs to be targeted. In this case, all the tissues had some phytotoxic effects, indicating that biomass may need to be removed or long-term management implemented.

Keywords: Allelopathy; Invasive Species; Species at Risk; Seed Germination; Seedling Growth

This work is dedicated to

My wonderful parents without whom I would not be the person I am today

My sister for her advice, support and friendship

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List of Acronyms

ERH	Enemy Release Hypothesis
NWH	Novel Weapons Hypothesis
SARA	Species at Risk Act

Chapter 1. Introduction

Invasive species are defined as a species that establish in new regions where their growth, spread, and persistence negatively impacts the environment (Mack et al., 2000). They can cause major losses in biodiversity, ecosystem structure, and function (Holmes et al., 2009). Invasive plant species have several characteristics that make them strong competitors in their new environment (Murrell et al., 2011). They can produce many seeds, have high germination rates, rapid seedling growth, and a strong ability to reproduce vegetatively. Seeds are often small and light weight making them easily transported to distant areas (Wang et al., 2011). Invasive species can also spread through the propagation of plant fragments and growth from rhizomes and stolons increasing their ability to spread locally (Gawronska & Golisz, 2006). One or a combination of these characteristics can be attributed to the success of invasive species (Wang et al., 2011).

There are several hypotheses that have been proposed to explain the success of invasive plant species. The most prominent and influential is the enemy release hypothesis (ERH) proposed by Keane and Crawley (2002). The ERH states that when a plant species is introduced to a new area there is a decrease in regulation by natural enemies such as herbivores and other competing plants (Keane & Crawley, 2002). In an invasive species' native range, it competes with other plants while also being eaten by specialist herbivores. The competitor species will also be eaten by specialist herbivores and both plants will be eaten by generalist herbivores (Keane & Crawley, 2002). Based on this, the ERH predicts that specialist herbivores for the introduced species will be absent from the new region, specialist herbivores from the new region switching to the introduced species will be rare and generalists will have a greater impact on native competitors compared to the introduced species. Thus, releasing the species from its natural enemies causes its distribution and abundance to increase rapidly in the introduced range.

There is evidence that the ERH is an explanation for the success of several plant invasions (Callaway & Ridenour, 2004; Kumar & Bais, 2010). Although, it should not be accepted as a universal reason invasive species are successful. There are several studies showing that some natural enemies have weak effects on invaders and

the consumer effects or damage from enemies are the same between its introduced and natural ranges (Callaway & Ridenour, 2004). This led to another hypothesis to explain the success of other invasive species; the novel weapons hypothesis (NWH) discussed by Callaway and Ridenour (2004). The NWH states that “some [introduced species] transform from native weaklings to invasive bullies by biosynthesizing biochemicals [that negatively impacts the] plants or soil microbes in the invaded communities, but relatively ineffective against natural neighbors that had adapted over time” (Callaway & Ridenour, 2004). There are various reasons plants produce biochemicals, including soil nutrient acquisition, defense against herbivory, antimicrobial protection, and some that have no particular function that are just metabolic by-products. In their native range a species has neighbours that coevolve to tolerate these biochemicals that are released into the environment. However, an introduced species in a new area proliferates where it coexists with other species that do not have a tolerance (Callaway & Ridenour, 2004). This brings in the concept that the invasion process of invasive species is aided by chemical means.

Allelopathy is the negative impact of a plant upon neighbouring plants and/or their associated microflora through the release of biochemicals into the environment (Chou, 2006; International Allelopathy Society, 2015; Wang et al., 2011). These chemicals are also called secondary metabolites, allelochemicals, natural products or phytogrowth-inhibitors (Lotina-Hennsen et al., 2006). Allelochemicals play an important role in plant dominance, succession, formation and regulation of plant communities, and maintenance of climax vegetation (Chou, 2006; Muller, 1969). Allelochemicals enter the environment through several pathways. They can be released by the leaching of living material and plant litter, the release of root exudates, volatilization through stomata, decomposition of plant material, and microbial activity (Chou, 2006; Kumar & Bais, 2010). Allelochemicals can be intraspecific or interspecific affecting the growth and development of neighbouring plants. They are a diverse class of compounds that can occur in any tissue, vary between species, and have different action mechanisms (Kumar & Bais, 2010; Lotina-Hennsen et al., 2006). Allelochemicals can be direct in their action by inhibiting the seed germination and plant growth of the target plant. There are a number of physiological pathways that are inhibited by allelochemicals that cause this response. These may include the inhibition of photosynthesis, respiration, cell division, nutrient and water uptake, enzyme activity and production, and molecular processes

such as protein and nucleic acid synthesis (Chou, 2006; Lotina-Hennsen et al., 2006; Seigler, 2006). Allelochemicals can also be indirect in their action by modifying the soil matrix and the availability of nutrients for plant growth. These can include inhibiting the growth of bacteria and fungi, altering pH, and reducing the amount of free ions in the soil available for plants (Seigler, 2006).

Phragmites australis ssp. *australis* (European Common Reed) is a tall perennial wetland plant from the family Poaceae (Mal & Narine, 2011) (Figure 1). *P. australis* culms can grow between 3-6 m in height from an extensive stolon and rhizome system (Lambert et al., 2010). The blue-green leaves are lanceolate shaped, 20-70 cm long, and taper to a long slender point (Mal & Narine, 2011). The inflorescence of *P. australis* is a 30 cm long terminal panicle that is dull purple to yellow with main branches bearing numerous spikelets. The fruit of *P. australis* is a caryopsis that is less than 2 mm long. Within an area, *P. australis* uses its rhizomes and stolons to expand horizontally (Invasive Species Centre, 2016). The spread between areas is a result of its ability to reproduce vegetatively through plant fragments that contain a node and the production of many small seeds. Seeds can be dispersed by the wind up to 10 km and plant fragments can be transported by vehicles and heavy equipment (Invasive Species Centre, 2016).



Figure 1. Dead *Phragmites australis* culms (C) forming a dense stand in a wetland near Leamington, Ontario during late spring. Inflorescences (I) can be seen at the apex of each culm.

P. australis was introduced to eastern North America from Eurasia in the 1800s (Invasive Species Centre, 2016). Since then it has become invasive and spread to all 10 provinces in Canada and 48 states in the United States (Figure 2) (Invasive Species Centre, 2016; Mal & Narine, 2011). In Ontario, *P. australis* is abundant across the southern part of the province and throughout the Great Lakes region. It is predicted that *P. australis* will be abundant across much of southern Canada within the next 20 years (Catling & Mitrow, 2011). *P. australis* aggressively invades wetlands, stream banks, lake shores, wet fields, ditches and roadsides, and can even survive in brackish water (Invasive Species Centre, 2016). With its rapid spread and growth, *P. australis* quickly outcompetes native plants for nutrients and water creating dense monocultures that decrease biodiversity and alter habitat for wildlife (Ontario Ministry of Natural Resources and Forestry, 2011). *P. australis* flourishes in recently disturbed habitats and is usually the first to colonize these areas (Mal & Narine, 2011). It prefers habitat with standing water but its rhizomes and roots can reach up to 1 m allowing it to survive in areas with low water. It also has the ability to drastically lower water levels as a result of its density and high rates of transpiration. In general, *P. australis* limits habitat for many plants and wildlife, including a number of species at risk.

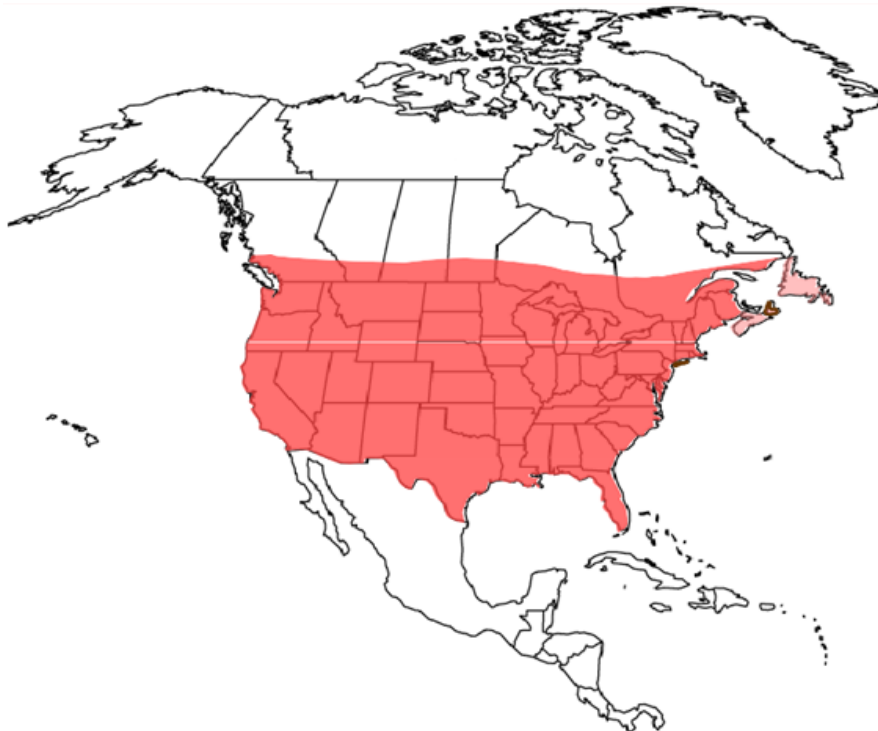


Figure 2. Approximate species distribution map for *Phragmites australis* in the United States and Canada. Saltonstall & Meyerson (2016).

Pre-2007, *P. australis* was thought to be allelopathic but no studies had tested this nor had there been an allelochemical isolated (Rudrappa et al., 2007). A study by Rudrappa et al. (2007) demonstrated that root exudates from *P. australis* caused a reduction of growth in *Arabidopsis thaliana*, *Nicotiana tabacum*, and *Lactuca sativa* *in vitro* and *A. thaliana* *in vivo*. They tested the root exudates and found that the active ingredient was 3,4,5-trihydroxybenzoic acid (gallic acid). Subsequent assays using gallic acid showed an inhibition in *A. thaliana* seedling growth demonstrating that it likely was the allelochemical responsible (Rudrappa et al., 2007). Other studies testing root exudates showed similar results in species such as *Melaleuca ericifolia*, a native species in *P. australis* invaded wetlands in Australia (Uddin et al., 2014c).

Another mechanism by which allelochemicals enter the environment is through the leaching of phytochemicals from litter (Chou, 2006; Kumar & Bais, 2010). Since *P. australis* stands produce an abundance of litter this could be occurring. Uddin et al. (2012) made aqueous extracts from fresh and dry leaves, rhizomes, roots, and stems of *P. australis* and applied them to seed germination and seedling growth assays. They demonstrated that leaf and rhizome (leaf > rhizome > root > stem) inhibited seed germination and seedling growth the most among model species (*L. lactuca* and *Raphanus sativus*) and associated species (*Juncus pallidus* and *Rumex conglomeratus*) (Uddin et al., 2012). It was also shown that dry tissues used to make the extracts inhibited germination the most. In a follow-up study the amount of gallic acid in each extract was determined. The results exhibited that the concentration of gallic acid in each tissue can be ranked as follows: leaf > inflorescence > rhizome > root > stem (Uddin et al., 2014a). This ranking is the same as the order of extracts that inhibited seed germination and seedling growth in the previous studies. It is important to also note that at least one of the test species in each study was not affected indicating that the allelopathic effect of *P. australis* is species-specific (Rudrappa et al., 2007; Uddin et al., 2012, 2014a, 2014c). It was also found that gallic acid was able to persist in the rhizosphere for an extended period of time giving *P. australis* a prolonged allelopathic effect (Uddin et al., 2012, 2014a).

Sida hermaphrodita (Virginia Mallow) is a tall perennial herbaceous plant in the Malvaceae family. *S. hermaphrodita* shoots can vary from 1-4.5 m in height (Figure 3) (Kasprzyk et al., 2013). *S. hermaphrodita* is a clonal species that forms stands from one or a few individuals that grow vegetatively (Spooner et al., 1985). Leaves are green

with 3-7 lanceolate lobes and irregularly serrate edges (Gleason & Cronquist, 1963). The inflorescences are corymbs bearing small white flowers with 5 petals about 8 mm long. Its native range is throughout the central Appalachian Mountains in the Northeast United States and Southern Ontario in Canada (Figure 4) (Spooner et al., 1985). The loss of floodplains and riparian areas have caused *S. hermaphrodita* to become rare with populations spread thinly throughout its range (Environment Canada, 2015). It is considered extremely rare in Canada because there are only 2 known populations located in the Niagara region of Southern Ontario. One of these populations is found in a conservation area that was restored into floodplain habitat and is the study site presented in this paper. *S. hermaphrodita* is listed as endangered under Schedule 1 of the Species at Risk Act (SARA) in Canada and is

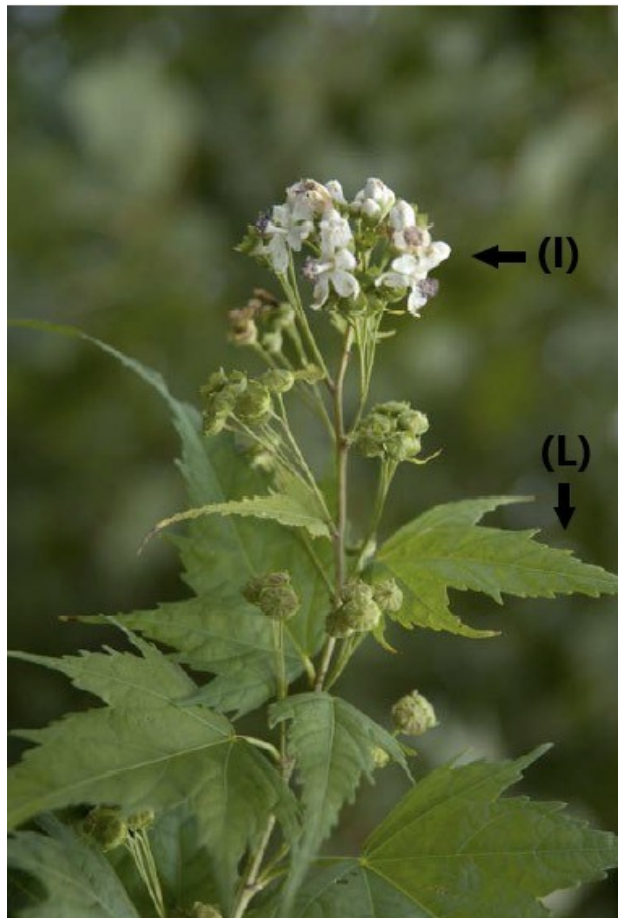


Figure 3. *Sida hermaphrodita* shoot apex showing the leaves (L), corymb inflorescence (I), and white flowers. Modified from COSEWIC (2010).

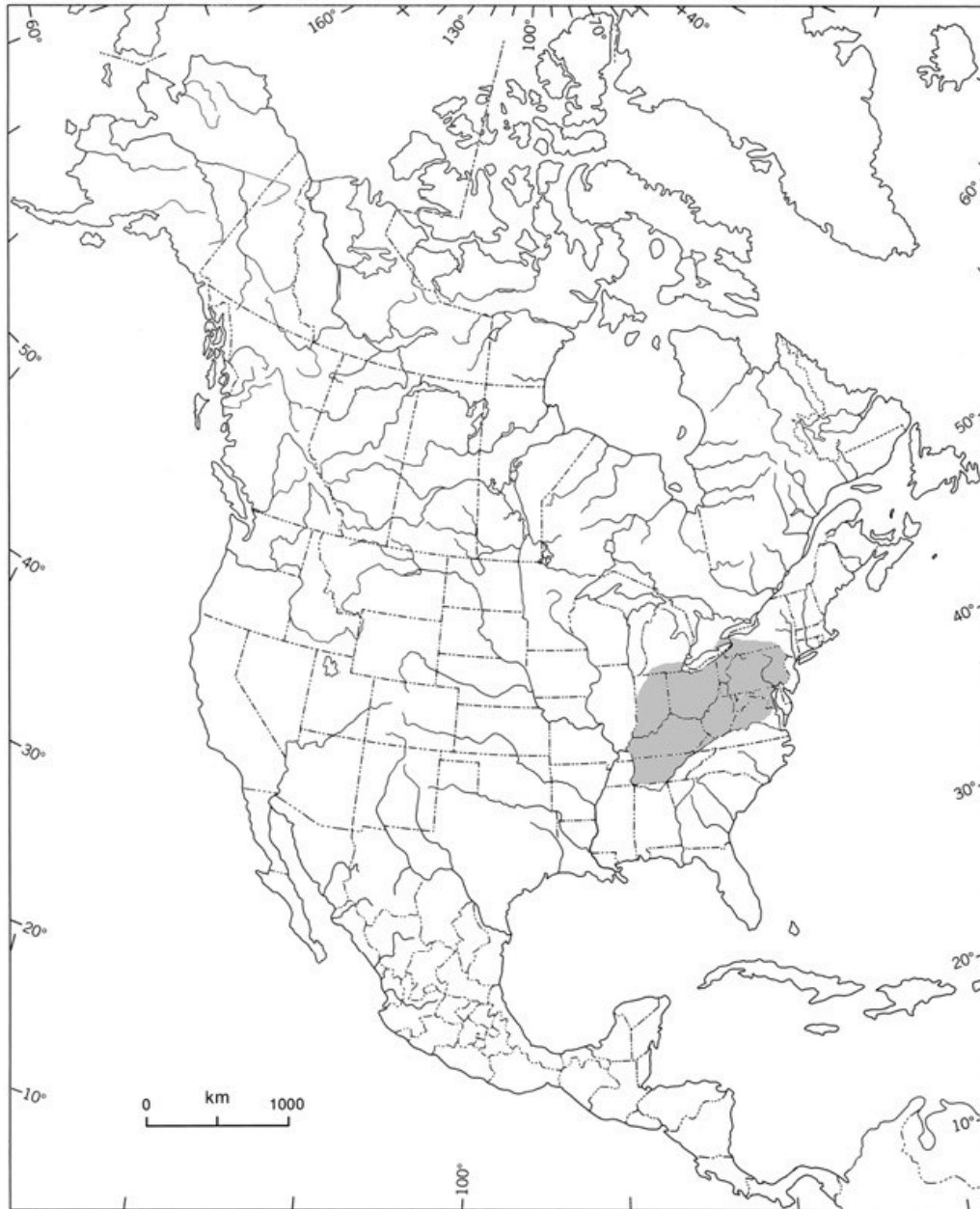


Figure 4. Approximate species distribution map for *Sida hermaphrodita* in North America with populations mostly in the United States and two in Southern Ontario. COSEWIC (2010).

considered vulnerable in the United States (Environment Canada, 2015). *S. hermaphrodita* occurs in many disturbed habitats and is thought to require periodic flooding. At the study site the population expanded after the dewatering of a reservoir. Subsequent plant colonization also expanded a population of *P. australis* onto the site. Now, *S. hermaphrodita* is thought to be threatened by the invasion of *P. australis*.

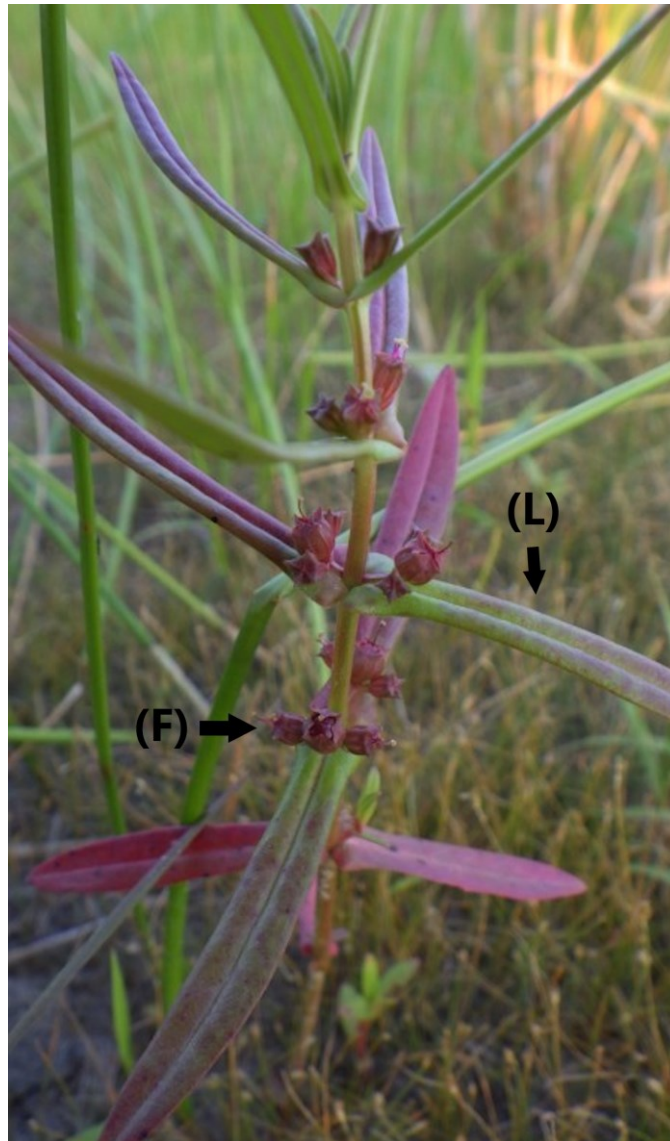


Figure 5. *Ammannia robusta* shoot showing the oblong-lanceolate leaves (L) and fruits (F) arranged in groups of 3-5 at the leaf axil. Modified from Reznicek et al. (2011).

Ammannia robusta (Scarlett Ammannia) is an annual herbaceous plant from the family Lythraceae that stands less than a metre tall (Douglas, 1999). *A. robusta* has 2-8 cm long oblong-lanceolate leaves which are clasping at the base (Figure 5). The flowers are arranged in groups of 3-5 at the leaf axils with petals that are rose-purple and 3-5 mm long. The fruits are globose in shape, 4-valved, and approximately 3-5 mm long (Douglas, 1999). In Canada, it occupies habitat in southwestern Ontario and south-central British Columbia (Ontario Ministry of Natural Resources and Forestry, 2017) (Figure 6). Historically there were 10 populations of *A. robusta* in Canada but now there are 6. It is listed as endangered under Schedule 1 of the SARA and is threatened by

habitat loss, changes in flood regimes, and invasive species (Ontario Ministry of Natural Resources and Forestry, 2017). In Canada, *A. robusta* is found in open, alkaline, sandy, or muddy shorelines, and semi-aquatic habitats where water levels are ephemeral (Ontario Ministry of Natural Resources and Forestry, 2017). This seasonal variation in water levels has been identified as critical habitat for the plant.

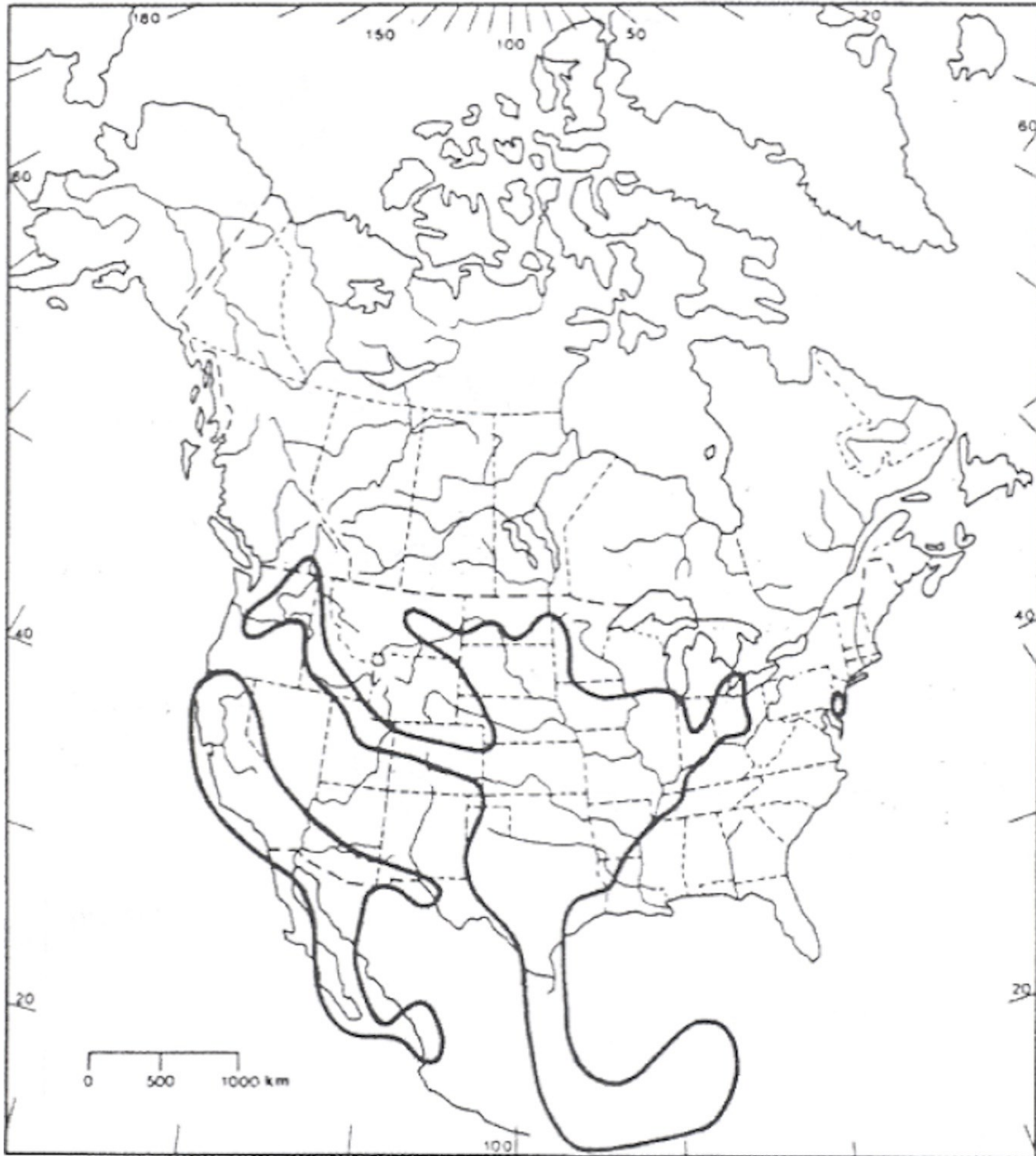


Figure 6. Approximate species distribution map for *Ammannia robusta* in North American with populations mostly in the United States and Mexico but also several in Southern British Columbia and Ontario. Argus et al. (1982).

Several studies have clearly shown the allelopathic capability of *P. australis* especially through root exudates and leaf and rhizome extracts, however there are some studies that contradict these findings. A study by Weidenhamer et al. (2013) found that exudation of gallic acid was present in amounts much smaller than previous studies and that its half-life in the soil was less than one day. They concluded that *P. australis* exudates could not be a primary explanation for its invasive success (Weidenhamer et al., 2013). Since allelopathy is species-specific, determining the effects on multiple species will provide more evidence that it is indeed allelopathic. Previously, the allelopathic effects of *P. australis* have not been tested on *S. hermaphrodita* and *A. robusta* in the field or laboratory. Understanding how invasive species are able to invade so aggressively is important in determining how to manage them and prevent the displacement of other species (Uddin et al., 2017). The main questions asked in this study were: 1. Do extracts made from *P. australis* inhibit seed germination of *S. hermaphrodita* and *A. robusta* in growth chamber studies? 2. Do extracts made from *P. australis* inhibit seedling growth of *S. hermaphrodita* and *A. robusta* in growth chamber studies? 3. Are *S. hermaphrodita* and *P. australis* numbers at the study site changing over time? 4. Is there a change in *S. hermaphrodita* population area and stand boundaries over time?

To address these questions I followed the methods of Uddin et al. (2012) and made several extracts from *P. australis* leaf, rhizome and root tissues at mass to volume concentrations of 10%, 5%, 2.5% and 1.25%, and deionized water as a control. These were used in seed germination and seedling growth assays for *S. hermaphrodita* and *A. robusta*. It is expected based on previous studies that there will be an inhibition of seed germination and seedling growth for both species in large part by the leaf and rhizome extracts. To quantify the effect of *P. australis* on *S. hermaphrodita* in the field, stem count data for *S. hermaphrodita* and *P. australis* were collected from 28 permanent vegetation plots over 3 years and *S. hermaphrodita* stands were mapped to monitor their changes over time. If *P. australis* is shown to have an effect on the seed germination and seedling growth of *S. hermaphrodita* it is expected that the area of *S. hermaphrodita* will decrease, the number of *S. hermaphrodita* stems will decrease and the number of *P. australis* stems will increase. The effect of *P. australis* on *A. robusta* in the field was not determined because it would be difficult to track the changes in the *A. robusta* population over time since it is an annual and the location of its stands varies from year to year.

Chapter 2. Assessing the allelopathic effects of *Phragmites australis* extracts on the seed germination and seedling growth of *Sida hermaphrodita* and *Ammannia robusta*.

2.1. Introduction

Invasive species are defined as a species whose growth, spread, and establishment in a new area negatively impacts the environment (Mack et al., 2000). Invasive species cause major losses in biodiversity, ecosystem structure, and function (Holmes et al., 2009). There are several characteristics and mechanisms that give invasive species a competitive advantage in their new environment. They have strong reproductive capabilities such as the ability to reproduce over a large area using small seeds and vegetative propagules (Wang et al., 2011). They also have high rates of seed germination, rapid seedling growth, and the ability to spread locally through rhizomes and stolons. Another mechanism by which invasive species can increase their abundance and distribution in their introduced region is through the release of biochemicals (Callaway & Hierro, 2003; Kumar & Bais, 2010).

Allelopathy is the negative effect that a plant has on another through the production and release of biochemicals into the environment (Chou, 2006; International Allelopathy Society, 2015). The action of allelochemicals can either be direct or indirect (Callaway & Hierro, 2003). Allelochemicals can directly affect neighboring plants by inhibiting seed germination and plant growth. They do this by affecting a number of physiological pathways such as photosynthesis, respiration, cell division, nutrient and water uptake, and enzyme activity among others (Seigler, 2006). Allelochemicals can also be indirect in their action by affecting bacteria and fungi, altering soil pH, and the availability of free ions. Allelochemicals can be released by the leaching of phytochemicals from living material or litter, root exudates, volatilization through stomata, decomposition, and microbial activity (Chou, 1988; Kumar & Bais, 2010; Uddin et al., 2012, 2014ab). Allelopathy plays an important role in plant dominance, succession, and the formation of plant communities and climax vegetation (Chou, 2006; Muller, 1969). In addition to the potential release from enemies, invasive plants that exhibit allelopathy could be especially good competitors in their new environment.

P. australis is a 3-6 m tall perennial wetland plant that is identified by its great height, 20-70 cm long blue-green coloured leaves, and 30 cm long purple to yellow inflorescences (Mal & Narine, 2011). Introduced from Eurasia, *P. australis* has become invasive and widespread throughout North America (Invasive Species Centre, 2016). The rapid growth and spread of *P. australis* outcompetes native plants creating dense monotypic stands that decrease biodiversity and alter wildlife habitat (Ontario Ministry of Natural Resources and Forestry, 2011). Evidence from several studies have shown that *P. australis* root exudates and aqueous extracts have inhibited seed germination and seedling growth in other species (Rudrappa et al., 2007; Uddin et al., 2012). In these studies, they isolated gallic acid from the exudates and extracts and determined it to be the allelochemical causing the inhibitory effects. *P. australis* uses these mechanisms to alter habitat for many plants and wildlife, including several species at risk.

S. hermaphrodita and *A. robusta* are two species at risk in Southern Ontario. They are both listed as endangered under Schedule 1 of the SARA (Environment Canada, 2015; Ontario Ministry of Natural Resources and Forestry, 2017). The threats to these species include habitat loss and invasive species. *S. hermaphrodita* can be identified by its 1-4.5 m shoots, 3-7 lanceolate lobed and irregularly serrate leaves, and corymb inflorescence with small white flowers (Gleason & Cronquist, 1963; Spooner et al., 1985). *S. hermaphrodita* is considered to be rare across its entire range with populations thinly scattered throughout (Environment Canada, 2015). Naturally, *S. hermaphrodita* is found in floodplains and riparian areas but due to habitat loss in these areas it is now mainly found in anthropogenically disturbed habitats (Spooner et al. 1985). *A. robusta* is a small herbaceous annual plant that grows less than 1 m tall (Douglas, 1999). It is identified by its 2-8 cm long oblong-lanceolate leaves, small rose-purple flowers in groups of 3-5 at the leaf axil, and globose valved fruits. *A. robusta* is found in open, alkaline, and sandy or muddy shorelines where water levels are ephemeral (Ontario Ministry of Natural Resources and Forestry, 2017). Both of these species are thought to be at risk from *P. australis* invasion.

Several studies have demonstrated that *P. australis* uses allelopathy as a possible mechanism for invasion, however a study by Weidenhamer et al. (2013) contradicts this. This study found that root exudates containing gallic acid had levels much lower than previous studies and had a half-life in the soil that was less than one day (Weidenhamer et al., 2013). Previously the effect of *P. australis* allelopathy on *S.*

hermaphrodita and *A. robusta* has not be tested. The main questions in this study are: 1. Do extracts made from *P. australis* inhibit seed germination of *S. hermaphrodita* and *A. robusta* in growth chamber studies? 2. Do extracts made from *P. australis* inhibit seedling growth of *S. hermaphrodita* and *A. robusta* in growth chamber studies? To understand these effects I followed the methods of Uddin et al. (2012) to make extracts from *P. australis* tissues (leaf, rhizome and root) at various concentrations (10%, 5%, 2.5%, and 1.25%). These were applied to seed germination and seedling growth assays to determine if *S. hermaphrodita* and *A. robusta* are inhibited. It is expected based on previous studies that there will be a clear inhibition in germination and seedling growth for both *S. hermaphrodita* and *A. robusta*.

2.2. Materials and methods

2.2.1. Plant material and seed collection

P. australis stems and leaves were collected from the study site in September 2017 and dried in cold storage. In June 2018 rhizomes and roots were collected, separated, and dried at room temperature in dark conditions. *S. hermaphrodita* seeds were collected from the study site in April 2013 and kept in a seed bank in cold storage. *A. robusta* plants were grown in the greenhouse and harvested for their seeds in May 2017. The seeds were separated from their capsule and placed in cold storage.

2.2.2. Extract preparation and analysis

The extracts were made following the methods of Uddin et al. (2012). These methods were used because they represent the standardized approach for preparing aqueous extracts for allelopathy experiments. Once dried, the leaves, rhizomes, and roots were cut into small pieces (<2 cm). Dried tissues were used to mimic the conditions in the field during the spring when the litter from winter die off is in high densities. Spring is also the period when seed germination and seedling growth would be most susceptible to inhibition from litter leachates. To make the extract, 150 g of each tissue was added to 1500 mL of deionized (DI) water to make a base solution with a concentration of 10% mass to volume ratio. For 24 hours the flask was shaken at room temperature and in dark conditions on an orbital shaker at a speed of 140 rpm (MaxQ™ 2000 Benchtop Orbital Shaker). Once completed the extract was separated from the

plant fragments using a funnel and cheese cloth. The extracts were centrifuged (Avanti™ J-30I Centrifuge) at a speed of 10,000 g at room temperature for 30 minutes to separate the dissolved solids from the aqueous extract. The extracts were filtered using vacuum filtration with successive filters of decreasing pore size (25 µm, 2.5 µm, and 0.45 µm). Filtration ensured that the extracts were free of any microbes that could have an effect on seed germination and seedling growth. The base extracts were divided and diluted with DI water to have equal amounts of solution with 10%, 5%, 2.5%, and 1.25% concentration. To compare each solution, a YSI Pro Plus Multi-Parameter water quality meter was used to measure pH, salinity, and conductivity. To prevent the degradation and efficiency of the extracts, they were stored in a refrigerator at 4 °C.

2.2.3. Seed germination assay

To determine if *P. australis* extracts have an effect on *S. hermaphrodita* and *A. robusta* seed germination, a petri dish assay was used. Blue blotter paper from Anchor Paper Co. was used as the medium for seed germination. It absorbs and holds water and provides a contrasting background to observe radicle emergence. Larger petri dishes (60 mm) were used for *S. hermaphrodita* and smaller petri dishes (35 mm) were used for *A. robusta*. *A. robusta* seeds are much smaller and do not require as much space as *S. hermaphrodita* so smaller petri dishes were used. From a sheet, solid and holed discs (50 holes) of blue blotter paper were cut using a laser cutter (BOSS LS-2436 Laser Cutter) to the appropriate size for each petri dish. In the petri dishes the disc with



Figure 7. Overview of the seed germination assay for *Sida hermaphrodita*. Seeds were placed on two discs of blue blotter paper and wetted with *Phragmites australis* extracts. The bottom disc was solid and the top disc had holes to contain each seed.

holes was placed on top of the solid disc of blotter paper. The holed disc provided an individual compartment where a single seed would be held in place to germinate without being in contact with any other seed in the petri dish (Figure 7). For each assay the designated lids for each petri dish was used as the base and the designated base was inverted and used as the lid. This provided a smaller amount of space between the medium and the lid, reducing evaporation.

The blotter paper was wetted with one of 5 concentrations (10%, 5%, 2.5%, 1.25%, and deionized water control) for each of the 3 tissue types (leaf, rhizome, and root) and sealed with paraffin wax. For each treatment combination 5 replicates were used. Germination counts started on the third day since not much germination occurs in either species until this time and then every 2 days after that. To prevent any degradation or loss of efficiency in the extracts they were replaced every 2 days. This was done by adding solution at the top of the petri dish while it was held on an angle. Gravity caused the replacement solution to flush the old solution out of the blotter paper. The old solution collected at the bottom of the petri dish and then was removed using a pipette. Germination was considered complete upon radicle emergence (Figure 8). The seeds were viewed under a Zeiss Stereo dissecting scope and photographs taken with a Zeiss Axiocam microscope camera. The duration of the experiment for *A. robusta* was 11 days and 21 days for *S. hermaphrodita*. Completion of each experiment was determined when germination had ceased for *S. hermaphrodita* and when all of the seeds had germinated for *A. robusta*.

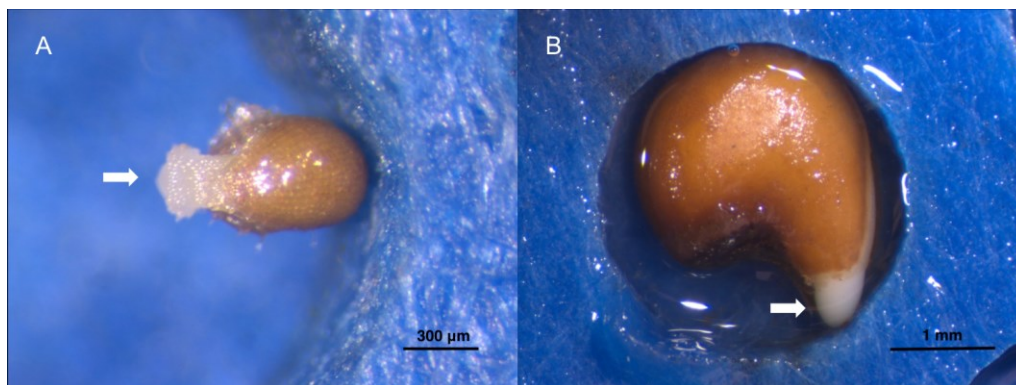


Figure 8. Radicle emergence in *Ammannia robusta* (A) and *Sida hermaphrodita* (B) depicted by the white arrows. For the seed germination assay the emergence of the radicle was the indicator that the seed had germinated.

The seed germination assay was performed in a growth chamber (Enconair AC-60 Ecological Chamber) on a 15h/9h light and dark cycle with temperature ranging from 25°C during the light cycle and 20°C during the dark cycle. These conditions were used to simulate field conditions during spring at the study site when seeds are germinating and seedlings are growing. Petri dishes were randomly placed in the growth chamber and re-randomized every two days to account for chamber variations in light and temperature.

2.2.4. Seedling growth assay

To measure extract effects on seedling growth, assays were set up using Coplin jars (Figure 9B). Blue blotter paper was cut and affixed to microscope slides using dentistry elastics. Two smaller pieces of blotter were inserted at the top of the microscope slide to reduce the pressure put on the seeds by the elastic (Figure 9A). Two microscope slides with *A. robusta* seeds and two with *S. hermaphrodita* seeds were randomly placed among the five slots within each Coplin jar. Since *A. robusta* seeds germinate readily they were placed on the slides without alteration. *S. hermaphrodita* seeds however require scarification. Before *S. hermaphrodita* seeds were placed on the slides they were scarified by poking a small hole into the seed coat to help initiate germination. To ensure that at least one seedling grew on each slide, two *S. hermaphrodita* and 3 *A. robusta* seeds were placed. Each Coplin jar was considered a replicate and there were three replicates per treatment. The growth chamber conditions and the treatments applied to the Coplin jars were the same as the seed germination experiment. Approximately 15 mL of extracts with one of 5 concentrations (10%, 5%, 2.5%, 1.25%, and deionized water control) for each of the 3 tissue types (leaf, rhizome, and root) were added to the Coplin Jars. The Coplin jar was topped with a petri dish lid with a slit in it and sealed with paraffin wax to prevent evaporation while still allowing air flow. To prevent the degradation and loss of efficiency the extract was changed every 2 days. The Coplin jars were randomly placed in the growth chamber and re-randomized every 2 days to account for chamber variations in light and temperature. Since *A. robusta* seeds are much smaller they were allowed to grow for 30 days while *S. hermaphrodita* was grown for 24 days. The experiment was concluded after 24 days and 30 days for *S. hermaphrodita* and *A. robusta* respectively.

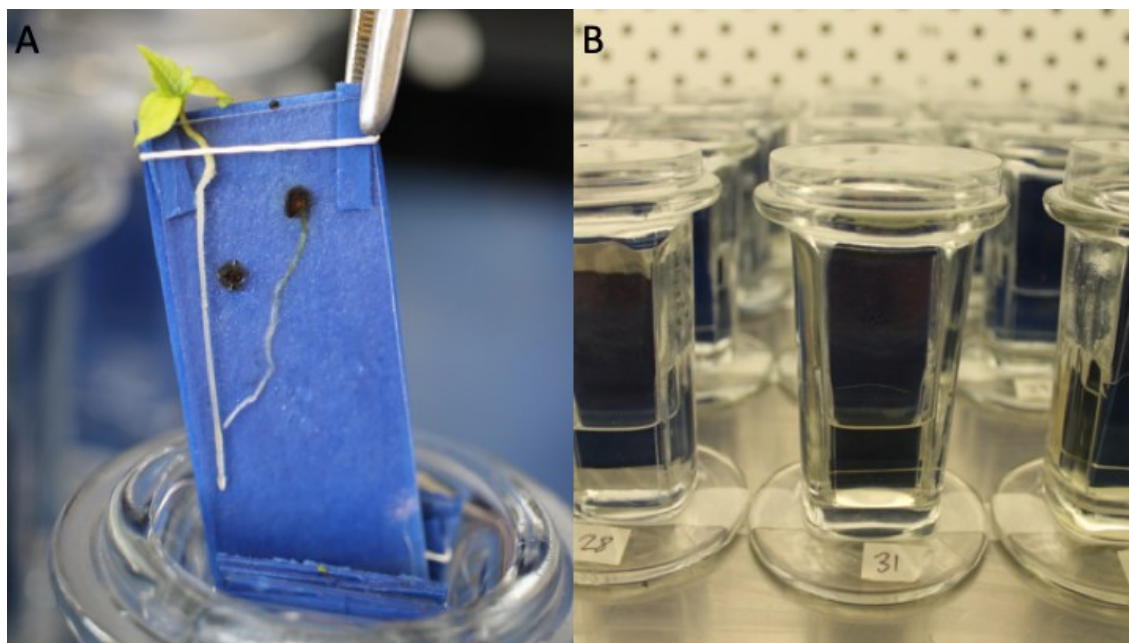


Figure 9. Overview of the seedling growth assay. Microscope slide set-up with *Sida hermaphrodita* seedling (A) and the experimental set-up of Coplin jars in the growth chamber (B). Microscope slides were placed in the Coplin jar that contained 15 mL of *Phragmites australis* extract.

Seedlings were collected at the end of the experiment and weighed using an analytical scale. Root length (cm), shoot length (cm), root surface area (cm²), shoot surface area (cm²), average root diameter (mm), and root volume (cm³) were quantified using a scanner and the WinRHIZO image analysis system (Regent Instruments Inc.). Because *A. robusta* seedlings were too small to obtain any meaningful data, only *S. hermaphrodita* data are presented.

2.2.5. Data analyses

The seed germination data was analyzed using the drc package in R (R Core Team, 2018; Ritz et al., 2013). It is a type of time-to-event modelling called dose-response analysis and is outlined in Ritz et al. (2013). It is considered a more appropriate and robust way to analyze seed germination compared to the commonly used intuition-based germination indexes or classical non-linear regression analyses (McNair et al., 2012; Ritz et al., 2013). This analysis accounts for right-censored data associated with seed germination (i.e. The seeds that have not germinated by the end of the experiment) and uses the probability of germinating in a time interval to account for

germination events that occur between observation times. The drc function outputs a log-logistic model with three parameters. Comparisons were made between treatments using the parameter “e” or the time it takes for 50% of the total proportion of seeds germinated throughout the experiment to germinate and “d” or the final proportion of seeds that germinate during the experiment. These comparisons were performed using the compParm command in the drc package which runs t-tests to determine significant differences between the treatments. In this case, the treatment is the tissue extract at a specific concentration.

For each metric measured for seedling growth the mean was determined for each treatment then compared using a two-way ANOVA with tissue type, concentration and the interaction of tissue type and concentration as sources of variation. Treatments were considered significantly different if $p < 0.05$. Normality and equal variance assumptions were checked using Q-Q and residual plots.

2.3. Results

2.3.1. Extract analysis

Between tissue type the pH, conductivity, and salinity all varied (Table 1). For pH the root extract at concentration 10% was the most basic (pH 7.75). This was followed by the control (pH 7.64) and leaf extracts (pH 7.64-7.41). The rhizome extracts (pH 6.11-5.47) and the remainder of root extracts (pH 6.75-6.47) were acidic. The leaf and rhizome extracts became more basic and the root extract became more acidic as concentration decreased. Conductivity and salinity both exhibited similar trends with rhizome extracts showing the highest conductivity and salinity (2561-417.6 $\mu\text{S}/\text{cm}$, 1.9-0.25 ppt), followed by root extracts (1316-191.9 $\mu\text{S}/\text{cm}$, 0.81-0.11 ppt) and then leaf extracts (850-171 $\mu\text{S}/\text{cm}$, 0.6-0.11 ppt). The control showed a conductivity of 1.4 $\mu\text{S}/\text{cm}$ and salinity of 0 ppt.

Table 1. Salinity (ppt), pH and conductivity ($\mu\text{S}/\text{cm}$) values for each extract (leaf, rhizome, and root), their varying concentrations (10%, 5%, 2.5%, 1.25%) and the deionized water control.

Tissue	Concentration (%)	pH	Conductivity ($\mu\text{S}/\text{cm}$)	Salinity (ppt)
Leaf	10	7.41	850	0.6
	5	7.53	481.3	0.33

Tissue	Concentration (%)	pH	Conductivity ($\mu\text{S}/\text{cm}$)	Salinity (ppt)
Rhizome	2.5	7.48	234.2	0.16
	1.25	7.64	171	0.11
	10	5.47	2561	1.9
	5	5.74	1491	0.97
	2.5	5.76	822	0.51
Root	1.25	6.11	417.6	0.25
	10	7.75	1316	0.81
	5	6.75	691	0.42
	2.5	6.68	352.5	0.21
	1.25	6.47	191.9	0.11
Control	0	7.64	1.4	0

2.3.2. Seed germination assay

Log-logistic models were created for the seed germination of *S. hermaphrodita* and *A. robusta*. In each plot the proportion of seeds germinated over time is shown. The dots represent the actual data points of proportion germinated during each observation time and the lines depict the model. The graphs for *S. hermaphrodita* (Figure 10) show a short initial lag followed by a rapid increase in germination until it plateaus at or less than 20% germination. The graphs for *A. robusta* (Figure 11) show a short initial lag in germination followed by a rapid increase in germination then a slight plateau before reaching around 100% germination. The models presented in the graphs output two parameters that were used for comparison between treatments (Table 2).

Time it takes to reach 50% germination (Model parameter “e”)

For *S. hermaphrodita* the time it took to reach 50% germination (Figure 12) was longer for seeds germinating in the rhizome (9.854 ± 7.171) extract at concentration 10% compared to the leaf (6.374 ± 4.132) and root (6.455 ± 6.335) extracts at the same concentration. The rhizome extract at concentration 10% (9.854 ± 7.171) took longer to reach 50% germination than the other rhizome extracts at concentrations 5% (6.288 ± 4.416), 2.5% (5.680 ± 2.368), 1.25% (5.948 ± 3.651), and the control (4.397 ± 2.245). The rhizome extract at concentration 5% (6.288 ± 4.416) also took longer to reach 50% germination compared to the control (4.397 ± 2.245).

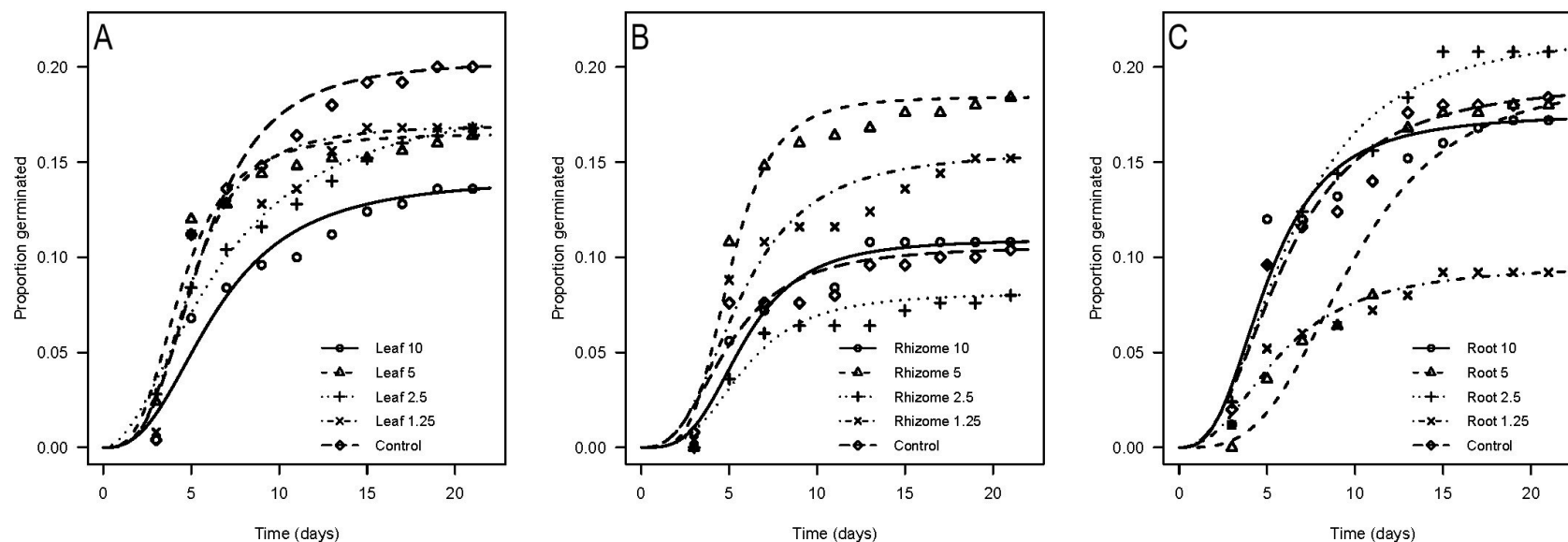


Figure 10. Proportion of *Sida hermaphrodita* seeds germinated over time (days) for each *Phragmites australis* tissue extract (leaf (A), rhizome (B), and root (C)), their varying concentrations (10%, 5%, 2.5%, 1.25%), and the control. The lines depict a log-logistic dose-response model created using the actual germination values obtained throughout the experiment. These actual values are represented by the plotted points.

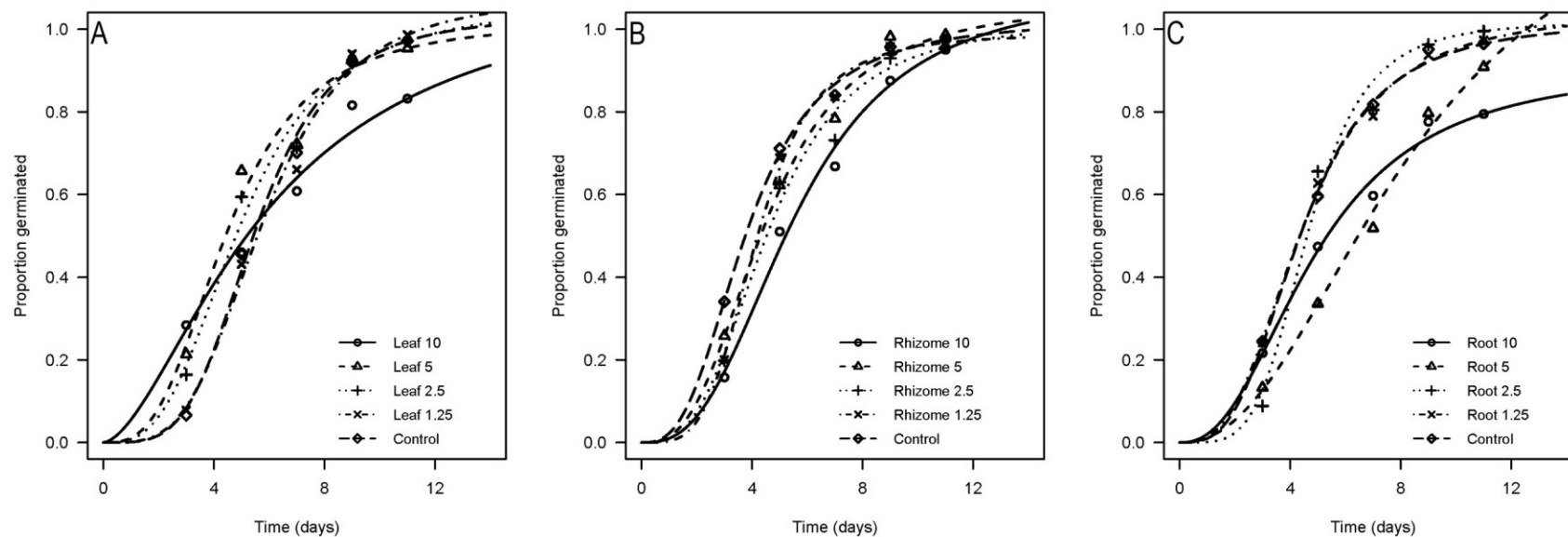


Figure 11. Proportion of *Ammannia robusta* seeds germinated over time (days) for each *Phragmites australis* tissue extract (leaf (A), rhizome (B), and root (C)), their varying concentrations (10%, 5%, 2.5%, 1.25%), and the control. The lines depict a log-logistic dose-response model created using the actual germination values obtained throughout the experiment. These actual values are represented by the plotted points.

Table 2. Seed germination log-logistic dose-response model parameter “e” and “d” estimates shown with their standard deviation for *Ammannia robusta* and *Sida hermaphrodita*.

Species	Tissue	Concentration	Parameter of Interest	
			e (time at 50% germinated)	d (total proportion of seeds germinated)
<i>Ammannia robusta</i>	Leaf	10	5.980 ± 7.220	1.134 ± 1.015
		5	4.590 ± 2.015	1.021 ± 0.233
		2.5	4.173 ± 1.064	0.988 ± 0.073
		1.25	3.815 ± 1.500	1.022 ± 0.163
		Control	4.921 ± 3.119	1.121 ± 0.449
	Rhizome	10	8.513 ± 13.461	1.423 ± 2.080
		5	4.652 ± 1.720	1.017 ± 0.105
		2.5	4.471 ± 1.854	1.044 ± 0.243
		1.25	4.409 ± 1.543	1.020 ± 0.165
		Control	4.473 ± 1.966	1.018 ± 0.239
	Root	10	4.980 ± 2.652	1.059 ± 0.313
		5	5.802 ± 2.822	1.079 ± 0.266
		2.5	5.514 ± 2.350	1.033 ± 0.177
		1.25	5.575 ± 3.734	1.100 ± 0.472
		Control	4.494 ± 1.941	1.064 ± 0.255
<i>Sida hermaphrodita</i>	Leaf	10	6.374 ± 4.132	0.142 ± 0.148
		5	5.805 ± 1.512	0.081 ± 0.087
		2.5	5.612 ± 3.137	0.156 ± 0.165
		1.25	4.938 ± 1.824	0.106 ± 0.114
		Control	5.032 ± 3.193	0.175 ± 0.219
	Rhizome	10	9.854 ± 7.171	0.195 ± 0.362
		5	6.288 ± 4.416	0.219 ± 0.215
		2.5	5.680 ± 2.368	0.096 ± 0.101
		1.25	5.948 ± 3.651	0.193 ± 0.186
		Control	4.397 ± 2.245	0.165 ± 0.224
	Root	10	6.455 ± 6.335	0.186 ± 0.199
		5	4.954 ± 2.448	0.170 ± 0.212
		2.5	5.608 ± 3.658	0.203 ± 0.233
		1.25	5.805 ± 2.196	0.109 ± 0.119
		Control	5.037 ± 2.578	0.184 ± 0.277

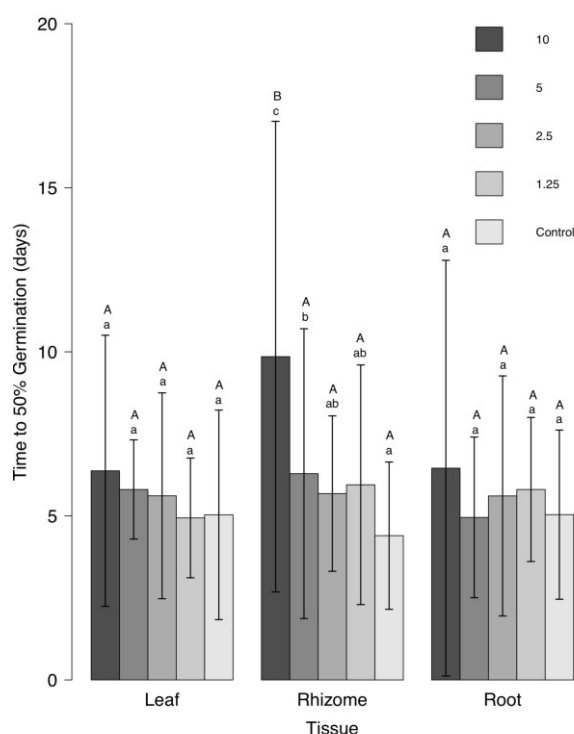


Figure 12. Effect of *Phragmites australis* tissue extract and concentration on the time it takes to reach 50% germination for *Sida hermaphrodita*. Different uppercase letters indicate significant differences of identical concentrations between tissue types. Different lowercase letters indicate significant differences between the concentrations within an individual tissue extract. Parameter estimates are shown here with \pm one standard deviation. ($p < 0.05$).

For *A. robusta* the time it took to reach 50% germination (Figure 13) was longer for seeds germinating in the root extract at concentrations 5% (4.652 ± 1.712), 2.5% (4.471 ± 1.854), and 1.25% (4.409 ± 1.543) compared to the leaf (4.590 ± 2.015 , 4.173 ± 1.064 , 3.815 ± 1.500 respectively) and rhizome (4.652 ± 1.720 , 4.471 ± 1.854 , 4.409 ± 1.543 respectively) extracts at the same concentrations. The rhizome extract at concentration 1.25% (4.409 ± 1.543) took longer to germinate to 50% compared to the leaf (3.815 ± 1.500) extract. At a concentration of 10% the rhizome (8.513 ± 13.461) extract took longer to germinate to 50% than the root (4.980 ± 2.652) extract. Within the leaf extracts the concentrations 10% (5.98 ± 7.220), 5% (4.590 ± 2.015), and the control (4.921 ± 3.119) took longer to germinate to 50% compared to the concentration 1.25% (3.815 ± 1.500). The rhizome extract at concentration 10% (8.513 ± 13.461) took longer to reach 50% germination than the other rhizome extracts at concentrations 5% ($4.652 \pm$

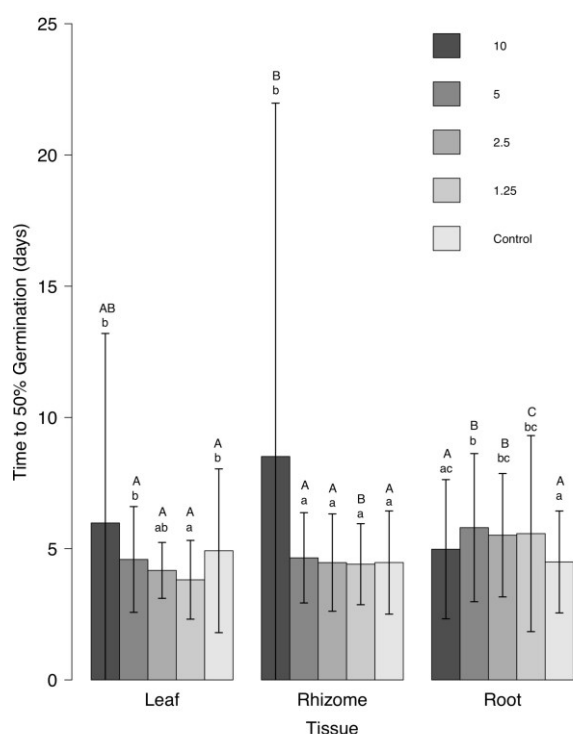


Figure 13. Effect of *Phragmites australis* tissue extract and concentration on the time it takes to reach 50% germination for *Ammannia robusta*. Different uppercase letters indicate significant differences of identical concentrations between tissue types. Different lowercase letters indicate significant differences between the concentrations within an individual tissue extract. Parameter estimates are shown here with \pm one standard deviation. ($p < 0.05$).

1.720), 2.5% (4.471 ± 1.854), 1.25% (4.409 ± 1.543), and the control (4.473 ± 1.966). Within the root extracts the concentrations 5% (4.652 ± 1.712), 2.5% (4.471 ± 1.854), and 1.25% (4.409 ± 1.543) took longer to germinate compared to the control (4.494 ± 1.941), although, concentration 5% (4.652 ± 1.712) took longer to germinate than concentration 10% (4.980 ± 2.652). Significant differences for parameter “e” in both species can be found in table 3.

Table 3. Significant differences between treatments for the seed germination log-logistic dose response model parameter “e” for *Sida hermaphrodita* and *Ammannia robusta*. Differences are shown with their standard error and p-values.

Species	Parameter of Interest		
	e (time at 50% germinated)		
	Extract Differences	Differences	p-value
<i>Sida hermaphrodita</i>	Leaf10-Rhizome 10	-3.480 ± 1.205	0.0038729 **

Species	Parameter of Interest		
	e (time at 50% germinated)		
	Extract Differences	Differences	p-value
<i>Ammannia robusta</i>	Rhizome 10-Rhizome 5	3.567 ± 1.113	0.0013531 **
	Rhizome 10-Rhizome 2.5	4.174 ± 1.305	0.0013831 **
	Rhizome 10-Rhizome 1.25	3.907 ± 1.105	0.0004087 ***
	Rhizome 10-Control	5.457 ± 0.943	7.276e-09 ***
	Rhizome 10-Root10	3.399 ± 1.454	0.0193801 *
	Rhizome 5-Control	1.890 ± 0.808	0.0193775 *
	Leaf10-Leaf1.25	2.164 ± 1.100	0.0490360 *
	Leaf5-Leaf1.25	0.774 ± 0.273	0.0046132 **
	Leaf5-Root5	-1.212 ± 0.313	0.0001067 ***
	Leaf2.5-Root2.5	-1.341 ± 0.241	2.646e-08 ***
	Leaf1.25-Control	-1.106 ± 0.397	0.0053050 **
	Leaf1.25-Rhizome 1.25	-0.594 ± 0.259	0.0217585 *
	Leaf1.25-Root1.25	-1.760 ± 0.389	6.151e-06 ***
	Rhizome 10-Rhizome 5	3.862 ± 1.378	0.0050796 **
	Rhizome 10-Rhizome 2.5	4.043 ± 1.388	0.0035937 **
	Rhizome 10-Rhizome 1.25	4.104 ± 1.384	0.0030300 **
	Rhizome 10-Control	4.040 ± 1.388	0.0036064 **
	Rhizome 10-Root10	3.533 ± 1.391	0.0110974 *
	Rhizome 5-Root5	-1.150 ± 0.270	2.082e-05 ***
	Rhizome 2.5-Root2.5	-1.043 ± 0.293	0.0003633 ***
	Rhizome 1.25-Root1.25	-1.166 ± 0.396	0.0032444 **
	Root10-Root5	-0.822 ± 0.330	0.0128024 *
	Root5-Control	1.308 ± 0.324	5.504e-05 ***
	Root2.5-Control	1.020 ± 0.300	0.0006666 ***
	Root1.25-Control	1.081 ± 0.415	0.0091464 **

Total proportion of seeds germinated (Model parameter “d”)

The total proportion of seed germinated for *S. hermaphrodita* (Figure 14) was lower for the leaf (0.081 ± 0.087) extract at concentration 5% compared the rhizome (0.219 ± 0.215) and root (0.170 ± 0.212) extracts at the same concentration. The total proportion germinated for the leaf (0.106 ± 0.114) extract at concentration 1.25% was lower than the rhizome (0.193 ± 0.186) extract at the same concentration. The rhizome (0.096 ± 0.101) extract with concentration 2.5% had a smaller proportion germinated compared to the root (0.203 ± 0.233) extract. For the leaf extract the concentration 5% (0.081 ± 0.087) had fewer germinated compared to the concentrations 10% ($0.142 \pm$

0.148), 2.5% (0.156 ± 0.165), and the control (0.175 ± 0.219). The concentration 1.25% (0.106 ± 0.114) also had fewer germinated than the control (0.175 ± 0.219) for the leaf extract. Within the rhizome extracts the concentration 2.5% (0.096 ± 0.101) had a lower proportion germinated compared to the concentrations 10% (0.195 ± 0.362), 5% (0.219 ± 0.215), 1.25% (0.193 ± 0.186), and the control (0.165 ± 0.224). Within the root extracts the concentration 1.25% (0.109 ± 0.119) had a lower proportion of seed germinated compared to the 10% (0.186 ± 0.199) and 2.5% (0.203 ± 0.233) concentrations and the control (0.184 ± 0.277).

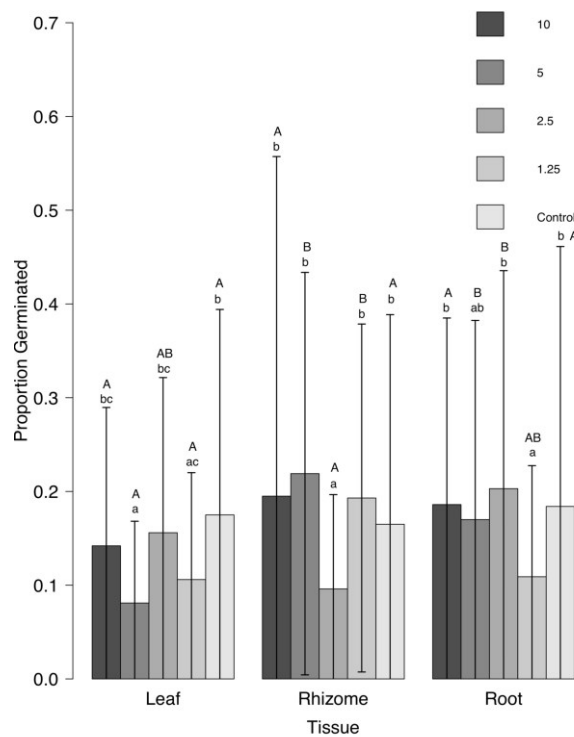


Figure 14. Effect of *Phragmites australis* tissue extract and concentration on the total proportion of *Sida hermaphrodita* seeds germinated by the end of the experiment. Different uppercase letters indicate significant differences of identical concentrations between tissue types. Different lowercase letters indicate significant differences between the concentrations within an individual tissue extract. Parameter estimates are shown here with \pm one standard deviation. ($p < 0.05$).

The total proportion of seeds germinated for *A. robusta* (Figure 15) was lower for the rhizome (1.017 ± 0.105) extract at a concentration of 5% compared to the root (1.079 ± 0.266) extract at the same concentration. Within the leaf extracts the concentration

2.5% (0.988 ± 0.074) had fewer germinating seeds compared to the control (1.121 ± 0.449). Significant differences for parameter “d” in both species can be found in table 4.

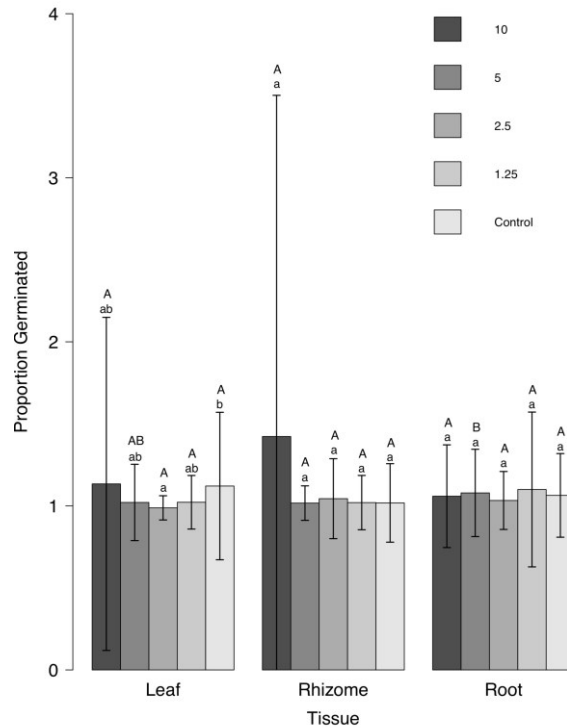


Figure 15. Effect of *Phragmites australis* tissue extract and concentration on the total proportion of *Ammannia robusta* seeds germinated by the end of the experiment. Different uppercase letters indicate significant differences of identical concentrations between tissue types. Different lowercase letters indicate significant differences between the concentrations within an individual tissue extract. Parameter estimates are shown here with \pm one standard deviation. ($p < 0.05$).

Table 4. Significant differences between treatments for the seed germination log-logistic dose response model parameter “d” for *Sida hermaphrodita* and *Ammannia robusta*. Differences are shown with their standard error and p-values.

Species	Parameter of Interest		
	d (total proportion of seeds germinated)		
	Extract Differences	Differences	p-value
<i>Sida hermaphrodita</i>	Leaf10-Leaf5	0.061 ± 0.029	0.0360687 *
	Leaf5-Leaf2.5	-0.075 ± 0.029	0.0107790 *
	Leaf5-Control	-0.094 ± 0.030	0.0017289 **
	Leaf5-Rhizome 5	-0.138 ± 0.033	2.691e-05 ***
	Leaf5-Root5	-0.088 ± 0.030	0.0028235 **

Species	Parameter of Interest		
	d (total proportion of seeds germinated)		
	Extract Differences	Differences	p-value
	Leaf1.25-Control	-0.069 ± 0.031	0.0273843 *
	Leaf1.25-Rhizome 1.25	-0.087 ± 0.033	0.0083433 **
	Rhizome 10-Rhizome 2.5	0.098 ± 0.034	0.0035819 **
	Rhizome 5-Rhizome 2.5	0.123 ± 0.034	0.0003000 ***
	Rhizome 2.5-Rhizome 1.25	-0.096 ± 0.033	0.0032107 **
	Rhizome 2.5-Control	-0.069 ± 0.031	0.0244316 *
	Rhizome 2.5-Root2.5	-0.107 ± 0.032	0.0009388 ***
	Root10-Root1.25	0.076 ± 0.035	0.0297427 *
	Root2.5-Root1.25	0.094 ± 0.033	0.0039735 **
	Root1.25-Control	-0.075 ± 0.032	0.0177076 *
<i>Ammannia robusta</i>	Leaf2.5-Control	-0.133 ± 0.053	0.011644 *
	Rhizome 5-Root5	-0.062 ± 0.030	0.036033 *

2.3.3. Seedling growth assay

The results for the two-way ANOVA (Table 5) indicated that there were no effects of tissue type, concentration or the interaction of tissue type and concentration on seedling weight, root length, shoot length, root surface area, shoot surface area, average root diameter, and root volume (Figure 16-22). Although the effect of tissue was not significant there was some effect at a higher significance level ($p=0.10$). The root surface area ($p=0.073$) and root volume ($p=0.062$) was lower for the leaf (0.330 ± 0.172 and 0.006 ± 0.003 respectively) extracts compared to the root (0.443 ± 0.201 and 0.009 ± 0.004 respectively) extracts.

Table 5. Two-way ANOVA results for the *Sida hermaphrodita* seedling growth assay for several measured biometrics. F statistic and p-values are shown for each biometric.

Source	df	Seedling Weight		Root Length		Shoot Length		Root Surface Area		Shoot Surface Area		Average Root Diameter		Root Volume	
		F	P	F	P	F	P	F	P	F	P	F	P	F	P
Tissue	2	0.607	0.548	1.963	0.148	0.171	0.843	2.713	0.073	1.308	0.279	0.882	0.418	2.892	0.062
Concentration	4	0.620	0.650	1.845	0.129	0.562	0.691	1.408	0.240	0.341	0.849	0.797	0.531	0.881	0.480
Tissue x Concentration	8	0.499	0.853	1.072	0.392	0.887	0.532	0.823	0.585	0.998	0.449	0.356	0.940	0.456	0.883

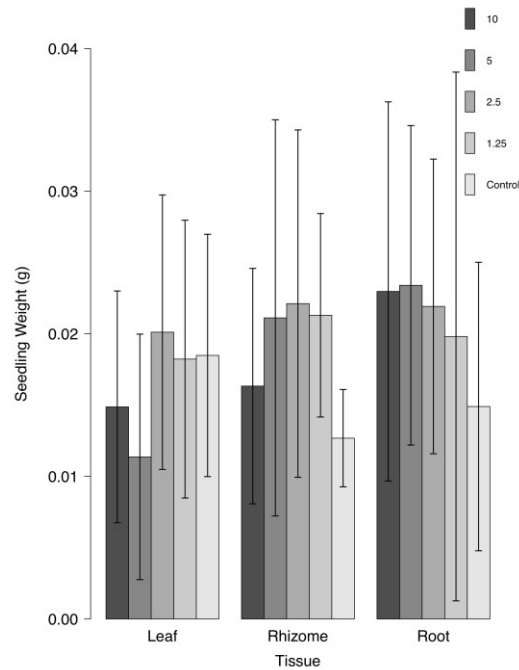


Figure 16. Effect of *Phragmites australis* tissues extract and concentration on *Sida hermaphrodita* seedling weight (g). Raw means are presented with \pm one standard deviation. ($p < 0.05$)

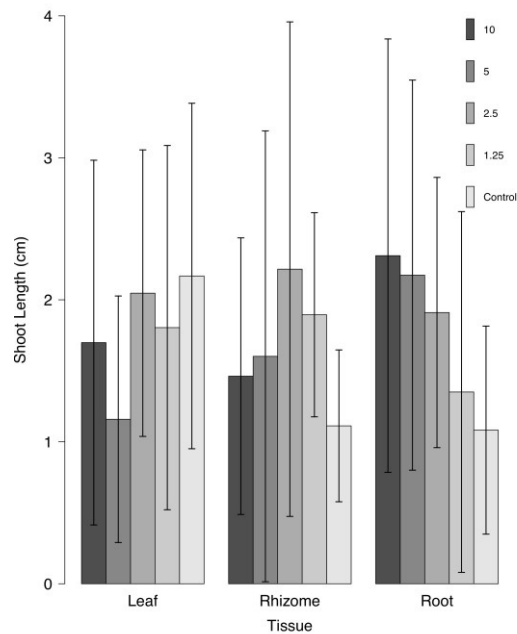


Figure 17. Effect of *Phragmites australis* tissues extract and concentration on *Sida hermaphrodita* seedling shoot length (cm). Raw means are presented with \pm one standard deviation. ($p < 0.05$).

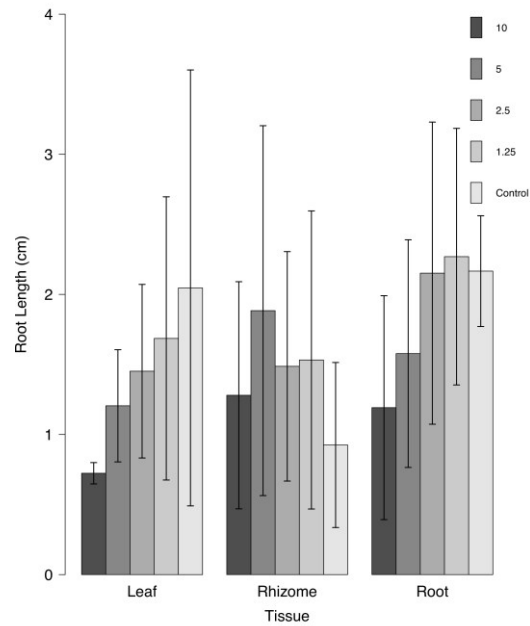


Figure 18. Effect of *Phragmites australis* tissues extract and concentration on *Sida hermaphrodita* seedling root length (cm). Raw means are presented with \pm one standard deviation. ($p < 0.05$).

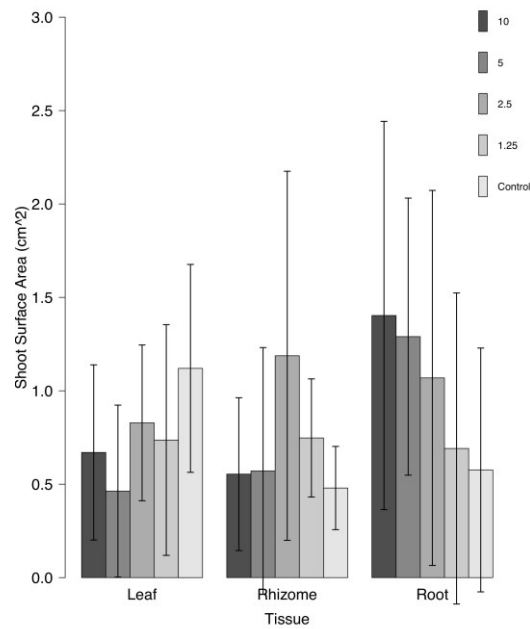


Figure 19. Effect of *Phragmites australis* tissues extract and concentration on *Sida hermaphrodita* seedling shoot surface area (cm²). Raw means are presented with \pm one standard deviation. ($p < 0.05$).

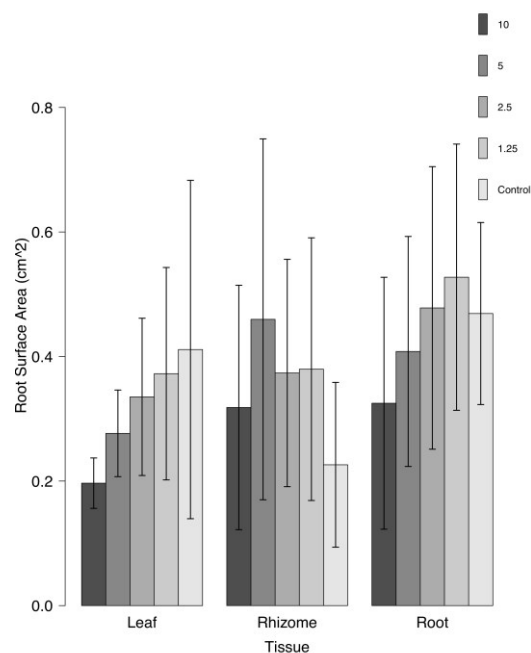


Figure 20. Effect of *Phragmites australis* tissues extract and concentration on *Sida hermaphrodita* seedling root surface area (cm²). Raw means are presented with \pm one standard deviation. ($p < 0.05$).

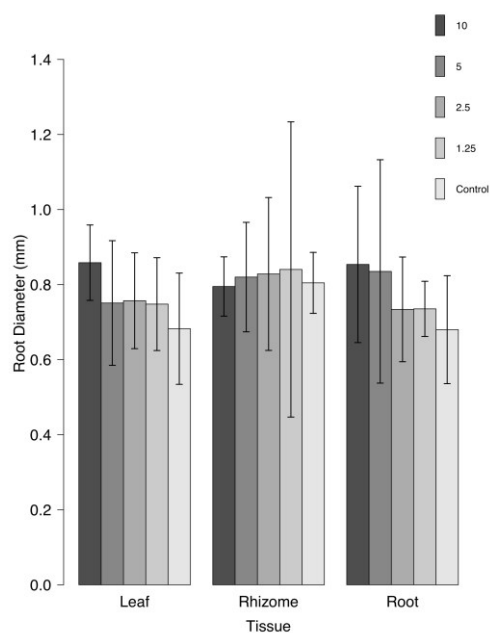


Figure 21. Effect of *Phragmites australis* tissues extract and concentration on *Sida hermaphrodita* seedling average root diameter (mm). Raw means are presented with \pm one standard deviation. ($p < 0.05$).

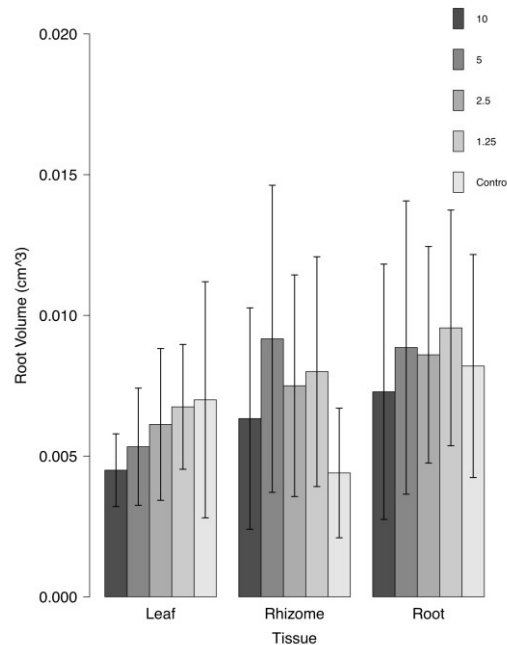


Figure 22. Effect of *Phragmites australis* tissues extract and concentration on *Sida hermaphrodita* seedling root volume (cm³). Raw means are presented with \pm one standard deviation. ($p < 0.05$).

2.4. Discussion

Invasive species are negatively affecting ecosystems around the world. Preceding habitat loss, invasive species are one of the leading causes of biodiversity loss (Bieberich et al., 2018). Through allelopathy, some invasive species are able to successfully establish in new areas and outcompete native plants (Callaway & Hierro, 2003; Callaway & Ridenour, 2004; Levine et al., 2003). It has been shown that the invasive species *P. australis* is one of these species that uses allelopathy as a mechanism for invasion (Rudrappa et al., 2007; Uddin et al., 2017). These studies also demonstrated that the allelochemical responsible for *P. australis* phytotoxicity was gallic acid (Rudrappa et al., 2007; Uddin et al., 2014a). Through *in vitro* and *in vivo* experiments, root exudates, aqueous plant extracts, and gallic acid itself were found to inhibit the seed germination and seedling growth of several species (Rudrappa et al., 2007; Uddin et al., 2012; Uddin et al., 2014a). Gallic acid was also found to persist in the rhizosphere for extended periods of time allowing it to have chronic effects on other plants (Rudrappa et al., 2007). However, other research indicated that allelopathy was unlikely a significant mechanism for the successful invasion of *P. australis* (Weidenhamer et al., 2013). They contradicted the findings of Rudrappa et al. (2007) by

showing that gallic acid concentrations within the leaves and rhizomes of *P. australis* were significantly lower than what was previously found. They also reported that the half-life of gallic acid in soil was less than one day so it is unlikely to be able to have an effect on other plants in the field. The controversy over the allelopathic potential of *P. australis* prompts more research on the topic.

In the present study, the goal was to determine if *P. australis* had an allelopathic effect on the two species at risk *S. hermaphrodita* and *A. robusta*. Both species are thought to be at risk of being displaced by *P. australis* so understanding the mechanism behind it is important in developing management strategies. To determine these effects a seed germination assay was performed using aqueous extracts from *P. australis* tissues (leaf, rhizome, and root) at varying concentrations (10%, 5%, 2.5%, 1.25%) and a control containing deionized water. The time it took to reach 50% germination and the total proportion of seeds germinated for each treatment were used as comparison parameters. The results of this study showed that *P. australis* extracts had an inhibitory effect on seed germination in both species. There were a number of treatments that showed an inhibition in germination compared to the controls. The treatments that showed this either had a larger lag in germination or had fewer germinated compared to the control. Studies also using aqueous extracts from *P. australis* showed that seed germination was inhibited compared to the control in model species *L. lactuca* and *R. sativus* and species associated with *P. australis* such as *R. conglomerates*, *J. pallidus* (Uddin et al., 2012), *Avena fatua*, *Convolvulus arvensis*, *Ammi visnaga*, *Rumex crispus*, *Asphodelus tenuifolius* (Khan et al., 2011), and *Spartina alternifolia* (Uddin et al., 2017). These findings show further evidence of the allelopathic potential of *P. australis*. However, the statistically significant differences shown were small. This means that a small number of seeds were affected by *P. australis*. This result may mean that the effect of *P. australis* allelopathy on *S. hermaphrodita* and *A. robusta* seed germination may not be enough to significantly reduce the recruitment from seeds in the field. Therefore, the results may not be ecologically significant. Further research would be needed to test this in the field.

For *S. hermaphrodita* the leaf and rhizome extracts significantly inhibited seed germination compared to root extracts. The leaf and rhizome extracts compared to the root extracts took longer to reach 50% germination and had fewer seeds germinated. The rhizome extract compared to the leaf extract had slower germination but did not

inhibit the total proportion of germinated seeds as much as the leaf extracts. Based on these results the allelopathic potential of *P. australis* tissues on *S. hermaphrodita* seed germination can be ranked as follows: leaf> rhizome> root. These results are consistent with the study by Uddin et al. (2012) which found that leaf and rhizome extracts inhibited seed germination of model species *L. lactuca* and *R. sativus* and associated species *R. conglomerates* and *J. pallidus* more than root extracts. The inhibition by *P. australis* extracts on *S. hermaphrodita* follows a common allelopathic response similar to other studies. This means that *S. hermaphrodita* is affected by *P. australis* allelopathy and could potentially be at risk in locations where it coexists.

For *A. robusta* the root and rhizome extracts took longer to germinate to 50% germination compared to the leaf extract. However, the leaf and rhizome extracts inhibited the total proportion of seeds that germinated more than the roots but only for one of the treatment concentrations. These results suggest that the allelopathic potential of *P. australis* tissues on *A. robusta* seed germination can be ranked as follows: root> rhizome> leaf. These results are inconsistent with other studies that tested the allelopathic potential of *P. australis* tissues on other species (Uddin et al., 2012). Their results showed that allelopathic potential of the tissues were as follows: leaf> rhizome> root. Since the *S. hermaphrodita* seed germination assay showed similar results to Uddin et al. (2012) the differing results shown for *A. robusta* are not a consequence of differences in experimental design but rather another explanation. There are several studies that have shown that the effects of allelochemicals may be species specific (Allaie et al, 2006; Burgos & Talbert, 2006; Uddin et al., 2012; Uddin et al., 2017). While some species showed an inhibitory effect of *P. australis* root exudates, aqueous extracts or gallic acid others did not. This species-specific characteristic of allelopathy could be the reason *A. robusta* differs from its response compared to *S. hermaphrodita* and other species. The differing levels of pH, conductivity, and salinity measured for each tissue extract suggests that the chemical composition may give an explanation for differential effects observed for different species (Uddin et al., 2012). The leaf extracts were found to be more basic compared to the rhizome and root extracts. Often *A. robusta* requires habitat found in wet, basic, and muddy shorelines (Ontario Ministry of Natural Resources and Forestry, 2017). Strong correlations between pH and phytotoxicity were observed in another allelopathy study testing the phytotoxicity of *Vulpia myuros* (An et al., 1997). This correlation could be an explanation of why *A. robusta* showed both no inhibition in

several of the leaf extract concentrations and an enhancement in germination from leaf 1.25% compared to other species since it prefers more basic conditions over acidic. However, further research is required to explore this further.

Within each *P. australis* tissue extract several concentrations (10%, 5%, 2.5%, and 1.25%) were used. For both *S. hermaphrodita* and *A. robusta* there was an inhibition in germination, however within each tissue extract some lower concentrations inhibited germination more than higher concentrations. This trend was seen for both parameters with the exception of the rhizome extract at concentration 10% for parameter “e”. This is inconsistent with other studies that have shown inhibition in germination and have tested for a dose response for *P. australis* and other species (Allaie et al., 2006; Batish et al., 2002; Romagni et al., 2000; Uddin et al., 2012). These studies show a clear dose response with higher concentrations inhibiting germination more than lower concentrations. In the Uddin et al. (2012) study the test species used was *L. sativa*. Due to the sensitivity of this species it is commonly used as a model species in phytochemical experiments. It was determined at what concentration each extract would inhibit seed germination by 50% (LC₅₀). The LC₅₀ for leaf and rhizome were 4.7% and 11.3% respectively. In the present study only one of the extracts (Rhizome 10%) inhibited seed germination by 50% for either *S. hermaphrodita* or *A. robusta*. To reach a point where seed germination is reduced to this degree you would need a higher concentration of extract. A possible explanation for the differences in results of the present study to other studies was the application of extract. Commonly, germination assays for allelopathy involved a one time addition of the extract. To account for any degradation or loss of efficiency in the extracts the germination assays in this study were subject to continuous replacement every two days. In a study by Rudrappa et al. (2009) the *P. australis* allelochemical gallic acid was found to photo-degrade into mesoxalic acid inducing higher mortality in seedlings. Photo-degradation could have occurred in the other studies causing the difference in results compared to the present study. Since it has been shown in other studies to inhibit seed germination in associated plant species to a higher degree it can be suggested that *S. hermaphrodita* and *A. robusta* seeds are not strongly affected by *P. australis* allelopathy, although, further study would be required.

Though there was an effect on seed germination, a significant effect of *P. australis* extracts on the seedling growth of *S. hermaphrodita* was not detected. This was

an unexpected result since previous studies showed that the extracts inhibited seedling growth parameters in several species (Uddin et al., 2012; Uddin et al., 2014b). The results did not find a significant effect based on tissue type, concentration or the interaction of the two, however there was some tissue effect observed for a significance level of 0.10. This showed that leaf extracts had an overall larger effect on inhibiting *S. hermaphrodita* seedling root surface area and root volume. Root metrics are important indicators of growth inhibition since roots are more sensitive to changes in the environment (Lotina-Hennsen et al., 2006). These results may also be attributed to the differences in experimental approaches between Uddin et al. (2012) and this study. The present study used a hydroponic system as the medium for growth where other study used soil (Uddin et al., 2012). A limitation to this studies' use of a hydroponic system was that the control contained deionized water. The amount of nutrients in the extract treatments could have enhanced the ability of the seedling to grow compared to the control (Jones, 1982). It was assumed that the seedlings in the control had enough nutrients to grow from the nutrient reserves in the seed. However, there could have been more nutrients in the extract treatments. This could be the reason a detectable effect between the control and other treatments was not observed. Since there was some sort of effect observed on a sensitive indicator of inhibition, albeit not significant, additional research using a different growth medium for the seedlings should be conducted. Another experimental design element that could be included into these allelopathy studies is the use of a soil and activated charcoal medium as the control. This way the control seedlings are exposed to the effects of each extract but the allelochemical is rendered inactive by the activated charcoal (Murrell et al., 2011; Rudrappa et al., 2007; Uddin et al., 2014a).

Due to the limitations of the seedling growth study design, the experiment should be conducted again using a different growth medium. Since there seemed to have been an effect of tissue redoing the experiment with an acceptable control treatment may tease out the inconsistencies in results between the present studies and other literature. Another future direction for research would be to test how the native microbial community is affected by *P. australis* and how this affects the growth *S. hermaphrodita* and *A. robusta*. Native plants rely on their soil biota and any alteration of this from allelopathy could indirectly affect their growth. In future laboratory and greenhouse studies they should be conducted to better mimic the conditions in the field. For

example, some of the other studies testing allelopathy use *in vivo* experiments with *P. australis* plants or litter at various densities to observe an effect. To understand the direct effects of *P. australis* I suggest that several greenhouse studies be done to better mimic field conditions.

The results here demonstrate that there is an inhibition from *P. australis* extracts on the seed germination of *S. hermaphrodita* and *A. robusta*. There was a difference in which tissues caused more of an inhibition between *S. hermaphrodita* (leaf> rhizome> root) and *A. robusta* (root> rhizome> leaf) indicating that the allelopathic effect of *P. australis* is species-specific. However, inhibition did not show a dose response and due to the lack of an observed LC₅₀ for these species a much higher concentration would be required to inhibit germination to a great deal. There was also no observed effect of tissue type, concentration or their interaction on any of the seedling growth parameters that were measured. These results give support to the allelopathic potential of *P. australis* against these species. However, the statistically significant differences are small indicating that they may not have an ecologically significant effect.

Chapter 3. Assessing the impact of *Phragmites australis* on the stand boundaries, density and total area of *Sida hermaphrodita* at the study site since 2014.

3.1. Introduction

Introduced species are considered one of the leading threats facing species at risk in Canada (Venter et al., 2006). *S. hermaphrodita* (Virginia Mallow) is one of these species at risk. In Canada, *S. hermaphrodita* is federally listed as endangered under Schedule 1 of the SARA (Environment Canada, 2015). Of the extremely rare populations found in Canada, one is at a conservation area and the other at a quarry site in Southern Ontario. It is also considered to be rare across its entire range which encompasses the Appalachian Mountain region of Northeastern United States (Spooner et al., 1985). *S. hermaphrodita* is a 1-4.5 m tall herbaceous plant from the Malvaceae (Mallow) family (Gleason & Cronquist, 1963; Kasprzyk et al., 2013). Populations of *S. hermaphrodita* are typically found on floodplains and riparian area that experience periodic flooding (Environment Canada, 2015). In 2006 a reservoir was dewatered at the conservation area leading to the spread of *S. hermaphrodita* at the site. The persistence of *S. hermaphrodita* on this site is mainly thought to be threatened by introduced species (Environment Canada, 2015).

An invasive species is defined as a species whose introduction to a new area has a strong negative impact on the environment (Mack et al., 2000). A site invaded by an invasive species is vulnerable to losses in biodiversity, ecosystem structure and function (Holmes et al., 2009). Locally, invasive species can spread quickly through the release of small and easily transported seeds and rapid vegetative growth (Gawronska & Golisz, 2006; Wang et al., 2011). There are several hypotheses that try to explain how introduced species become invasive. The ERH states that when a plant is introduced to a new area there is a decrease in regulation by their natural enemies causing its distribution and abundance to rapidly increase (Keane, & Crawley, 2002). This may be true for some invasive species, however, it does not apply to all. Callaway and Ridenour (2004) proposed that the NWH is another way introduced species become invasive. Through the NWH it is thought that the release from adapted neighbours in the native

range allows the species' previously harmless biochemicals to transform into novel biochemical weapons in the introduced range (Callaway & Ridenour, 2004). This brings in the concept that some introduced species are invasive through chemical means.

Allelopathy occurs when plants negatively impact the growth of neighboring plants through the release of biochemicals into the environment (Chou, 2006; International Allelopathy Society, 2015). The leachate from living material or litter, root exudates, chemical release through stomata, plant decomposition, and microbial activity can be sources of allelochemicals (Chou, 2006; Kumar & Bais, 2010). Allelopathy can affect other plants directly or indirectly (Seigler, 2006). Allelochemicals directly inhibit seed germination and plant growth by inhibiting photosynthesis, respiration, cell division, nutrient and water uptake, enzyme activity and production, and other molecular processes such as protein and nucleic acid synthesis (Chou, 2006; Lotina-Hennsen et al., 2006; Seigler, 2006). Allelochemicals can also modify the soil matrix and the availability of nutrients by inhibiting the growth of bacteria and fungi, altering pH, and reducing the amount of free ions which indirectly affect the growth of neighbouring plants (Seigler, 2006). They also play an important role in plant dominance, succession, and the formation of climax plant communities (Chou, 2006; Kumar & Bais, 2010; Muller, 1969).

P. australis is a tall perennial grass that can grow up to 6 m in height (Lambert et al., 2010). It is identified by its great height, 30 cm purple to yellow terminal panicle inflorescence, and its 20-70 cm long blue-green leaves (Mal & Narine, 2011). It is widespread across much of the United States and Southern Canada and is still spreading to new areas (Invasive Species Centre, 2016). *P. australis* invades wetlands, stream banks, lakes shores, disturbed habitat, and along roadsides and ditches choking out other plants creating monotypic and dense stands (Ontario Ministry of Natural Resources and Forestry, 2011). *P. australis* thrives in disturbed habitats and colonizes these areas very quickly. Its invasiveness can be attributed to its fast growth, rapid spread, and ability to outcompete plants. There is also evidence that it uses gallic acid as an allelochemical released from root exudates and through the leaching of litter to inhibit seed germination and seedling growth of other plants (Rudrappa et al., 2007; Uddin et al., 2012; Uddin et al., 2014c). One particular study attributed the decline in the associated wetland species *M. ericifolia* in the field to the inhibition of its seed germination and seedling growth by *P. australis* in the lab (Uddin et al., 2014c).

However, there is contradicting evidence that shows that gallic acid released from *P. australis* has a very short half-life in the soil and that allelopathy is not a primary mechanism for its invasion success (Weidenhamer et al., 2013).

The conservation area where *S. hermaphrodita* has one of its two populations in Canada is the study site presented in this chapter. Once the dam was removed and the reservoir dewatered the *S. hermaphrodita* population expanded into this area. But with this so did the expansion of the *P. australis* population. While initially their populations were isolated, they are now encroaching upon each other with edges of each overlapping (Figure 23). To understand the dynamic between *P. australis* and *S. hermaphrodita* a field study was conducted. The main questions asked here are: 1. Are *S. hermaphrodita* and *P. australis* numbers at the study site changing over time? 2. Is there a change in *S. hermaphrodita* population area and stand boundaries over time? Based on the studies conducted showing that *P. australis* is allelopathic and in general the known invasiveness of species, it is expected that there will be a reduction of *S. hermaphrodita* and increase in *P. australis* numbers in the permanent vegetation plots over time. It is also expected that *P. australis* is encroaching upon *S. hermaphrodita*, so the total population area will decrease over time.



Figure 23. *Sida hermaphrodita* stand (left) adjacent to a stand of *Phragmites australis* (right) at the study site. The two stands here are seen overlapping.

3.2. Methods

3.2.1. Study site

Field work took place at a conservation area north of Lake Erie in Southern Ontario (Figure 24). The area has one of the two populations of *S. hermaphrodita* in Ontario (Environment Canada, 2015). Prior to 2006 a reservoir was present on the site until it was dewatered. An effort to create a cold-water stream, pairing ponds, and marshes throughout the site followed. As a result of dewatering the reservoir, new habitat allowed for the expansion of the *S. hermaphrodita* population (Environment Canada, 2015). The new habitat also allowed the further invasion of *P. australis*, potentially threatening the persistence of *S. hermaphrodita* at the site. The study area, located at the northwest of the property, was chosen to encompass the sites at the conservation area where *P. australis* and *S. hermaphrodita* stands were next to each other. Compared to the rest of the conservation area this area was likely to show encroachment of the two species within the following survey years.

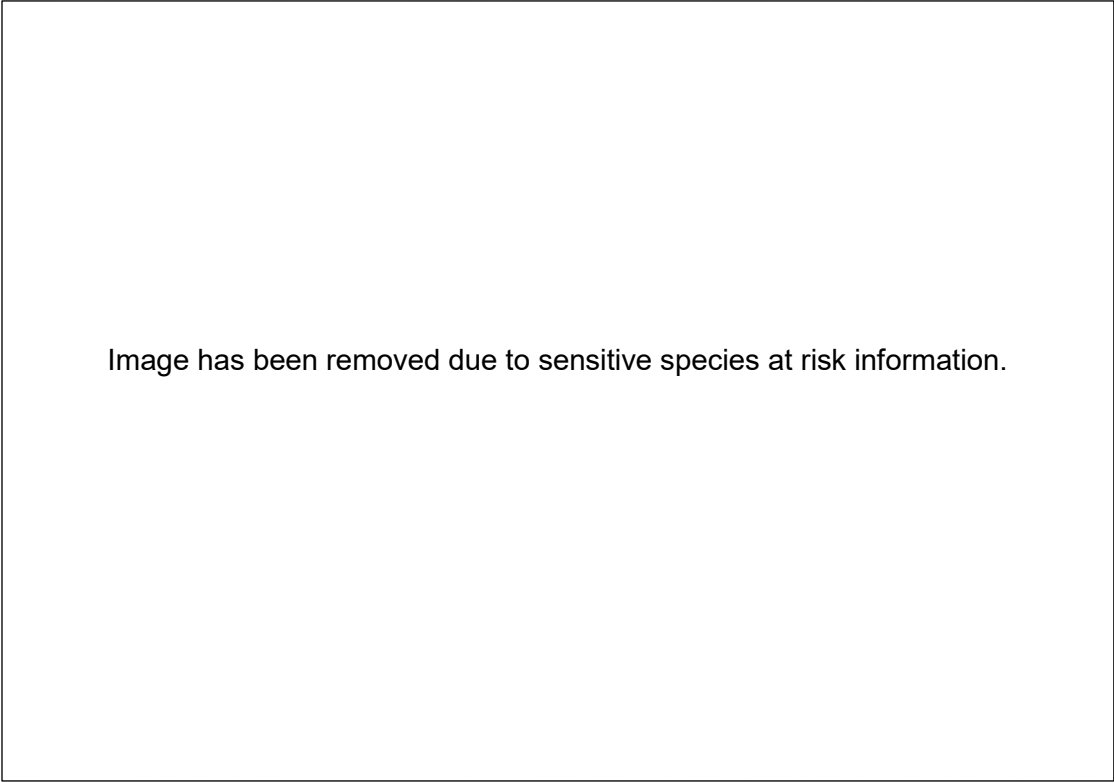


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Figure 24. Property boundaries of the conservation area with an outline of the study area, dewatered reservoir, and the locations of the decommissioned dam and creek.

3.2.2. Vegetation surveys and mapping

Twenty-eight permanent 1 m by 1 m vegetation plots were set up to measure the changes in *S. hermaphrodita* stand boundaries over time. Plots were placed next to the stands of *S. hermaphrodita* where seedlings could still be found. Vegetation surveys were conducted in July of 2016, 2017, and 2018. At each plot *S. hermaphrodita* and *P. australis* stems were counted. To obtain density (stems/m²) the total number of stems for *P. australis* and *S. hermaphrodita* in each year was divided by the number of 1 m by 1 m vegetation plots (28). Vegetation mapping of the *S. hermaphrodita* stands was conducted during the summer in 2014, 2016, and 2018 using a SX Blue II GPS. The stand boundaries were mapped by walking the perimeter of each stand. Stands were considered to be separate if there was greater than one-metre distance between two plants. Area was obtained by analyzing the total area of all stands in ArcMap.

3.3. Results

In 2016, 2017, and 2018 density of *S. hermaphrodita* was 6 stems/m², 10.8 stems/m², and 10.6 stems/m² and 4.7 stems/m², 2.2 stems/m², and 2.1 stems/m² for *P. australis* respectively. These results indicated an increase in *S. hermaphrodita* density and decrease in *P. australis* density over time (Figure 25). Total population areas of the *S. hermaphrodita* population at the study site for 2014, 2016, and 2018 were 1574 m², 2611 m², and 3828 m² respectively. Total area showed an overall increase in *S. hermaphrodita* area over time (Figure 26). Mapping of the *S. hermaphrodita* stand boundaries at the study site were shown to increase or expand from 2016 (yellow) to 2018 (purple) (Figure 27).

3.4. Discussion

Since the dewatering of the reservoir in 2006, *P. australis* and *S. hermaphrodita* have expanded onto the site (COSEWIC, 2010). Before 2014 *P. australis* and *S. hermaphrodita* populations were isolated but still expanding. There was concern that these populations would begin encroaching upon one another and *P. australis*, given its invasiveness, would threaten the persistence of *S. hermaphrodita* at the conservation area. *S. hermaphrodita* is extremely rare and endangered in Canada so its conservation is required under SARA. It is also important to protect so biodiversity is not lost

(Environment Canada, 2015). The present study was conducted to determine the impact *P. australis* had on *S. hermaphrodita*. At the study site, 28 permanent vegetation plots were placed on the periphery of *S. hermaphrodita* stands to observe how they were changing over time. Population mapping also occurred to measure the total area of *S. hermaphrodita* to see how it changes over time.

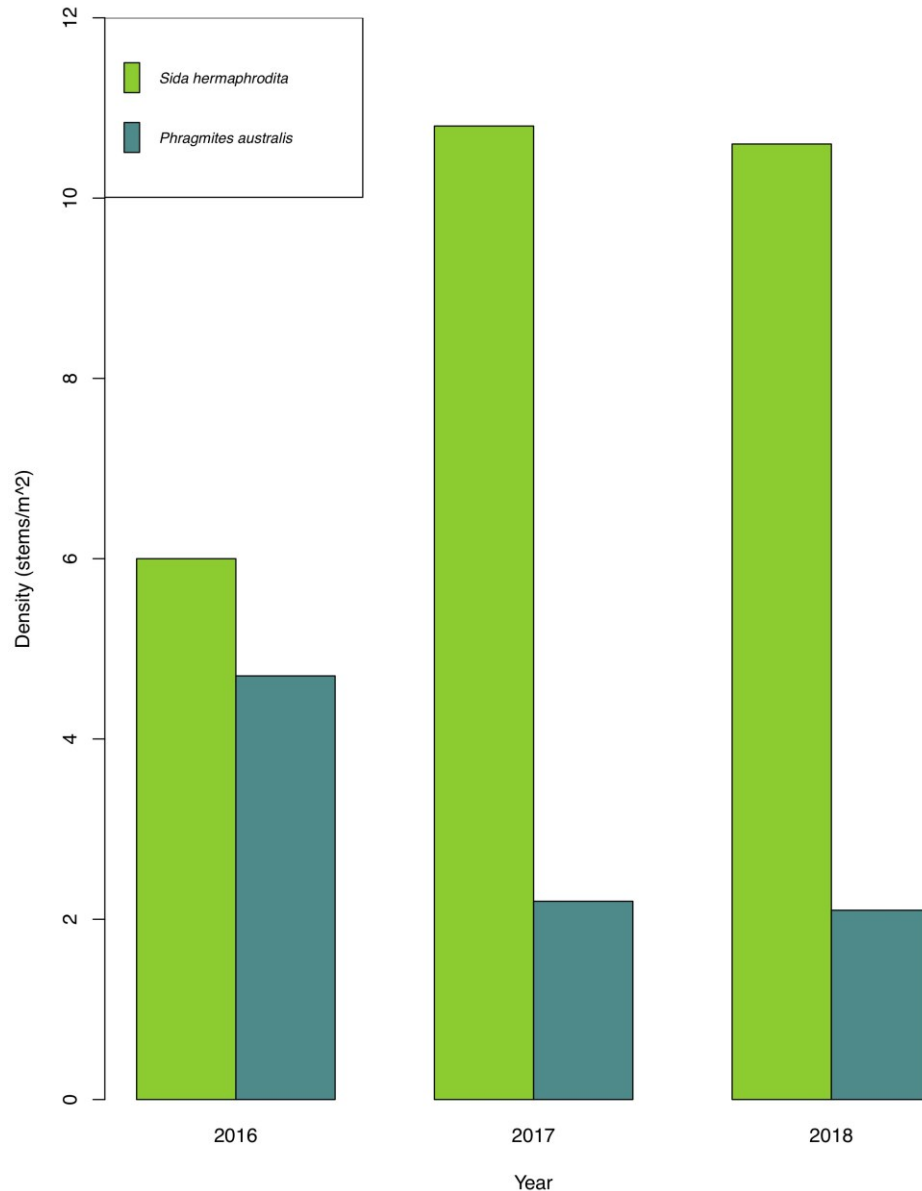


Figure 25. *Sida hermaphrodita* (light) and *Phragmites australis* (dark) density from 2016, 2017, and 2018 at the study site. (density=stems/m²).

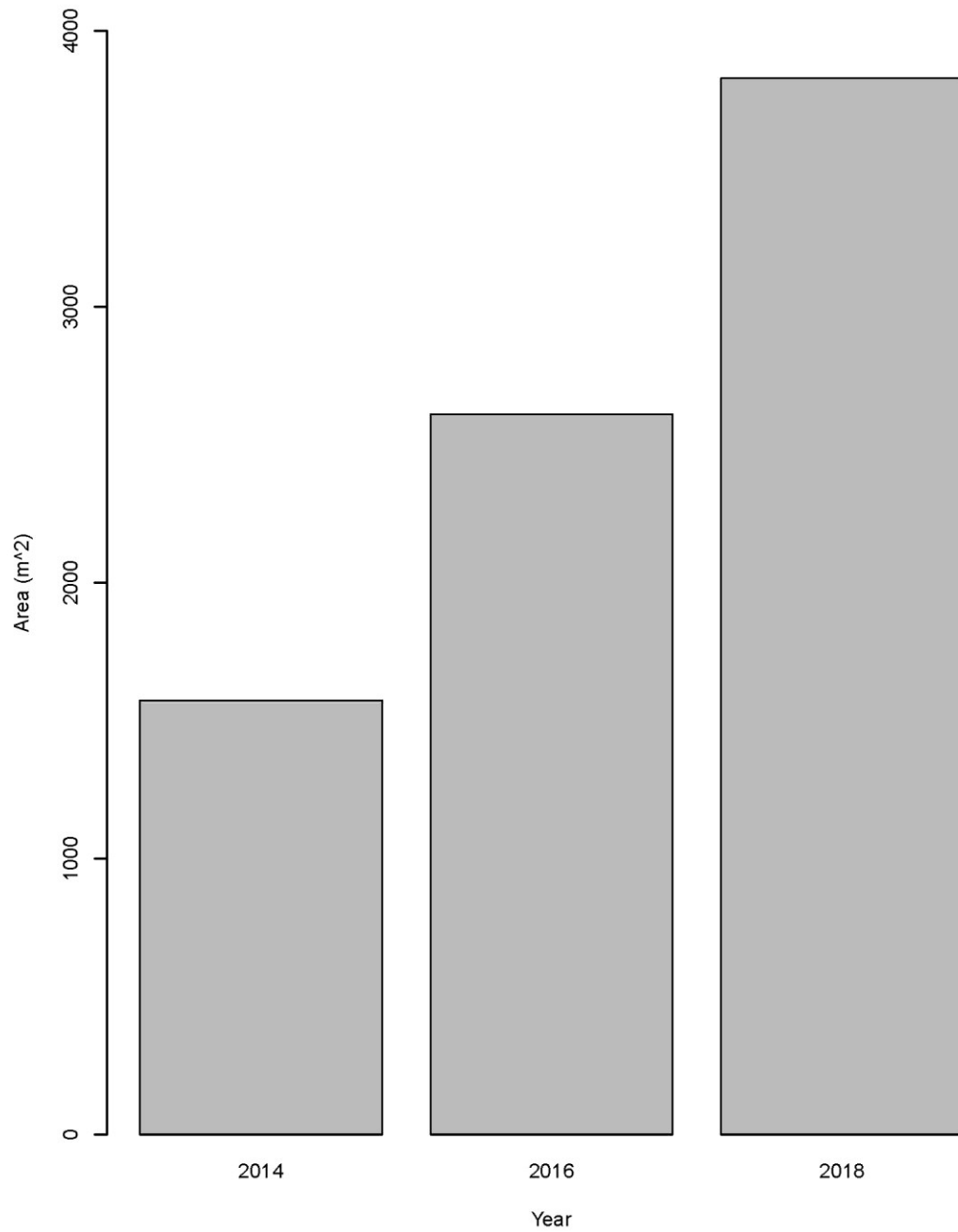


Figure 26. Total *Sida hermaphrodita* stand area (m²) in 2014, 2016 and 2018 at the study site.

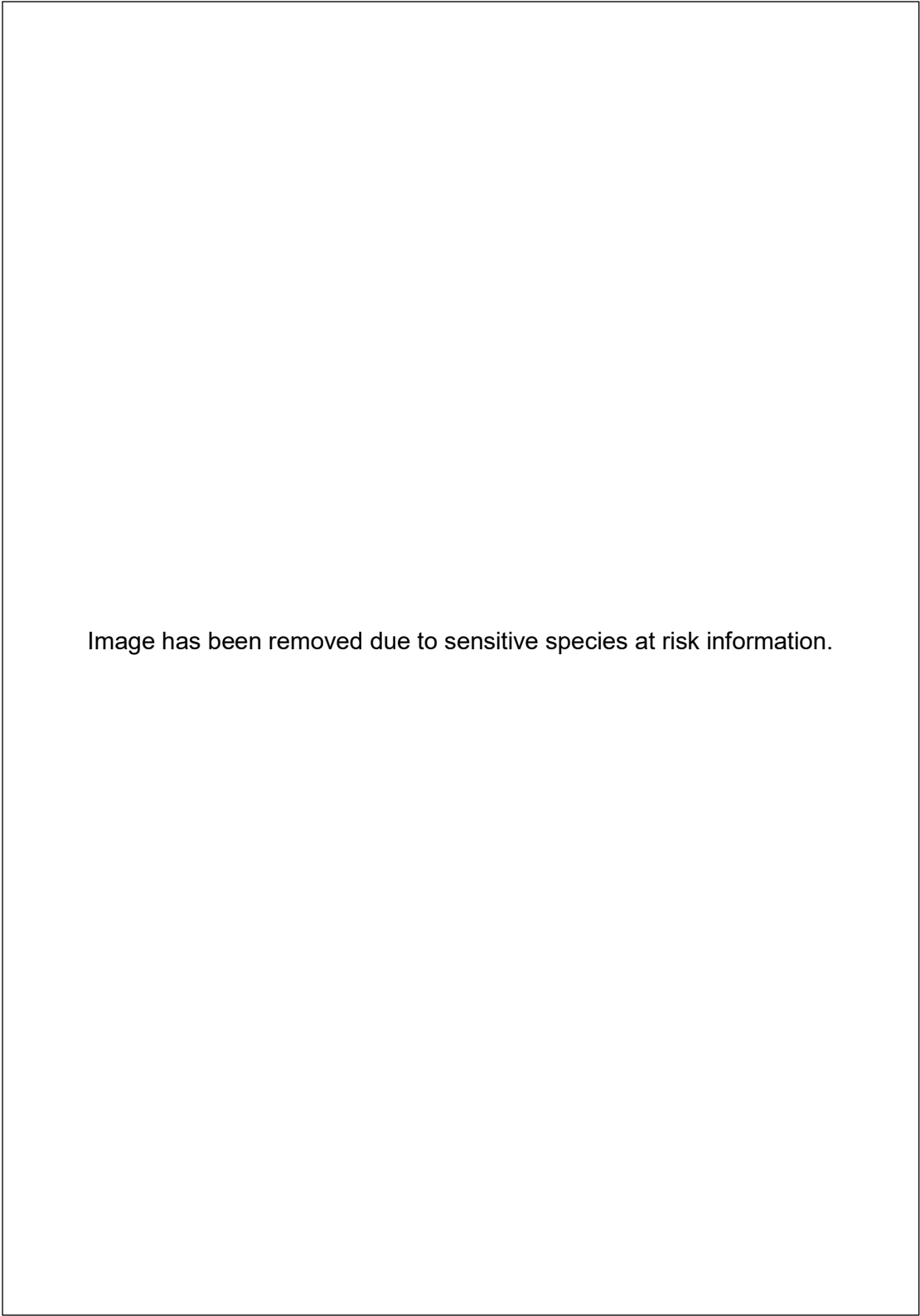


Image has been removed due to sensitive species at risk information.

Figure 27. *Sida hermaphrodita* 2016 stand mapping outlined in yellow and the 2018 stand mapping overlayed on top in purple within the study area in Southern Ontario.

The results showed that since 2014 the total area of *S. hermaphrodita* has increased despite the overlap between itself and *P. australis*. The mapping data shows that the *S. hermaphrodita* stands in the study area are expanding outwards. The vegetation plots also confirmed this observation. Overall, the density of *S. hermaphrodita* stems within the plots have increased since 2016 and the density of *P. australis* stems have decreased. Based on the invasiveness (Catling & Mitrow, 2011; Invasive Species Centre, 2016; Mal & Narine, 2011) of *P. australis* and the recent evidence of its use of allelopathy (Rudrappa et al., 2007; Uddin et al., 2012), the results of the present study were unexpected.

In the field a number of different interactions may be occurring all at the same time. Resource competition, allelopathy, nutrient mobilization, and microbial influence may all operate simultaneously (Bhowmik & Inderjit, 2003; Inderjit & Del Moral, 1997). With respect to plant species interaction any one or a combination of these can influence plant dominance. The trade-offs and asymmetry between native and invasive species of these biotic interactions have been shown to facilitate their coexistence (Heard & Sax, 2013). Coexistence is likely to occur in invaded communities when the invasive species exhibit functional or niche differences compared to the other species (Adler, 1999; Daehler, 2003; Shea & Chesson, 2002). In this case *S. hermaphrodita* and *P. australis* could be functionally different, have different niches or competition trade-offs explaining their coexistence at the study site. However, more research would be required to determine these differences. The expansion of *P. australis* and *S. hermaphrodita* onto the study site is relatively recent. The plant community is likely still establishing and may change over time. A limitation here is the timeframe of this study. To fully understand the interspecific population dynamics, more long-term data would be beneficial.

There is also an opportunity to address the controversy in the *P. australis* allelopathy here. Many studies have shown that invasive species, including *P. australis* are very genetically diverse among and within their new range (Lankau, 2012; Lavergne & Molofsky, 2007). Across the introduced range of *Alliaria petiolate* (Garlic Mustard) populations have been shown to vary in their investment to allelopathic traits depending on invasion history (Lankau, 2012). This leads to allelopathic gradients of impact on native species. The variation in allelochemicals seen by Weidenhamer et al. (2013) and Uddin et al. (2012) could be result of the genetic diversity observed in invasive species. It is also important to note that the Weidenhamer et al. (2013) and Uddin et al. (2012)

studies were conducted in the United States and Australia respectively. Differences in results may be caused by these variations in invasion history. Since *P. australis* is abundant around the world the allelopathic potential of this species may vary at a global, landscape or local level. However, additional research specifically on *P. australis* would be required to understand these variations.

At the study site *S. hermaphrodita* is currently not in decline as a result of *P. australis* encroachment. From the vegetation surveys and mapping it was shown that the *S. hermaphrodita* population at the study site is increasing in size and number. Although, *P. australis* is allelopathic the effect on *S. hermaphrodita* in the field was not enough to displace it. This likely was due to the ability of these two species to coexist through occupying different niches, having different functional traits or interaction trade-offs.

Chapter 4. Conclusion

The main purpose of this project was to determine if the species at risk *S. hermaphrodita* and *A. robusta* are affected by *P. australis* allelopathy. *P. australis* is a highly invasive grass that is widespread and abundant throughout much of the United States and southern Canada (Mal & Narine, 2011). Several studies have shown that *P. australis* exhibits allelopathy on several species through the release of root exudates and the leaching of litter (Rudrappa et al., 2007; Uddin et al., 2012). Using allelopathy and other mechanism such as rapid spread and growth, *P. australis* aggressively invades an area creating a dense monotypic stand that decreases biodiversity (Mal & Narine, 2011). For many species and the species at risk discussed in this study the effect of allelopathy could threaten their persistence in an area invaded by *P. australis*. Understanding these complex interactions is beneficial for determining management option for both invasive species and species at risk. The questions answered in this project were: 1. Do extracts made from *P. australis* inhibit seed germination of *S. hermaphrodita* and *A. robusta* in growth chamber studies? 2. Do extracts made from *P. australis* inhibit seedling growth of *S. hermaphrodita* and *A. robusta* in growth chamber studies? 3. Are *S. hermaphrodita* and *P. australis* numbers at the study site changing over time? 4. Is there a change in *S. hermaphrodita* population area and stand boundaries over time?

From this study it was found that *P. australis* had an inhibitory effect on the seed germination of both *S. hermaphrodita* and *A. robusta*. However, the severity of inhibition for each tissue type was different for *S. hermaphrodita* (leaf > rhizome > root) compared to *A. robusta* (root > rhizome > leaf) indicating a species-specific effect. Inhibition of germination also did not follow the dose-response seen in other species and few extracts inhibited germination past LC₅₀. This indicated that higher concentrations were would be required to produce more inhibition. Although seed germination was inhibited there was no effect of tissue type, concentration of the extract or their interaction on *S. hermaphrodita* seedling growth. From these results it is likely that *S. hermaphrodita* and *A. robusta* populations would not be strongly affected by *P. australis* allelopathy in field conditions. If there was a strong effect of *P. australis* allelopathy it would be expected that populations of *S. hermaphrodita* that were present on sites with *P. australis* would be in decline. The results of this project show that *S. hermaphrodita* is in fact increasing

in population size and density at the study site. The idea of trade-offs is a likely explanation for this observation. From the seed germination assays it was found that there is an inhibition of seed germination so *S. hermaphrodita* is susceptible to *P. australis* allelopathy. In the field however, there may be an interaction between *S. hermaphrodita* and *P. australis* where the former is doing better, trading-off with *P. australis* allelopathy and causing them to coexistence. The absence of a strong effect in laboratory and field studies should not lessen the need for intense management of *P. australis*.

4.1. Implications for Restoration

As the spread and abundance of *P. australis* is rapidly increasing across North America, there is a need for viable management options (Catling & Mitrow, 2011). The invasion of *P. australis* causes local extirpation of some species including species at risk (Silliman & Bertness, 2004). Specific to this study it seems that *S. hermaphrodita* is currently not at risk from *P. australis* invasion. Although the nature of their interaction at the study site in the future is unknown and control for *P. australis* should be a priority. The Ontario Ministry of Natural Resources released protocol for the best management practices in 2011 reporting on the most effective ways to manage *P. australis* (Ontario Ministry of Natural Resources and Forestry, 2011). Herbicide treatment is considered one of the most effective ways to manage *P. australis*. Typically, application occurs in early fall when resources are being allocated to the roots. By waiting until fall however, *P. australis* seeds have already matured so multi-year management may be necessary (Ontario Ministry of Natural Resources and Forestry, 2011). Herbicide treatment is frequently followed by mowing or rolling and prescribed burns. These methods are not effective alone but in tandem with herbicide treatment are very effective.

At the study site, a multiple year management program for *P. australis* is in effect. In the first year of management, managers applied herbicide then rolled the aboveground biomass onto the ground. The location encompassing our study area was managed after the field study presented here was completed. In their management they also opted to not include prescribed burns in their management plan since they would put *S. hermaphrodita* at risk. So, once the *P. australis* is sprayed it is rolled on the ground and left alone. Not only would this block out any light for the germination of seeds but based on the results of this study it may also inhibit the seed germination of *S.*

hermaphrodita and seed germination and seedling growth of other plants. The large density of litter could likely produce high amounts of leachate that potentially have an allelopathic effect. Typically burning following rolling would be suggested but even if this was feasible at the site there has been evidence that even burned *P. australis* litter has the same allelopathic ability to inhibit seed germination and seedling growth compared to litter that has not been burned (Uddin & Robinson, 2017).

From the study results I suggest that the above ground biomass be removed from the site following herbicide treatment. The results of this study also showed that rhizomes inhibit seed germination and other results showed it inhibits seedling growth in some species (Uddin et al., 2012). Furthermore, the *P. australis* rhizosphere contains phenolics that potentially accumulate and persist for an extended period of time. This information suggests that removal of rhizomes after treatment would be the best option to counteract allelopathy. However, it may do more harm than good to remove the belowground parts of the plant, so a long-term management plan may be required. Multiple years of treatments may be required to control *P. australis* followed by multiple years of seeding to counter the allelochemicals that potentially remain in the soil. Some other things to consider when managing for *P. australis* include regulatory information on herbicide use, timing of herbicide application, and wildlife assessments.

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Appendix A.

Laboratory Photographs



Figure A1. Dried *Phragmites australis* tissue used to make the 10% base extract for the seed germination and seedling growth assays. This image depicts *P. australis* dried rhizome segments.



Figure A2. Mixing vessel used for the dried plant material and deionized water to make the 10% base extract used in the seed germination and seedling growth assay. This image depicts *Phragmites australis* rhizomes.



Figure A3. Apparatus used to separate the plant material from the aqueous extract after 24 hours on the shaker. The vacuum pump on the right pulled the aqueous extract through successive filters of decreasing pore size (25 μm , 2.5 μm and 0.45 μm) into the side arm flask.



Figure A4. *Phragmites australis* rhizome extracts after separation from plant material, filtration using successive filters of decreasing pore size (25 μm , 2.5 μm and 0.45 μm) and dilution to different concentrations (10% base concentration to 5%, 2.5% and 1.25%) using deionized water.



Figure A5. *Phragmites australis* leaf extracts after separation from plant material, filtration using successive filters of decreasing pore size (25 μm , 2.5 μm and 0.45 μm) and dilution to different concentrations (10% base concentration to 5%, 2.5% and 1.25%) using deionized water.



Figure A6. *Phragmites australis* root extracts after separation from plant material, filtration using successive filters of decreasing pore size (25 μm , 2.5 μm and 0.45 μm) and dilution to different concentrations (10% base concentration to 5%, 2.5% and 1.25%) using deionized water.

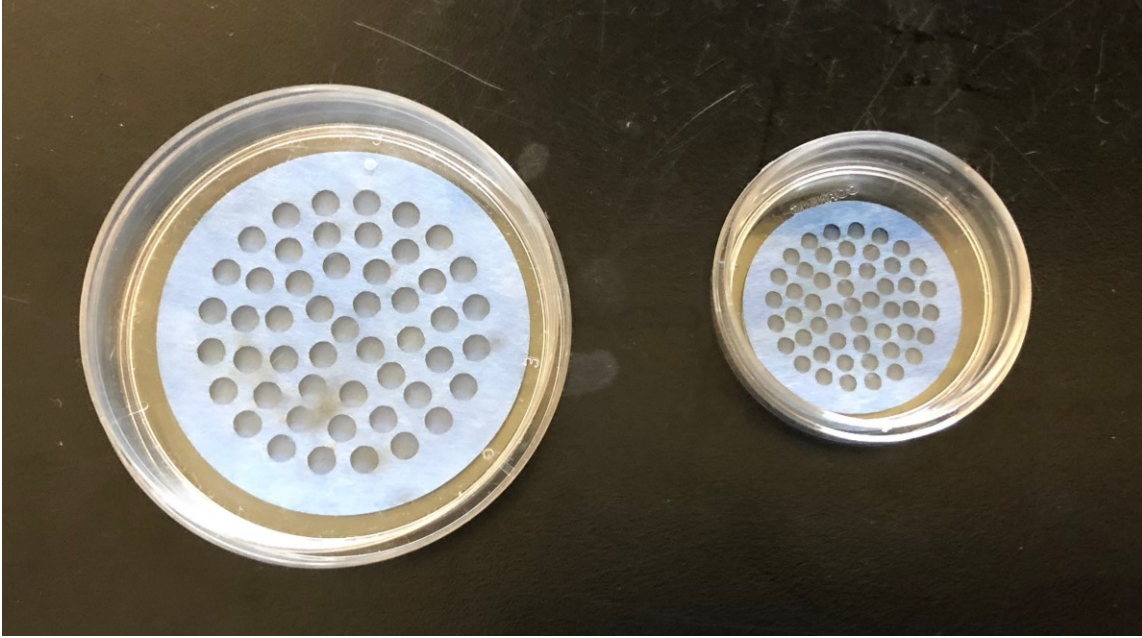


Figure A7. Petri dishes lined with a bottom layer of solid blue blotter paper and a top layer of blotter paper with 50 holes used in the *Sida hermaphrodita* (left) and *Ammannia robusta* (right) seed germination assays.

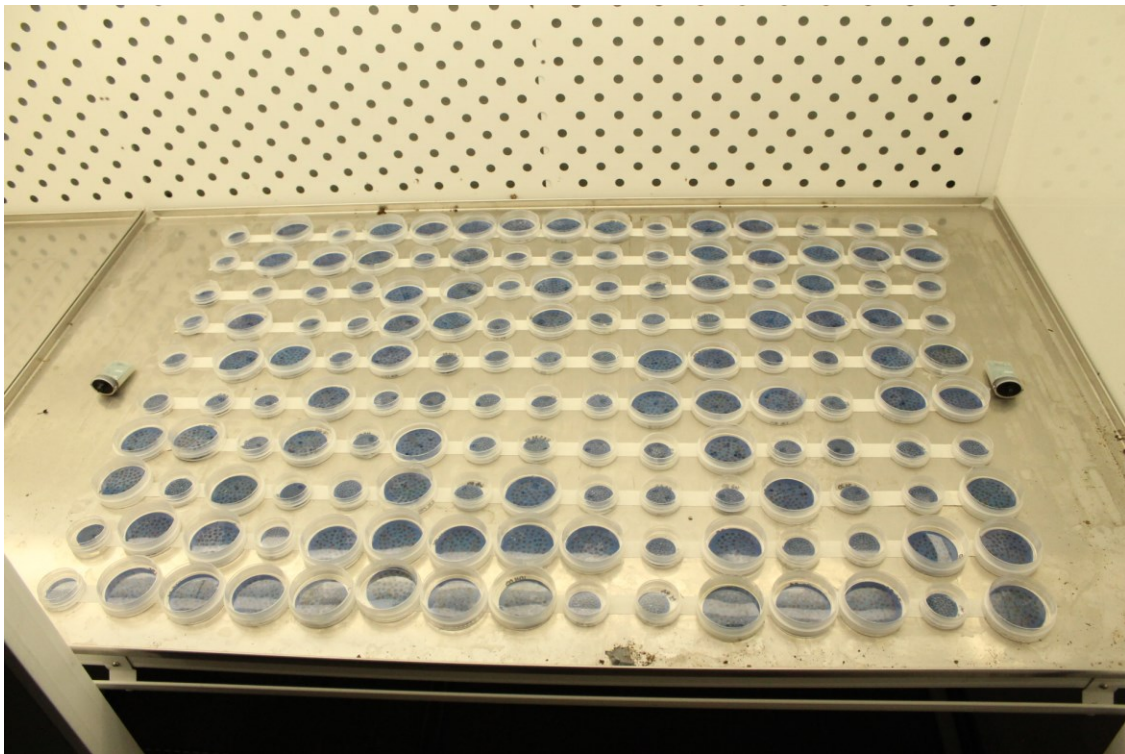


Figure A8. Petri dish arrangement in the growth chamber for the *Sida hermaphrodita* and *Ammannia robusta* seed germination experiment.

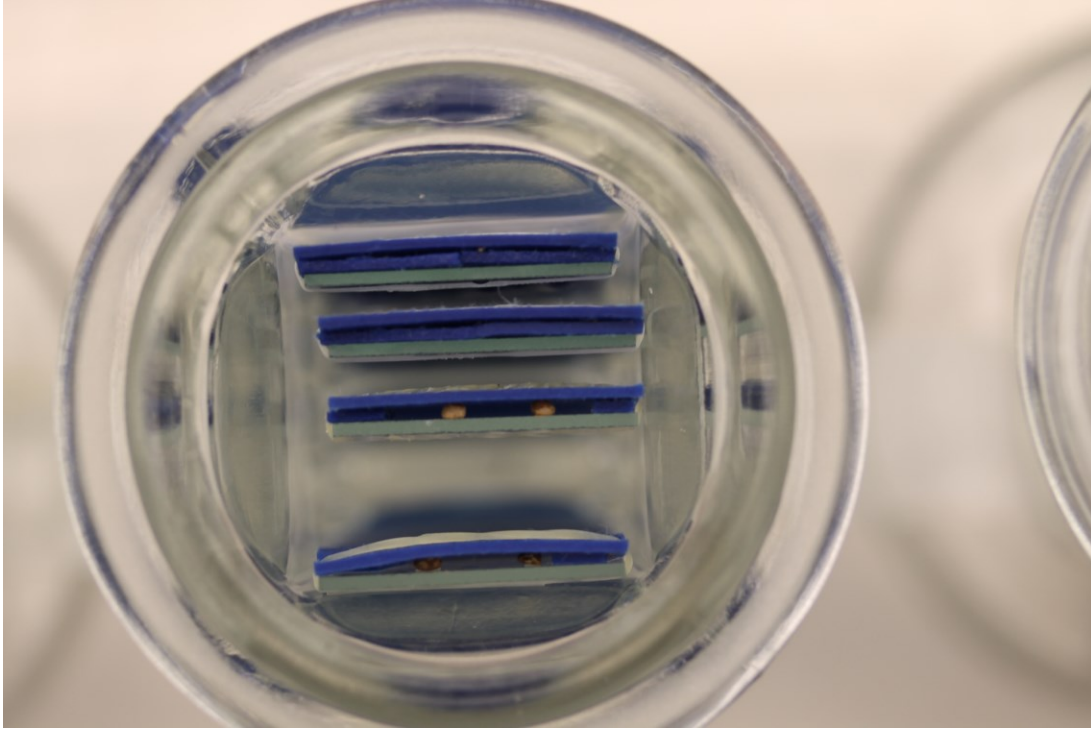


Figure A9. Microscope slide arrangement in a Coplin jar for one of the *Sida hermaphrodita* seedling growth assays.

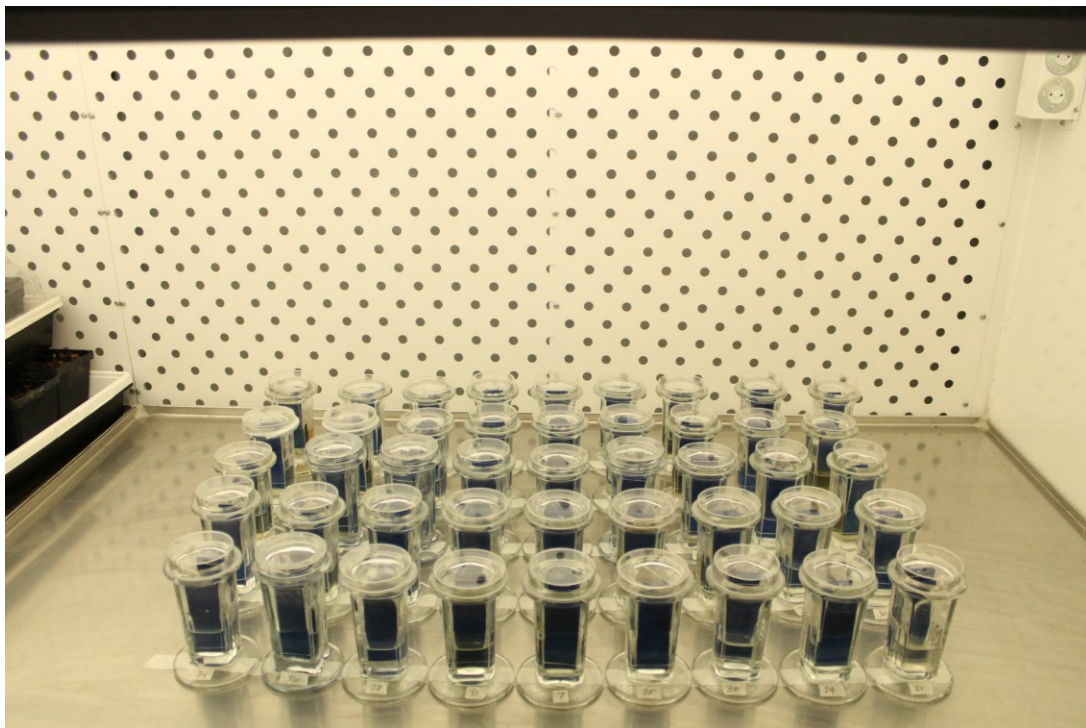


Figure A10. Coplin jar arrangement in the growth chamber for the *Sida hermaphrodita* seedling growth experiment.

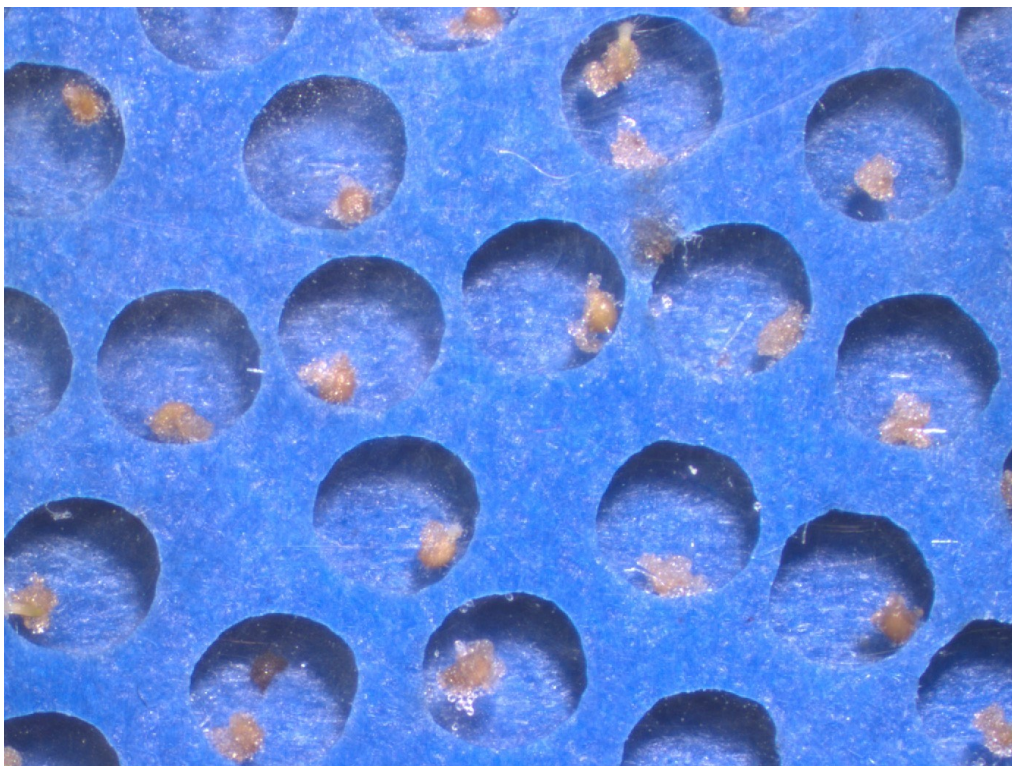


Figure A11. *Ammannia robusta* seeds in the seed germination assay (10X magnification).

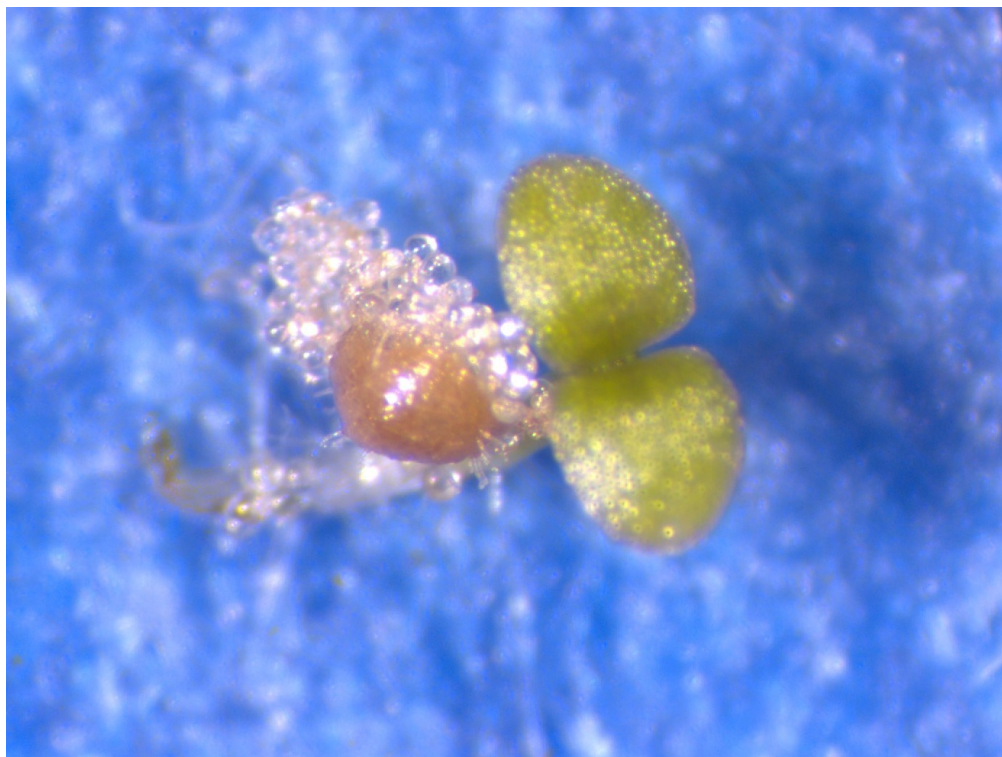


Figure A12. *Ammannia robusta* seedling escaped from the seed coat (50X magnification).

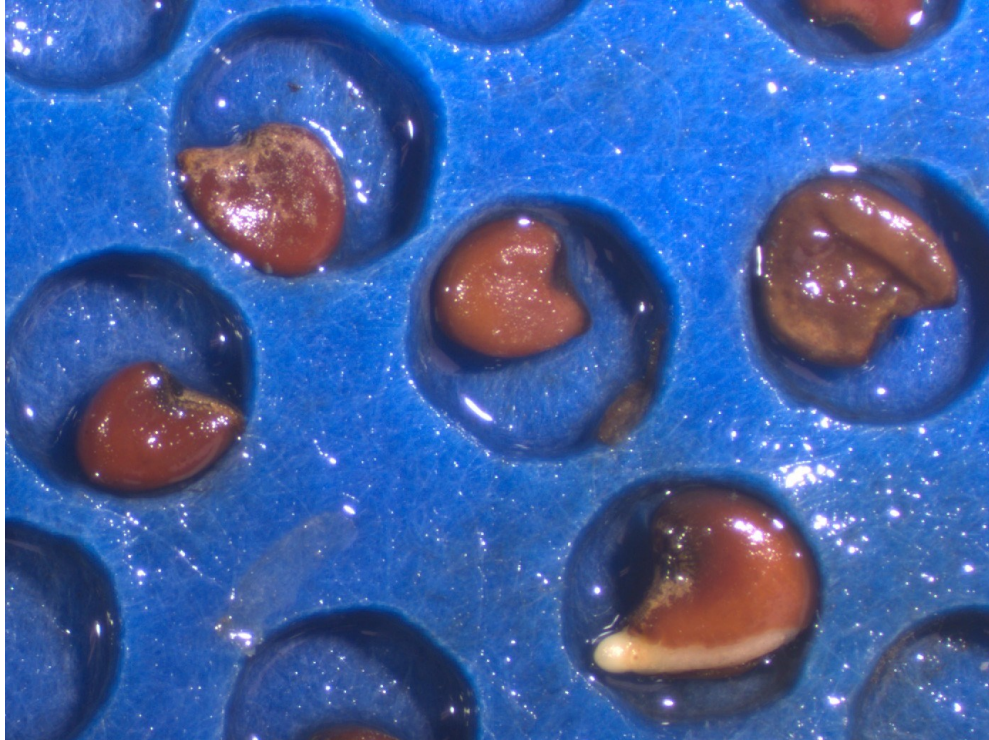


Figure A13. *Sida hermaphrodita* seeds in the seed germination assay (10X magnification)

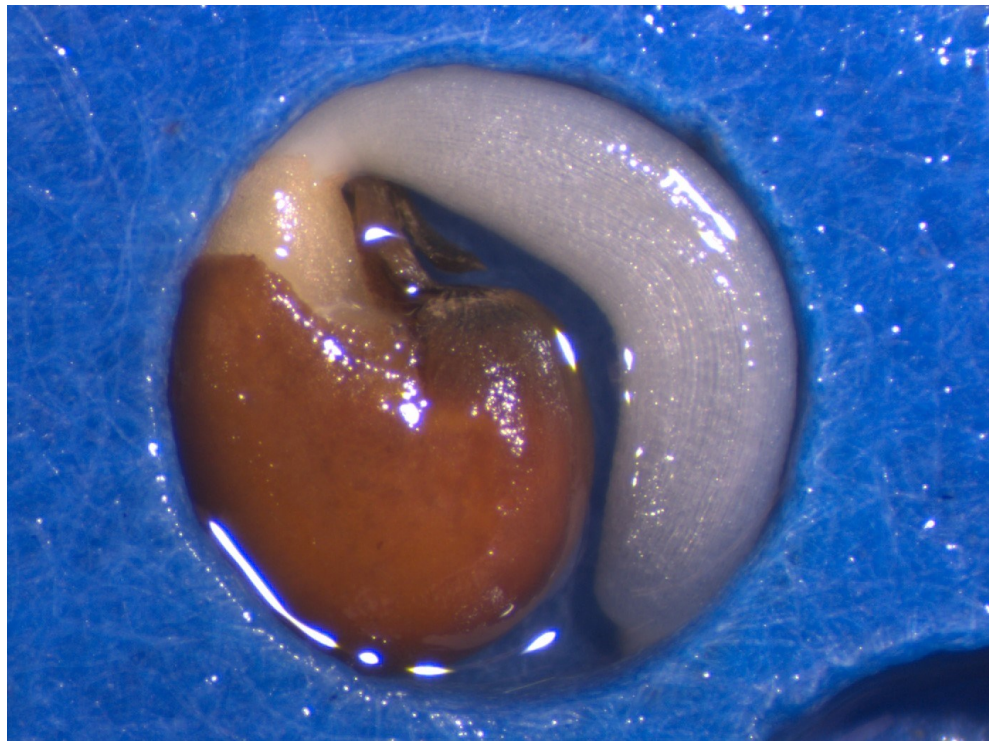


Figure A14. *Sida hermaphrodita* seed germinated with radicle protrusion (25X magnification).

Appendix B.

Field Photographs



Figure B1. 1 m x 1 m permanent vegetation plot at the study site.



Figure B2. *Phragmites australis* (left) and *Sida hermaphrodita* stands (center and right) at the study site.



Figure B3. *Phragmites australis* stand with myself as a scale (1.88 m).



Figure B4. *Sida hermaphrodita* stand with myself as a scale (1.88 m).

Appendix C.

Vegetation survey data collected in July of 2016, 2017 and 2018 from 28 permanent vegetation plots at the study site. The table shows the number of *Sida hermaphrodita* vegetative stems and *Phragmites australis* stems per plot and totals for each year.

Plot	Year					
	2016		2017		2018	
	<i>Sida hermaphrodita</i> Vegetative Stems	<i>Phragmites australis</i> Stems	<i>Sida hermaphrodita</i> Vegetative Stems	<i>Phragmites australis</i> Stems	<i>Sida hermaphrodita</i> Vegetative Stems	<i>Phragmites australis</i> Stems
1	1	0	0	0	0	0
2	2	0	1	0	1	0
3	39	0	22	0	3	0
4	7	0	0	0	0	0
5	6	0	24	0	3	0
6	1	0	30	0	29	0
7	43	0	23	0	36	0
8	2	0	21	0	20	0
9	10	0	9	0	11	0
10	2	0	37	0	42	0
11	8	0	5	0	0	0

Plot	Year					
	2016		2017		2018	
	<i>Sida hermaphrodita</i> Vegetative Stems	<i>Phragmites australis</i> Stems	<i>Sida hermaphrodita</i> Vegetative Stems	<i>Phragmites australis</i> Stems	<i>Sida hermaphrodita</i> Vegetative Stems	<i>Phragmites australis</i> Stems
12	0	0	12	0	8	0
13	0	0	0	0	0	0
14	0	0	0	0	0	0
15	9	0	23	0	20	0
16	15	19	2	3	2	3
17	0	0	0	0	9	0
18	0	0	5	0	4	0
19	7	1	6	20	5	23
20	0	13	8	0	2	5
21	5	8	8	27	7	24
22	0	2	6	0	5	0
23	0	0	12	0	10	0
24	1	0	25	0	31	0
25	0	0	3	9	7	1
26	1	0	18	2	31	3
27	1	0	3	0	11	0
28	8	88	0	0	1	0
Total	168	131	303	61	298	59