# THE GERMINATION OF *HELONIAS BULLATA* L. (SWAMP PINK) IN RESPONSE TO FLOODED, SATURATED, AND DRY CONDITIONS

A thesis presented to the faculty of the Graduate School of
Western Carolina University in partial fulfillment of the
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# PREFACE

This thesis is structured in a manuscript format. Chapter one includes the intent and purpose of this project. Chapter two contains a broad literature review related to the biological concepts in Chapter three. Chapter three includes the manuscript organized for submission to *Aquatic Botany*. The inclusive literature review cites references from all three chapters in the manuscript.

# ABSTRACT

THE GERMINATION OF *HELONIAS BULLATA* L. (SWAMP PINK) IN RESPONSE TO FLOODED, SATURATED, AND DRY CONDITIONS

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Poor sexual recruitment is a major conservation concern for the federally threatened, obligate wetland species *Helonias bullata* L. (swamp pink). *Helonias* predominately occurs in forested wetlands amongst hummock-hollow topography where water levels fluctuate spatially and temporally. This patchiness in moisture creates a high level of unpredictability in suitable microsites for germination and subsequent establishment. Germination percentages and rates were compared among seeds placed in flooded, saturated, and dry conditions in a controlled and natural environment for 1-35 days to determine how moisture conditions influence germination. In a controlled and natural environment, *Helonias* exhibited high final germination percentages (>50%) after all treatments, except dry conditions in the growth chamber. In addition, longer exposure to dry conditions both in a controlled and natural environment increased lag time before germination onset. Conversely, germination onset occurred earlier for seeds exposed to moisture treatments in a controlled (floating and submerged) and natural environment (stream margin, floating, and stream margin) for 3-30 days. For instance, after 13 days in a controlled environment, seeds floating (90%) and submerged (77%) germinated while in treatment. In the field, seeds floating (58%), submerged (3%), and in the stream margin (49%) germinated while in treatment after 20 days. Conversely, seeds "dry"

(only exposed to rainwater) in the field after 20 days did not germinate while in treatment. Water dispersal may be an important part of *Helonias* regeneration niche because seeds exhibited high floating capability. Also, final germination percentages were significantly higher for seeds floating in comparison with seeds dry in the field. Overall, *Helonias* seeds exposed to moisture conditions (floating, submerged, and stream margin) germinated within a short time frame (10-30 days). Rapid germination when conditions are appropriate may be important for *Helonias* since it occurs in forested wetlands where spatial and temporal heterogeneity can create a narrow window for regeneration.

# CHAPTER ONE: INTRODUCTION

Many plant species are threatened because seed production, seed dispersal or germination is hindered or restricted (Brown et al. 2003; Chen and Xie 2007). Conservation efforts are improved by understanding factors that limit a rare plant's ability to colonize new suitable habitat, increase in population density, or to maintain population density (Chen and Xie 2007). Fundamental to any rare plant conservation plan is a basic knowledge of both seed dispersal and germination ecology (Ikeda and Itoh 2001). Dispersal for rare plants may be restricted due to a lack of effective seed dispersal agents (Primack 1995). Also, the distance the seed has to travel to colonize new suitable habitat may be too far from the existing population due to the rarity of the specialized habitat (Primack 1995; Ellison and Parker 2002). For some rare species, germination may be highly dependent upon specialized locations within the habitat, e.g. safe sites, or the timing of certain events, e.g. flooding (Primack 1995).

Low seedling recruitment is a major conservation concern for the federally threatened, obligate wetland species *Helonias bullata* L. (swamp pink) (U.S. Fish and Wildlife Service 1991). *Helonia*s *bullata* is the only species within the genus *Helonias*; therefore, it is referred to as *Helonias* throughout this thesis. This species is an evergreen liliaceous perennial that occurs predominantly in forested wetlands, such as *Chamaecyparis thyoides* (Atlantic white cedar) swamps, inland stream corridors, spring seepage areas, and swamp forest-bog complexes (Murdock 1994; Dodds 1996). The geographic range of *Helonias* is from the coast of New Jersey, Delaware, Maryland, and Virginia to the mountainous regions of Virginia, North Carolina, South Carolina, and

Georgia (U.S. Fish and Wildlife Service 1991). Across this species' range, inflorescence production appears low (Sutter 1984; U.S. Fish and Wildlife Service 1991). However, *Helonias* has highly self-compatible flowers that produce a copious amount of viable seeds (Sutter 1984; U.S. Fish and Wildlife Service 1991). Despite high seed viability, results from demographic monitoring have revealed low seedling recruitment in the field (Sutter 1984; Peterson 1992; Gary Kauffman, personal communication).

*Helonias* occurs amongst hummock-hollow topography (Laidig et al. 2009), where flooded and drought conditions can occur (Ehrenfeld 1995b). Hummocks formed by the accumulation of woody debris, organic matter, and peat typically occur 50 cm to 100 cm above the forest floor, and hollow or depressions occur at base elevation generally with a water table at or above the soil surface (Ehrenfeld 1995b; Duberstein and Conner 2009). The difference in elevation across microtopography greatly influences moisture and saturation levels (Ehrenfeld 1995a), which determines microsite suitability for germination (Vivian-Smith 1997). Because *Helonias* reportedly has short-lived seeds (U.S. Fish and Wildlife Service 1991), germination and seedling establishment may depend on suitable conditions at time of seed arrival (van der Valk 1981). Laidig et al. (2009) demonstrated *Helonias* rosettes were commonly associated with hummocks in and along poorly defined stream channels. Thus, seeds could land in a variety of microsites with different moisture conditions. For instance, seeds could land in the stream channel, float and disperse by water or sink and become submerged. On the other hand, seeds could land on the upper emergent portions of hummocks where drier conditions occur or remain dry in dehiscent capsules for several days before dispersal. Currently, the germination response of *Helonias* seeds after exposure to various moisture conditions remains undetermined.

The objective of this study was to determine how different moisture conditions, including flooded, saturated, and dry influence the germination of *Helonias*. To accomplish this objective, three experiments in a controlled environment were used to test the germination response of seeds: (1) floating, (2) submerged, and (3) kept dry without water. In addition, one experiment was conducted in field conditions to test the germination response of seeds placed in flooded (floating and submerged), saturated, and dry conditions in a small headwater stream. Questions addressed were: (1) how does seed contact with water, floating and/or submerged, affect the germination response (percentages and rates) of *Helonias* seeds, (2) what is the germination response (percentages and rates) of seeds placed in a saturated stream margin, and (3) what is the germination response (percentages and rates) of *Helonias* seeds that remain dry or in dehiscent capsules?

# CHAPTER TWO: LITERATURE REVIEW

## Water Dispersal

In wetland habitats, many plants depend on water dispersal or hydrochory for colonizing new suitable microsites (Soomers et al. 2010). Water dispersal appears to play an important role in structuring wetland plant communities (Schneider and Sharitz 1988; Brock et al. 1989; Nilsson et al. 1991; Edwards et al. 1994; Middleton 2000; Pollux et al. 2009; Soomers et al. 2010) because plant composition seems more dependent upon propagule colonization in comparison with competition (Nilsson et al. 1991). Hydrochory serves as an important source of species immigration to sites near or in water (Nilsson et al. 2010). Seeds disperse by a variety of water bodies, including rivers (Nilsson et al. 1991), drainage ditches (Soomers et al. 2010), streams, swamps (Schneider and Sharitz 1988; Edwards et al. 1994; Middleton 2000), lakes, tidal marshes, and oceans (Nilsson et al. 2010). After release from the parent plant, seeds enter water bodies either directly or indirectly (Chambert and James 2009). Many wetland plants inhabit the land-water interface; therefore, high percentages of gravity/wind dispersed seeds directly enter water bodies (Nilsson et al. 2010). Seeds that land on the ground can indirectly enter water by wind, rain, animals, or lateral water flow (Chambert and James 2009; Nilsson et al. 2010). Water dispersal is directional, with most hydrochorous (water dispersed) seeds dispersed downstream (Schneider and Sharitz 1988).

### *Buoyancy Potential*

The buoyancy potential of hydrochorous seeds varies, ranging from a couple of hours to several months (Griffith and Forseth 2002). Seed characteristics that increase the surface area to volume ratio appear to enhance buoyancy potential (Nilsson et al.

2010). Small and light seeds stay afloat due to water's high surface tension (Ikeda and Itoh 2001). In addition, some seeds have corky tissue with air spaces that enables them to float for extended periods of time (van der Pijl 1972; Nilsson et al. 2010). Other seed adaptations that appear to enhance buoyancy potential include enlarged seed appendages, such as hairs (Cronk and Fennessy 2001) and oil covering the seed testa (Ikeda and Itoh 2001). Edwards et al. (1994) found that the wetland species *Asclepias perennis* Walter had greater expanded seed margins that contributed to enhanced buoyancy over time in comparison with the wind-dispersed upland *A. exaltata* Bull. Ikeda and Itoh (2001) found *Penthorum chinese* Pursh remained buoyant due to seed surface oil and warts covering the seed testa.

Emergent wetland plants generally have seeds with greater floating capability compared to aquatic, semi-aquatic, and terrestrial species (Boedeltje et al. 2003). Lopez (2001) demonstrated that species from seasonally flooded forests appeared adapted for water dispersal because seeds remained buoyant for longer periods in comparison with upland species. For instance, 53% of species inhabiting periodically flooded forests remained buoyant for 60 days in water. In contrast, species from upland sites could not remain buoyant for more than 13 days. In general, floating capability decreases the likelihood seeds become waterlogged and sink into hypoxic conditions (Schneider and Sharitz 1988; McKevlin et al. 1998); increases the chances seeds find elevated 'safe' sites for germination and establishment (Schneider and Sharitz 1988); and allows seeds to travel away from the competitive parental habitat (Sculthorpe 1967).

*Hydrochorous Seed Deposition*

Seed morphology (Schneider and Sharitz 1988) along with the hydrology and geomorphology of the water body determine the final deposition of hydrochorous seeds (Chambert and James 2009; Nilsson et al. 2010). Water velocity in fast flowing water, such as rivers and streams determines the speed and rate of hydrochorous seed transport (Soomers et al. 2010). In slow flowing or stagnant water bodies, net wind speed may determine the direction and transport rate of hydrochorous seeds more than water velocity (Soomers et al. 2010). Nilsson et al. (1991) found a high concentration of experimental hydrochorous seeds (wooden cubes) in eddies downstream from rapids and along the outer curves of slow glides/pools. Schneider and Sharitz (1988) demonstrated that the seeds of *Taxodium distichum* (L.) Rich (bald cypress) and *Nyssa aquatica* L. (water tupelo) deposited in different locations in a swamp forest due to differences in seed shape. Smaller, angular bald cypress seeds deposited more frequently on knees; in contrast, larger, ellipsoid tupelo fruits were more concentrated in open stagnant areas (Schneider and Sharitz 1988). Other factors influencing the final deposition of hydrochorous seeds include emergent substrates, and aquatic and riparian vegetation (Soomers et al. 2010). Soomers et al. (2010) determined that the presence of vegetation along drainage ditches was positively correlated with seed deposition. Schneider and Sharitz (1988) illustrated in a forested floodplain that hydrochorous seeds were predominately trapped by logs and knees.

**Germination Ecology of Wetland Plants**

Wetlands are highly diverse ecosystems due to fluctuating hydrological regimes that vary across space and time (Cronk and Fennessy 2001). The hydrological regime, including depth, duration, frequency, and timing of flooded conditions (Leck and Brock 2000) determines available microsites for germination and establishment of wetland plants (Conti and Gunther 1984). Depth and duration of flooding influence oxygen and light levels and determine microsite suitability for germination of wetland plants (Casanova and Brock 2000). For instance, in prairie potholes and Carolina bays, many herbaceous plants germinate and establish during episodic dry periods when oxygen and light levels increase due to the removal of standing water (Sharitz and Pennings 2006). In forested wetlands, prolonged flooding events can limit seed germination and seedling establishment of woody plants and eventually forests may be replaced by wet meadows or marshes (Sharitz and Pennings 2006). Britton and Brock (1994) demonstrated that the timing of flooding influenced the germination of wetland plants; more species germinated post spring flooding events in comparison with flooding in the summer. Gerritsen and Greening (1989) demonstrated that the frequency of inundation periods affected the germination and subsequent establishment of wetland plants in the Okefenokee marsh. For instance, high inundation periods resulted in complete dominance of wet-adapted species (e.g. floating-leaved aquatic plants), with a low frequency of inundation resulting in a high occurrence of dry adapted species (e.g. sedges).

In wetland systems, the interaction between topography and the hydrological regime creates a mosaic of moisture conditions that range from anaerobic to aerobic. Different germination patterns in response to anaerobic, hypoxic, and aerated conditions often reflect where mature wetland plants occur (Conti and Gunther 1984). For instance, the floating-leaved aquatic plant *Nuphar luteum* L. (spatterdock) required anaerobic conditions for high germination; in contrast, the emergent plant *Xyris smalliana* Nash (small's yellow-eyed grass) exhibited an inability to germinate in anaerobic conditions (Conti and Gunther 1984).

The timing of germination for many emergent wetland plants coincides with the hydrological fluxes of the wetland (Walls et al. 2005). In forested wetlands, many plant species germinate during summer drawdown when oxygen levels increase and soil temperatures rise (Dubarry 1963; Conti and Gunther 1984; Middleton 2000; Cronk and Fennessy 2001; Geissler and Gzik 2008; Rudinger and Dounavi 2008). For floodplain species *Fraxinus pennsylvanica* Marshall (green ash) and *Quercus palustris* Muench (pin oak), germination was inhibited for seeds in stagnant flooded conditions, but germination occurred once seeds were removed (Walls et al. 2005). In addition, woody swamp species *Taxodium distichum* (L.) Rich (bald cypress) and *Nyssa aquatica* L. (water tupelo) could not germinate underwater; however, seeds retained viability while submerged and germinated during drawdown conditions (Dubarry 1963; Rudinger and Dounavi 2008). Overall, the timing of germination during favorable conditions is important in wetland systems with seasonal or annual flooding events where suitable conditions can change rapidly.

***Helonias bullata*** **L. (swamp pink)**

Due to population decline and habitat degradation, *Helonias bullata* L. (swamp pink) was designated a federally threatened species in 1988 (U.S. Fish and Wildlife Service 1991). *Helonias* is a perennial herb that inhabits a variety of wetland ecosystems, such as *Chamaecyparis thyoides* swamps, meadows, inland stream corridors, swamp forest-bog complexes and spring seepage areas (U.S. Fish and Wildlife Service 1991; Murdock 1994; Dodds 1996). Its geographic range extends from the coast of New Jersey south to Virginia and into the mountainous regions of Virginia, North Carolina, South Carolina, and Georgia. *Helonias* forms basal rosettes composed of evergreen liliaceous parallel-veined oblong-spatulate leaves (Godfrey and Wooten 1979; U.S. Fish and Wildlife Service 1991). *Helonias* produces a fragrant, terminal raceme inflorescence composed of 30-50 perfect individual flowers (Sutter 1984) with pink tepals and distinctive blue anthers. Flower production occurs from March to May with a peak bloom period in mid-April. The flowers mature into dehiscent capsules containing an average of 25-35 individual seeds. After pollination and during fruit maturation, *Helonias* has a hollow scape that bolts to a height of approximately 1.5 meters. The capsules fully mature and release seeds in late May/early June (Chafin 2007). Mature seeds are linear with a fatty appendage (U.S. Fish and Wildlife Service 1991) along the entire seed edge that terminates into a 2 mm hook/appendage on the distal seed edge and 1 mm on the proximal (April Punsalan, personal observations).

A stable, constant water supply at or near the ground surface is the most important factor that determines habitat suitability for *Helonias* (U.S. Fish and Wildlife Service 1991). Some of the largest *Helonias* populations occur in areas with high water availability. In southern New Jersey, where over half of the world's populations occur, *Helonias* occurs in Atlantic white cedar swamps within the Delaware River Basin (Laidig et al. 2009). These sites are characterized by mucky soils with constant seepage and lateral ground-water movement (Laidig et al. 2009). Optimal water levels for suitable habitat for *Helonias* in this region range from 5 to 10 cm below the ground surface (Laidig et al. 2009).

In North Carolina, the largest *Helonias* population occurs in the Pisgah National Forest in an area called the "Pink Beds". This site receives surface water from several headwater streams that feed into the bottom of the valley from surrounding ridges. *Helonias* subpopulations occur in “swamp forest-bog complexes” (Schafale and Weakley 1990) situated along the headwaters of the South Fork Mills River and associated streams, small tributaries, and seepage areas. Rosettes typically occur in "boggy" depressions that are covered with *Sphagnum* mats with a water table at or slightly below the ground surface. In the overstory, woody dominant species include *Acer rubrum* L. (red maple), *Pinus strobus* L. (eastern white pine), and *Tsuga canadensis* (L.) Carrière (Canadian hemlock) (many of these trees are dead in the overstory due to *Adelges tsugae* (Annad), hemlock woolly adelgid). In the understory, shrubs include *Alnus serrulata* (Aiton) Willdenow (tag alder), *Rhododendron maximum* L. (great laurel), and *Kalmia latifolia* L. (mountain laurel) (April Punsalan, personal observations).

Habitat loss and degradation is the primary threat to existing *Helonias* populations (U.S. Fish and Wildlife Service 1991). Hydrological changes from wetland drainage and urban development have contributed to the degradation of *Helonias* habitat (Dodds 1996) and even led to the extirpation of some populations in New Jersey (Laidig et al. 2009).

Habitat degradation due to an increase in sedimentation and siltation from off-site development has decreased rosette density at some sites (U.S. Fish and Wildlife Service 2007). Windham and Breden (1996) illustrated that *Helonias* populations were positively associated with an increase in percent forest cover, but negatively associated with an increase in urban development via GIS land use/land cover.

Poor sexual recruitment is another conservation concern for *Helonias* (U.S. Fish and Wildlife Service 1991). Factors possibly hindering the sexual recruitment and the colonization of new sites for *Helonias* include low inflorescence production, limited seed dispersal, and poor seedling recruitment (Sutter 1984; U.S. Fish and Wildlife Service 1991). Across this species' range, inflorescence production appears low (Sutter 1984; Peterson 1992; U.S. Fish and Wildlife Service 1991). However, *Helonias* has highly self-compatible flowers that produce a copious amount of viable seeds (Sutter 1984; U.S. Fish and Wildlife Service 1991). Despite high seed viability, results from demographic monitoring have revealed low seedling recruitment in the field (Sutter 1984; Gary Kauffman, personal communication).

Restricted seed dispersal distances may hinder seedling recruitment and the colonization of new sites for *Helonias*. Germination and seedling establishment may be limited in close proximity to parent plants because basal rosettes are often densely clustered (Sutter 1984) due to rhizomatous asexual reproduction. In the Pink Beds, Sutter (1984) noted seedlings occurred only on the periphery of the population, suggesting restricted seedling establishment close to the parent plant. Water may serve as a more effective dispersal agent for *Helonias* by dispersing seeds further distances than wind or gravity. In addition, since *Helonias* seeds exhibit high floating capacity (U.S. Fish and

Wildlife Service 1991; Peterson 1992) and rosettes typically occur on hummocks along and within slow flowing streams (Laidig et al. 2009), water could disperse seeds.

The large number of *Helonias* occurrences in New Jersey swamps may be due to reliable seed dispersal by water. In 2007, U.S. Fish and Wildlife Service reported a total of 140 extant occurrences of *Helonias* in New Jersey. In contrast, in the southern Appalachian region, there are only 16 extant occurrences in North Carolina, one in South Carolina, and one in Georgia (U.S. Fish and Wildlife Service 2007). The fragmentation of *Helonias* populations in the southern Appalachian region may have led to dispersal limitations within and between bogs or nutrient poor fens. Many southern Appalachian bogs are degraded and isolated due to agricultural and urban development (Murdock 1994). Seed dispersal by water unlikely occurs between isolated bogs (Ellison and Parker 2002). *Helonias* populations in the southern Appalachian region may be more threatened because fragmentation increases edge effects, which leads to lower reproductive success (Middleton et al. 2006). Moreover, isolated populations with seed dispersal limitations are at a higher risk of extirpation due to possible climatic shifts and stochastic events (Primack and Miao 1992). Hydrological restoration (ground water and surface water) should be a top conservation priority for *Helonias* populations in the southern Appalachian region, especially because these populations contain the greatest genetic diversity (Godt et al. 1995). Currently, the U.S. Fish and Wildlife Service propose to protect 23, 478 acres of southern Appalachian bogs scattered across 13 counties in North Carolina and Tennessee. *Helonias* occurs in four of the North Carolina counties with proposed refuge acreage.

Recovery objectives for *Helonias* include restoring populations in areas where it has been extirpated and establishing new colonies at sites in decline (U.S. Fish and Wildlife Service 1991; Laidig et al. 2009). Primack and Miao (1992) suggest that direct seeding should be used for rare plant restoration instead of vegetative planting because it mimics the natural dispersal process and a greater number of genotypes are utilized. However, restoration efforts from direct seeding at degraded sites in southern New Jersey have been unsuccessful regardless of planting location (Dodds 1996). Results of my research will help with *Helonias'* conservation efforts by determining how moisture conditions seeds encounter during the dispersal phase influence germination.

# CHAPTER THREE: MANUSCRIPT

## Introduction

In forested wetlands, variation in microtopography creates a mosaic of microhabitats (Huenneke and Sharitz 1986; Vivian-Smith 1997; Duberstein and Conner 2009; Rossell et al. 2009). The microtopographic mosaic in forested wetlands, often referred to as hummock-hollow topography, consists of hummocks formed by the accumulation of woody debris, peat, and organic matter and interwoven tree roots (heights range from 50 cm to 100 cm) (Ehrenfeld 1995b) and hollows or depressions where the water table is typically at or above the soil surface (Duberstein and Conner 2009). Microtopographic relief across hummock-hollow topography affects soil moisture, soil nutrients (Rossell et al. 2009), litter accumulation (Vivian-Smith 1997), microclimate and emergent substrate variability (Huenneke and Sharitz 1990). Seasonal differences in precipitation, inflow/outflow of surface water, and infiltration/discharge of sub-surface water further differentiate microsites across microtopography in forested wetlands (Duberstein and Conner 2009).

The spatial and temporal interaction of microtopography and seasonal hydrological fluctuations produces a wide variety of microsites for germination (Scarano et al. 1997; Vivian-Smith 1997) and the "window of opportunity" (Eriksson and Froborg 1996), or germination window for wetland plants can be narrow or wide across both space and time (Middleton 2000). This degree of patchiness creates a high level of unpredictability for germination and subsequent establishment (Scarano et al. 1997). In forested wetlands, seedling recruitment for many wetland plants appears limited to hummocks or other emergent substrates above the forest floor, suggesting restricted germination in flooded anaerobic conditions in hollows (Huenneke and Sharitz 1986; Huenneke and Sharitz 1990; Jordan and Hartman 1995).

*Helonias bullata* L. (swamp pink) is a federally threatened, obligate wetland species of forested wetlands, such as *Chamaecyparis thyoides* (Atlantic white cedar) swamps, inland stream corridors, spring seepage areas, and swamp forest-bog complexes (Murdock 1994; Dodds 1996). Poor sexual recruitment is a conservation concern for *Helonias* because it appears to limit the colonization of new sites (U.S. Fish and Wildlife Service 1991). Factors possibly hindering the sexual recruitment of *Helonias* include low inflorescence production, limited seed dispersal distances, and poor seedling recruitment (U.S. Fish and Wildlife Service 1991). Across this species' range, inflorescence production appears low (Sutter 1984; U.S. Fish and Wildlife Service 1991). However, *Helonias* has highly self-compatible flowers that produce a copious amount of viable seeds (Sutter 1984; U.S. Fish and Wildlife Service 1991). Despite high seed viability, results from demographic monitoring have revealed low seedling recruitment in the field (Sutter 1984; Peterson 1992; Gary Kauffman, personal communication).

*Helonias* occurs amongst hummock-hollow topography where flooded and drought conditions can occur (Ehrenfeld 1995b; Vivian-Smith 1997). Since *Helonias* reportedly has short-lived seeds (U.S. Fish and Wildlife Service 1991), germination and seedling establishment may depend on suitable conditions at time of seed arrival (van der Valk 1981). Laidig et al. (2009) demonstrated that *Helonias* rosettes were commonly associated with hummocks in and along poorly defined steam channels. Thus, seeds could land in a variety of microsite conditions. For instance, seeds could land in the stream channel, float and disperse by water and/or sink and become submerged. On the other hand, seeds could remain dry in dehiscent capsules or land on the upper, emergent portions of hummocks where drought conditions can occur (Ehrenfeld 1995b; Vivian-Smith 1997). Currently, the germination response of *Helonias* seeds after exposure to various moisture conditions remains undetermined. Because direct seeding efforts at degraded sites in southern New Jersey have been unsuccessful regardless of planting location (Dodds 1996), determining the germination of *Helonias* seeds in response to moisture conditions they may encounter during dispersal could help with future recovery objectives.

The objective of this study was to determine how different moisture conditions, including flooded, saturated, and dry influence germination. To accomplish this objective, three experiments in a controlled environment were used to test the germination response of seeds: (1) floating, (2) submerged, and (3) dry without water. In addition, one experiment was conducted in a natural environment to test the germination response of seeds placed in flooded, saturated, and dry conditions in a small headwater stream. Questions addressed were: (1) how does seed contact with water, floating and/or submerged, affect the germination response (percentages and rates) of *Helonias* seeds, (2) what is the germination response (percentages and rates) of *Helonias* seeds placed in a saturated stream margin, and (3) what is the germination response (percentages and rates) of *Helonias* seeds that remain dry or in dehiscent capsules?

**Methods**

*Species Description*

*Helonias bullata* L. (swamp pink) is a liliaceous, herbaceous perennial with evergreen parallel-veined oblong-spatulate or oblanceolate leaves that form a basal rosette (Godfrey and Wooten 1979; U.S. Fish and Wildlife Service 1991). *Helonias*' geographic range spans from the coast of New Jersey, Delaware, Maryland, and Virginia to the mountainous regions of Virginia, North Carolina, South Carolina, and Georgia. Flower production occurs from March to May with peak bloom in mid-April. *Helonias* produces a fragrant, terminal raceme composed of 30-50 perfect individual flowers (Sutter 1984) with pink tepals and distinctive blue anthers. The flowers mature into dehiscent capsules that contain approximately 25-35 individual seeds. During flower development, *Helonias* has a hollow scape approximately 2-9 dm. tall that grows to a height of 1.5 m during seed maturation. Capsules mature and release seeds in late May/Early June (Chafin 2007). Mature seeds are linear, 5 mm long and have a fatty appendage along the entire seed edge (U.S. Fish and Wildlife Service 1991).

*Study Site*

This study was conducted at the "Pink Beds" in the Pisgah National Forest (Figure 1). The Pink Beds contains the largest *Helonias* population in North Carolina (Sutter 1984). At this site, *Helonias* rosettes occur in “swamp forest-bog complexes” (Schafale and Weakley 1990) located along the headwaters of the South Fork Mills River and associated streams, small tributaries, and seepage areas. Rosettes typically occur on hummocks covered with *Sphagnum* mats and in hollow or depressions with mucky soil (April Punsalan, personal observations). The flooding frequency of the Pink Beds is unknown, but periodic flooding undoubtedly occurs due to the proximity of streams and reported beaver activity (David Danley, personal communication). Overstory woody dominants include *Acer rubrum* L. (red maple), *Pinus strobus* L. (eastern white pine), and *Tsuga canadensis* (L.) Carrière (Canadian hemlock) (many of these trees are dead in the overstory due to *Adelges tsugae* (Annad), hemlock woolly adelgid). In the understory, shrubs include *Alnus serrulata* (Aiton) Willdenow (tag alder), *Rhododendron maximum* L. (great laurel), and *Kalmia latifolia* L. (mountain laurel). The herbaceous layer in "boggy" openings includes *Chelone lyonii* Pursh (pink turtlehead), *Osmundastrum cinnamomeum* (L.) C. Presl (cinnamon fern), *Osmunda regalis* L. *var. spectabilis* (Willdenow) A. Gray (royal fern), *Solidago patula* Muhlenburg ex Willdenow (rough-leaved solidago), and *Carex spp.* (April Punsalan, personal observations).

In 2012, a field study was conducted along Pigeon Branch (35º21' 4.9278"N/ 82º46' 36.4794"W), a small headwater stream in the Pink Beds (Figure 1). Pigeon Branch was chosen due to the occurrence of *Helonias* rosettes along the stream. In addition, the length and depth of Pigeon Branch was sufficient for the 2012 field treatments. During the 2012 field treatments (May 30th-June 29th), the water depth in Pigeon Branch ranged from 4-8 cm and the average water temperature was 16.6ºC.

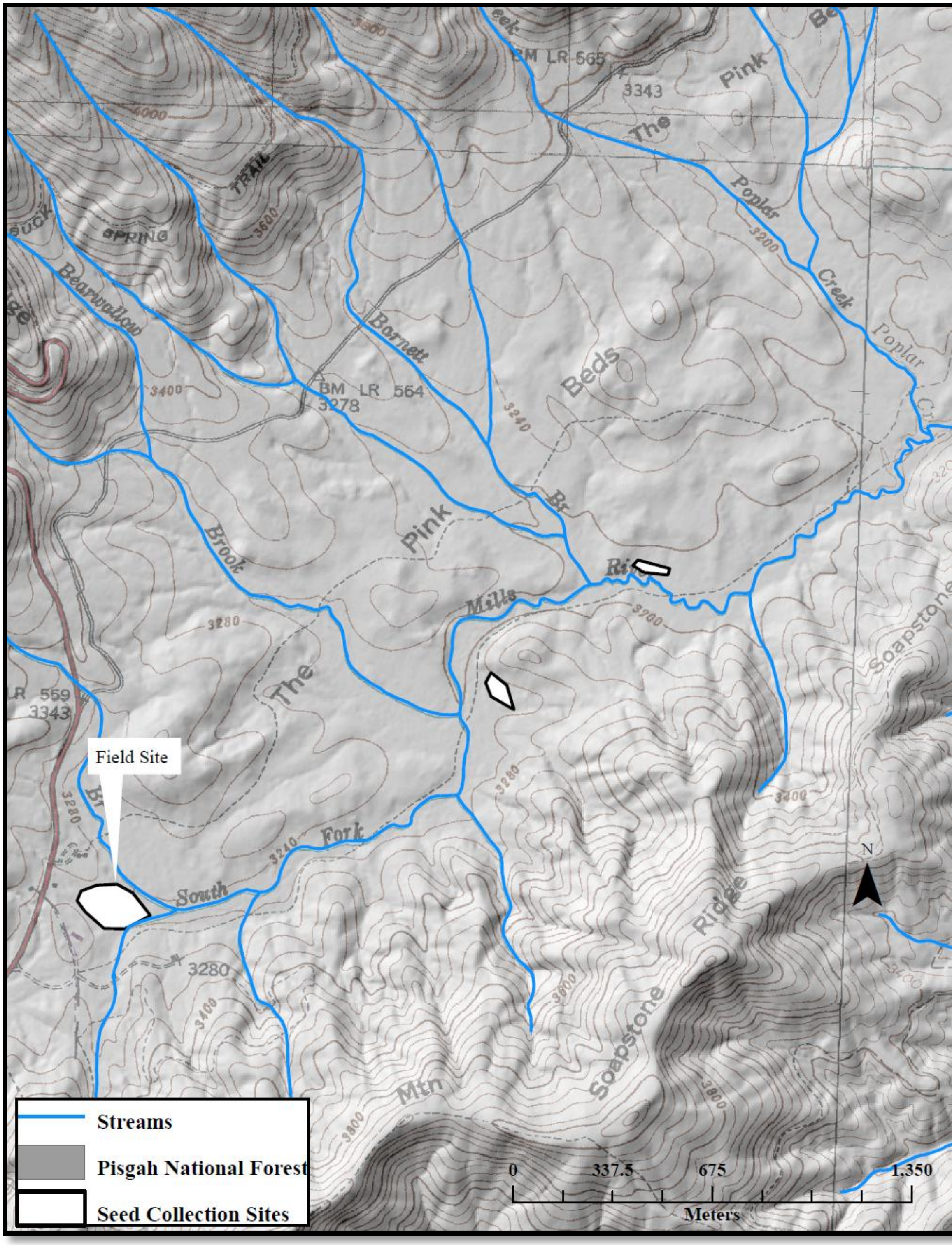


Figure 1: Map of seed collection sites and field site (35º21'4.9278"N/ 82º46'36.4794"W) where 2012 field treatments were conducted (floating, submerged, stream margin, and dry) in the Pink Beds, Pisgah National Forest, North Carolina.

*Seed Collection*

To reduce any negative impacts to the population due to seed collection, three different sites within the Pink Beds population were chosen based on high rosette density and inflorescence production. Five inflorescences per site were randomly selected (inflorescences were numbered and numbers were randomly selected) and covered with nylon mesh to prevent seed loss. Inflorescences were collected when dehiscent capsules first started to split open during the last week of May in 2011 and 2012. In each year, seeds were removed from inflorescences and mixed together to produce a homogeneous seed lot. Experiments commenced within 48 hours of seed collection.

*Controlled Environment*

To determine the germination response of *Helonias* seeds after exposure to different moisture conditions, three experiments were conducted in a controlled environment: seeds placed floating in water (floating), seeds submersed in water (submerged), and seeds kept dry (dry). To test the germination response of seeds exposed to dry conditions, seeds were placed in cylindrical 88 ml glass containers without any water. To test the germination response of floating seeds, seeds were placed in 946 ml round glass containers (14 cm diameter) filled with distilled water. Distilled water was added daily to the glass containers to maintain 6 cm water depth, which was within the 4-8 cm range of field water depths for *Helonias*. To test the germination response of seeds submersed, seeds were placed in nylon mesh bags weighted with marbles and submersed beneath 5 cm of distilled water in 946 ml glass containers (12 cm x 12 cm x 6 cm high).

For each experiment, seeds were exposed to conditions for 1, 2, 3, 5, 8, 13, 21, and 35 days. Containers were repositioned daily in growth chambers to exclude position effects. Experiments were conducted in one growth chamber at 60% relative humidity at 27.7°C for 14 hr. daylight at a photon flux of 670μm $m^{-2}$ $s^{-1}$and 14.4°C for 10 hr. darkness to approximate average field conditions in Transylvania County for June.

Germination was tested for the floating, submerged, and dry experiments using six replicates of 25 seeds. Seeds were placed in petri dishes (25 seeds per dish) filled with a soil mixture (3 parts peat moss: 2 parts milled sphagnum: 1 part builder’s sand (Ron Determann, personal communication)). Petri dishes were checked daily for moisture content and kept saturated for a 50-day germination period. On a daily basis, seeds that germinated were recorded and removed when a radicle 1 mm emerged. Seeds that were moldy, mushy, or shriveled were removed from dishes.

*Natural Environment*

To determine the germination response of *Helonias* seeds after exposure to different moisture conditions in a natural environment, four treatments were used: dry, floating, stream margin, and submerged. For each treatment, 25 seeds were placed into a polystyrene ring constructed from the bottom of an 8 oz. styrofoam cup. Nylon mesh was placed around each ring and fastened with a zip tie. The containers (dry, stream margin, floating, and submerged) were tied to pin flags and placed into Pigeon Branch. Containers for the dry treatment were kept above the water by attaching them directly to the top of the pin flag. For the stream margin treatment, containers were positioned on the pin flag below the dry treatment containers and placed in the stream margin using fishing line. To secure the containers on the stream margin, soil was compacted around the container rim and marbles were placed inside containers for weight. The floating treatment containers were attached to the pin flags below the stream margin treatment and placed on the surface water using fishing line. The polystyrene ring ensured these containers remained buoyant for the length of the experiment. Containers for the submerged treatment were placed at the bottom of the pin flag under 4-8 cm of water in Pigeon Branch; marbles were placed inside containers for weight.

The above treatments remained in Pigeon Branch for 1, 3, 5, 8, 12, 16, 20, and 30 days. The water temperature of Pigeon Branch was recorded at each time interval listed above. Containers removed at each treatment interval were placed in plastic bags and taken to the growth chamber to assess germination percentages and rates.

Seeds were removed from containers and sanitized using a 2% hypochlorite solution to remove potential microorganism contamination. Seeds were rinsed three times with deionized water. Germination was tested using 25 seeds with six replicates. Seeds (25) were placed in petri dishes on Whatman # 1 filter paper. Seeds from all treatments were germinated in one growth chamber at 27.7°C for 14 hr. daylight at a photon flux of 670µm $m^{-2}$ $s^{-1}$ and 14.4°C for 10 hr. darkness at 60% humidity for a 30-day germination period. On a daily basis, seeds that germinated were recorded and removed when a radicle 1 mm emerged. Dishes were checked every other day for moisture content and kept saturated. To determine if non-germinated seeds were viable, non-germinated seeds were tested with tetrazolium at the end of a 30-day germination period. Most seeds germinated or were infected by mold during the experiment; therefore, only an average of 1-2 seeds per treatment was tested for viability at the end of the experiment.

*Data Analyses*

For all experiments, final mean germination percentages were calculated as the total number of seeds that germinated at the end of the experiment divided by the total number of seeds (25) placed in a petri dish. In addition, germination rates were calculated at 5-day intervals by dividing the cumulative number of germinated seeds by the total number of seeds (25) placed in a petri dish. Germination rates were plotted against time to illustrate how the length of time in each treatment affected the timing of germination.

To determine how moisture conditions affected the germination response of *Helonias* seeds after different periods of time, analysis of variance was used to compare final germination percentages (mean ± S.D.) among time intervals. Data (percentages) were arcsine transformed to conform to normality. *Post hoc* comparisons between days for each experiment were made using Bonferroni t-tests ($p<0.05$).

To determine how moisture conditions affected the germination rate of *Helonias* seeds after different time periods, a repeated measures analysis of variance (RMANOVA; mixed model procedure) was performed to compare mean germination percentages at 5-day intervals. SAS 9.2 was used for all analyses (SAS Institute Inc., 2008). Non-transformed germination percentages were displayed for results.

**Results**

*Controlled Environment*

Exposure of *Helonias* seeds to dry conditions for eight days significantly (p=0.0001) decreased germination by about 50%, from 48% to 25% (Figure 2). Exposure to dry conditions for 13 days significantly (p=0.0001) decreased germination approximately 25%, from 25% to 14% (Figure 2). No germination occurred for seeds exposed to dry conditions for 35 days (Figure 2). Exposure of seeds to dry conditions for 8-21 days significantly reduced (RMANOVA; p=0.0001) the onset of germination as compared with seeds exposed to dry conditions for 1-5 days (Table 1; Figure 3).

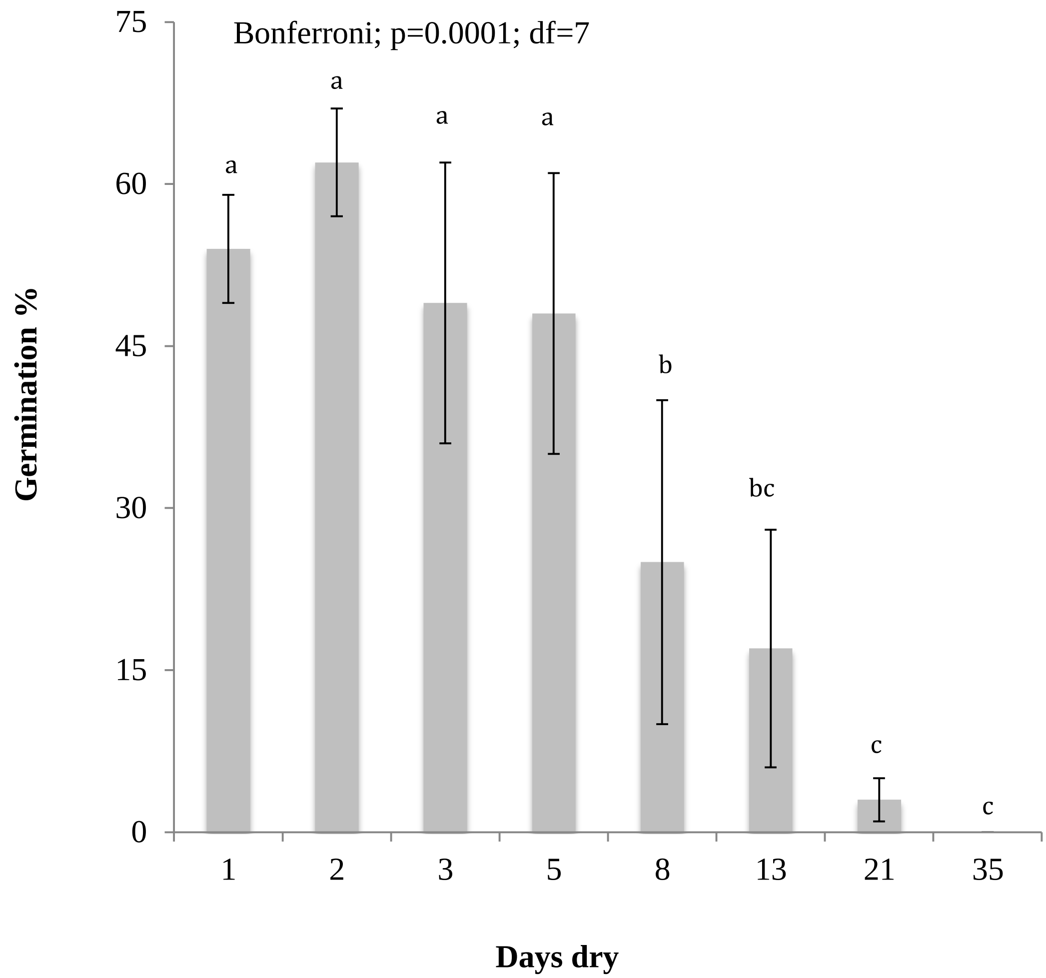


Figure 2. Final mean germination percentages of *Helonias bullata* seeds exposed to dry conditions in a controlled environment. Significant differences (p=0.0001) in final germination percentages among dry exposure are indicated by different lowercase letters.

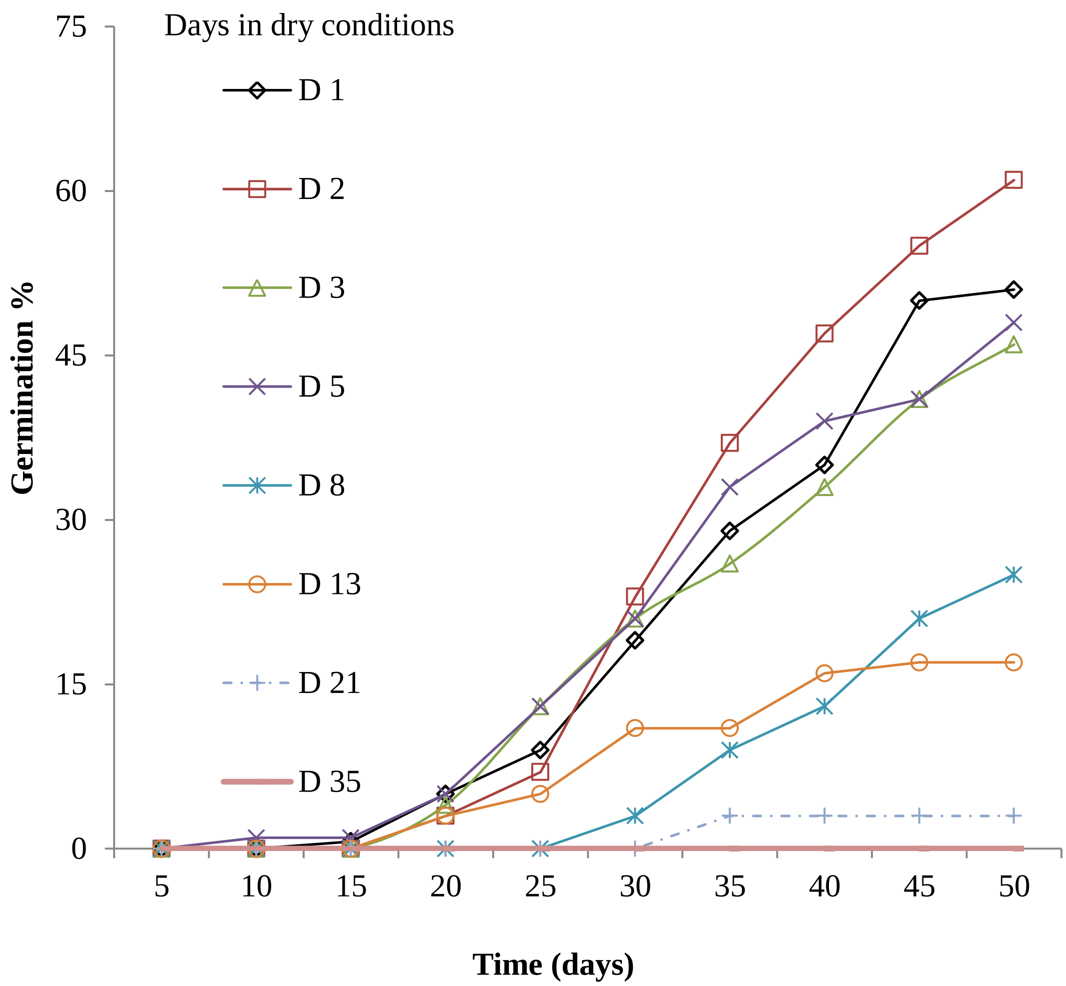


Figure 3. Cumulative germination rate of *Helonias bullata* seeds after 1, 2, 3, 5, 8, 13, 21, and 35 days of exposure to dry conditions in a controlled environment. Each point represents the cumulative mean germination percent for six replicates of 25 seeds.

Table 1. Results from a repeated measures of analysis of variance (RMANOVA; mixed model procedure) showing the effects of dry conditions after 1, 2, 3, 5, 8, 13, 21, and 35 days (day) on mean cumulative germination percentages at 5-day intervals for a 50-day germination period for *Helonias bullata* seeds.

| Effect | Numerator DF | Denominator DF | F Value | Pr>F |
|---|---|---|---|---|
| Day | 7 | 40 | 11.69 | <0.0001 |

After 13 days in water, 90% of seeds germinated while floating (mean calculated from 401/443 germinated seeds across six replicates). Thus, final germination percentages and rates were only compared for seeds floating for 1, 2, 3, 5, and 8 days. Floating duration (1, 2, 3, 5, and 8 days) did not affect final germination percentages of *Helonias* seeds (ANOVA; p=0.12). Mean germination was high (mean±S.D:78%±19) regardless of the length of time seeds were floating. However, the length of time seeds were floating significantly (RMANOVA; p=0.0001) affected the germination rate of *Helonias* seeds (Table 2; Figure 4). Seeds floating for 3-8 days germinated earlier and took less time to reach >60% germination in comparison with seeds floating for only 1-2 days (Figure 4). For instance, seeds floating on water for eight days germinated to 65%±24 after five days. In contrast, seeds floating on water for one day took 45 days to reach 65%±18 germination.

After 21 days, 100% of seeds germinated while floating (mean calculated from 429/429 germinated seeds across six replicates). Additionally, at day 21, germinated seeds developed a cotyledon and primary root axis approximately 2-5 cm long. After 35 days, 94% of seedlings were still floating (mean calculated from 422/450 floating seedlings across six replicates).

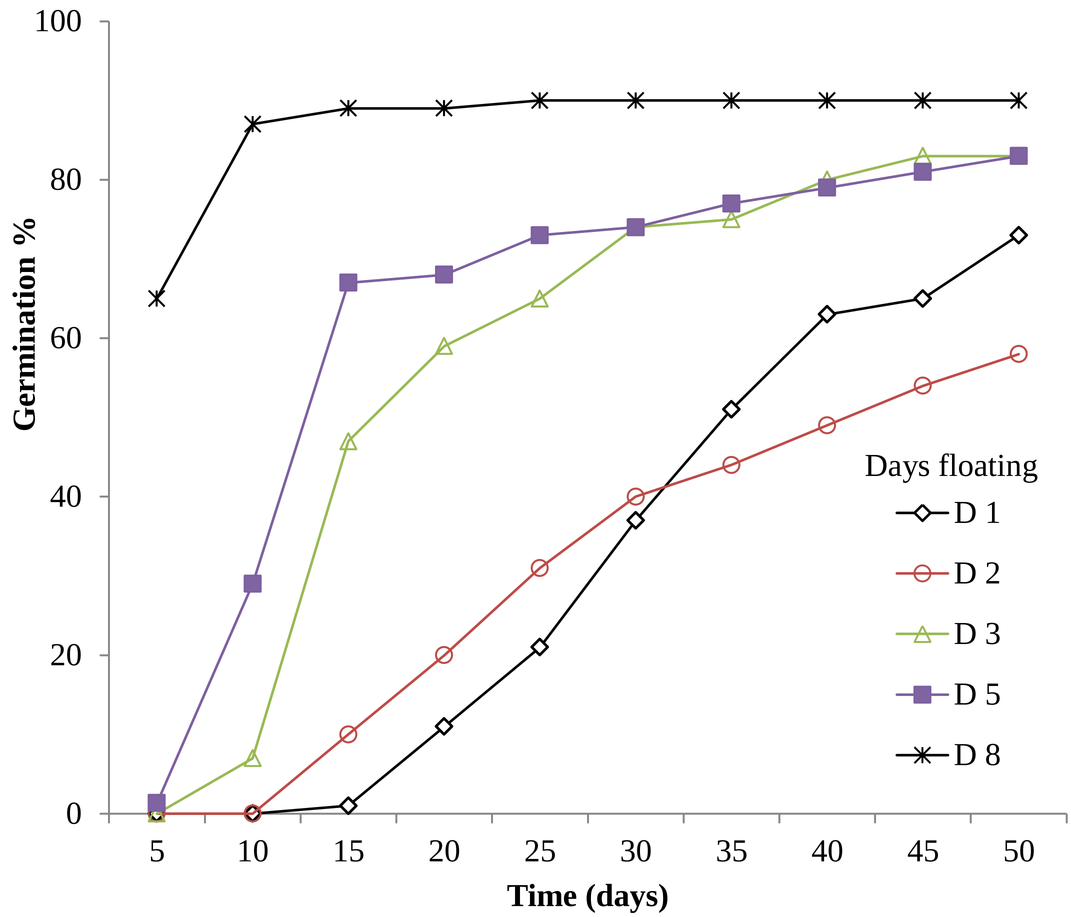


Figure 4. Cumulative germination rate of *Helonias bullata* seeds after 1, 2, 3, 5, and 8 days of floating on water in a controlled environment. Each point represents the mean germination percent for six replicates of 25 seeds.

Table 2. Results from a repeated measures of analysis of variance (RMANOVA; mixed model procedure) showing the effects of seeds floating on water after 1, 2, 3, 5, 8, 13, 21, and 35 days (day) on mean cumulative germination percentages at 5-day intervals for a 50-day germination period for *Helonias bullata*.

| Effect | Numerator DF | Denominator DF | F Value | Pr>F |
|---|---|---|---|---|
| Day | 4 | 25 | 12.43 | <0.0001 |

For the submerged experiment, 77% of seeds germinated (mean calculated from 348/450 germinated seeds across 6 replicates) after 13 days of treatment. Thus, final germination percentages and rates were only compared among seeds submerged for 1, 2, 3, 5, and 8 days. Mean germination was high (mean±S.D:76%±18) irrespective of the length of time seeds were submerged.

The length of time seeds were submersed significantly (RMANOVA mixed model procedure; p=0.0001) affected the germination rate of *Helonias* seeds (Table 3; Figure 5). Germination onset occurred earlier for seeds submersed for 3-8 days and seeds took less time to reach >50% germination in comparison with seeds submersed for only 1-2 days (Figure 5). For example, seeds submersed for eight days germinated to 63%±21 after five days. In contrast, seeds submersed for only one day took 35 days to germinate to 47%±10.

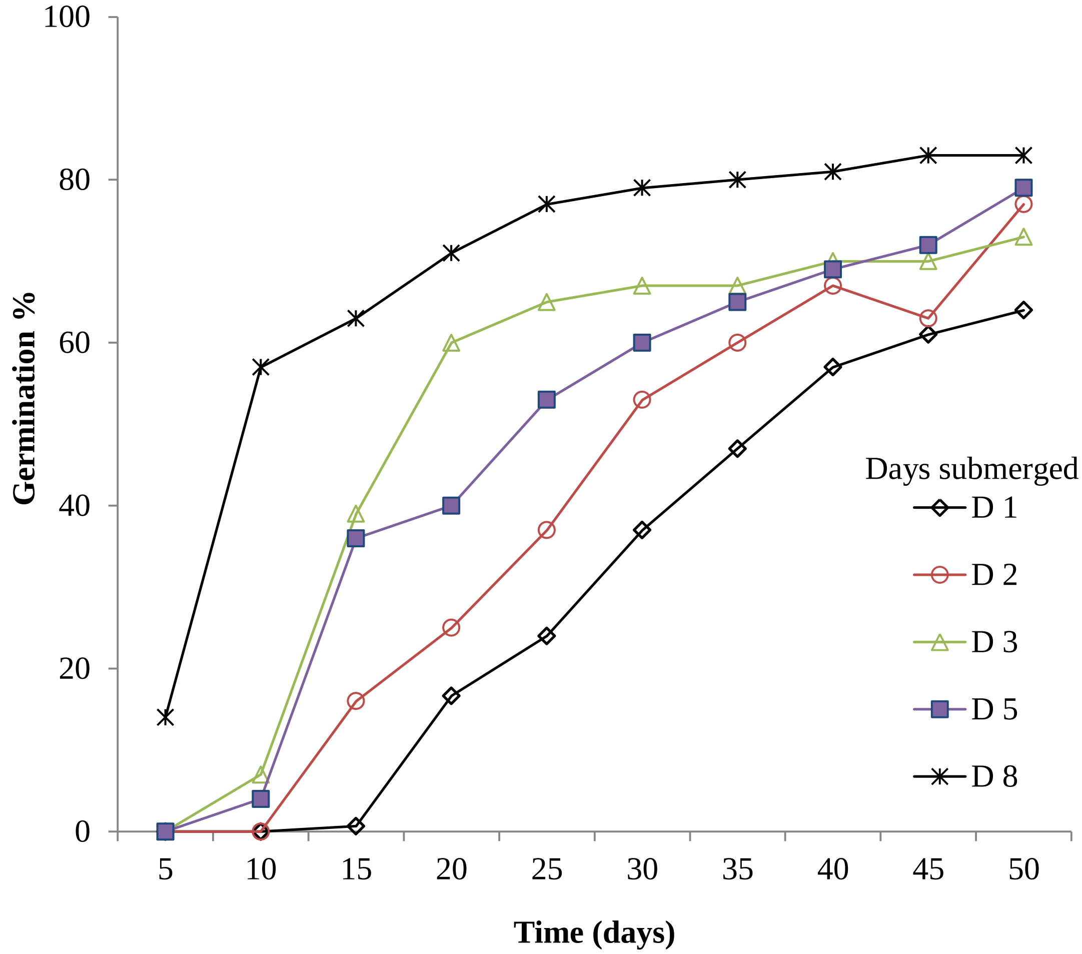


Figure 5. Germination rate of *Helonias bullata* seeds after 1, 2, 3, 5, and 8 days of submergence in a controlled environment. Each point represents the cumulative mean germination percent for six replicates of 25 seeds.

Table 3. Results from a repeated measures of analysis of variance (RMANOVA; mixed model procedure) showing the effects of submerged conditions after 1, 2, 3, 5, 8, 13, 21, and 35 days (day) on mean cumulative germination percentages at 5-day intervals for a 50-day germination period for *Helonias bullata* seeds.

| Effect | Numerator DF | Denominator DF | F Value | Pr>F |
|---|---|---|---|---|
| Day | 4 | 25 | 3.76 | <0.016 |

*Natural Environment*

Results from *post hoc* comparisons using Bonferroni t-tests ($p<0.05$) revealed that the length of time (1, 3, 5, 8, 12, 16, 20, and 30 days) seeds were floating, submerged, in the stream margin, and kept “dry” (only exposed to rainwater) did not affect the final germination percentage of *Helonias* seeds. However, final germination percentages were significantly ($p=0.0004$) affected by treatment (dry, floating, stream margin, and submerged) (Table 4). Comparisons among treatments revealed that seeds floating in the stream had significantly higher germination (82%±9) than seeds kept "dry" in the field (59%±6) (Table 4; Figure 6).

Table 4. Results of from a two-way analysis of variance displaying the effect of treatment type (dry, stream margin, floating, and submerged), day in treatment (day), and treatment x day interaction on final mean germination percentages of *Helonias bullata* seeds.

| Source | DF | SS | Mean Square | F Value | Pr>F |
|---|---|---|---|---|---|
| Treatment | 3 | 1.40 | 0.46 | 6.40 | 0.0004 |
| Day | 7 | 1.90 | 0.27 | 3.72 | 0.0009 |
| Treatment x day | 21 | 2.05 | 0.10 | 1.34 | 0.1546 |
| Error | 159 | 11.56 | 0.073 | | |

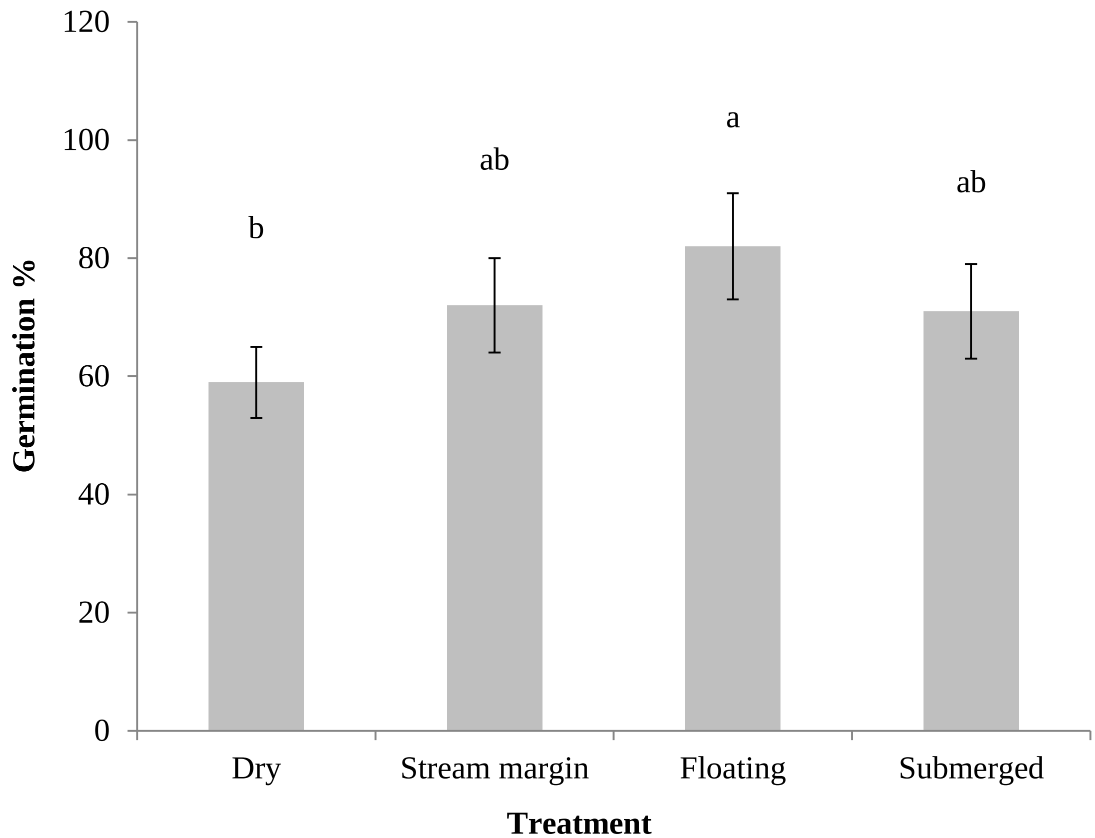


Figure 6. *Helonias bullata* final germination percentages (mean ± S.D.) for four treatments (dry, stream margin, floating, and submerged) placed in Pigeon Branch, Transylvania County, North Carolina.  Significant differences (p=0.0004) between final mean germination percentages among treatments are indicated by different lowercase letters.

Results from the RMANOVA mixed model procedure revealed treatment type (p=0.0001), duration in treatment (p=0.0001), and a treatment x duration interaction (p=0.0001) significantly affected the germination rate of *Helonias* seed (Table 5). Germination onset occurred sooner for seeds exposed to moisture treatments in the Pigeon Branch (floating, submerged, and stream margin) in comparison with seeds kept "dry" in the field (Figure 7). In addition, germination onset occurred faster for seeds in moisture treatments for longer periods of time (8-30 days) in comparison with seeds kept "dry" (Figure 7). For instance, after 20 days, 49%±37 seeds germinated in the stream margin, 58%±1 germinated floating in the stream, and 3%±0.05 germinated submerged while in treatment (Figure 7). In addition, 79%±21 of seeds floating and 77%±23 of seeds in the stream margin had developed a cotyledon and primary root length of approximately 3-5 cm while in treatment after 30 days. Conversely, seeds kept "dry" for 30 days did not germinate while in the field and required a 30-day germination period post treatment to reach >60% germination.

Table 5. Results from a repeated measures of analysis of variance (RMANOVA; mixed model procedure) showing the effects of treatment (dry, stream margin, floating, and submerged), and day in treatment (1, 3, 5, 8, 12, 16, 20, and 30 days), and treatment x day interaction on mean cumulative germination percentages at 5-day intervals for a 30-day germination period for *Helonias bullata* seeds.

| Effect | Numerator DF | Denominator DF | F Value | Pr>F |
|---|---|---|---|---|
| Treatment | 3 | 159 | 23.5 | <0.0001 |
| Day | 7 | 159 | 15.4 | <0.0001 |
| Treatment * Day | 21 | 159 | 3.2 | <0.0001 |

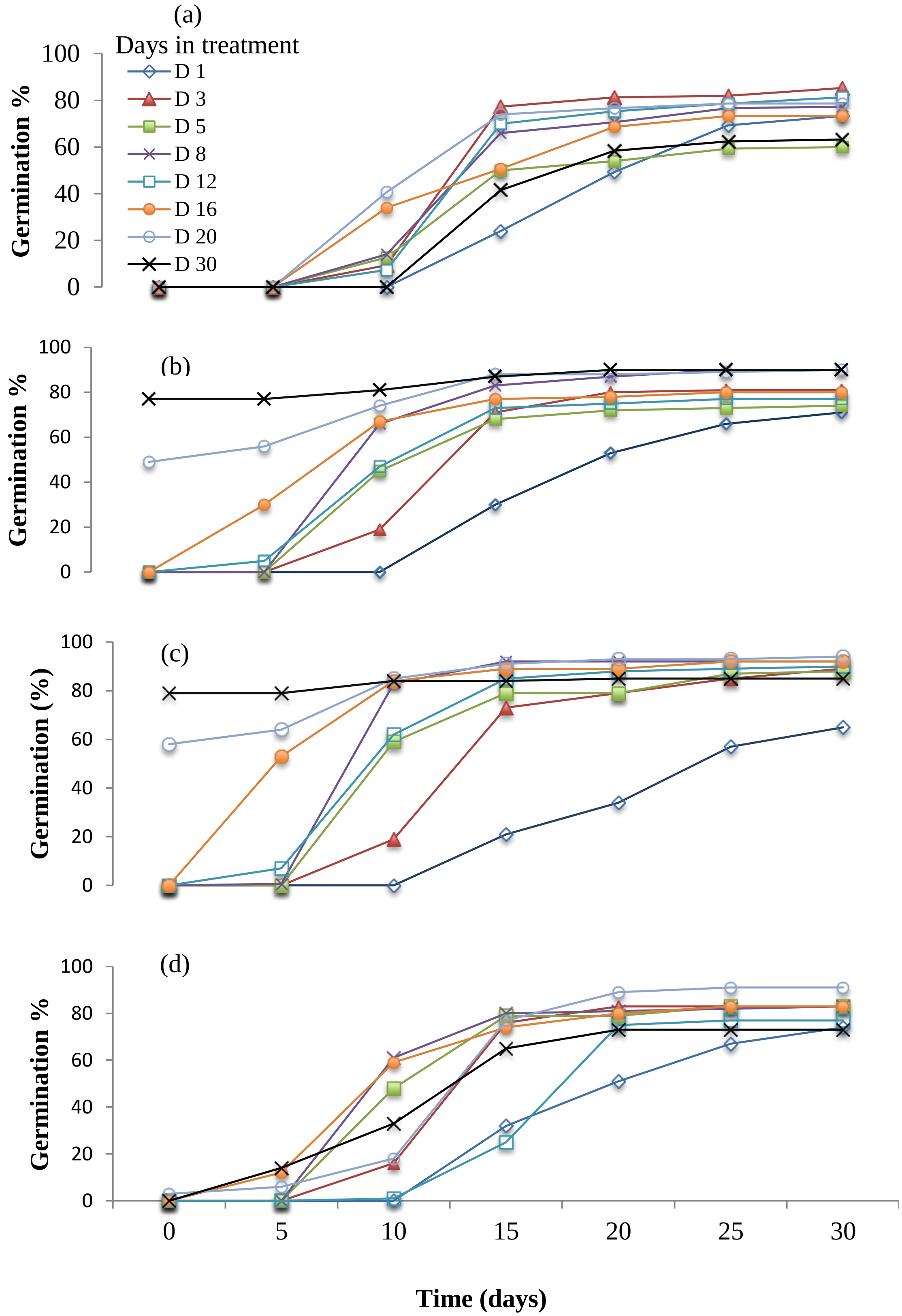


Figure 7. Cumulative germination rate of *Helonias bullata* seeds after 1, 3, 5, 8, 12, 16, 20, and 30 days (a) dry, (b) in the stream margin, (c) floating, and (d) submerged in Pigeon Branch in the Pink Beds, Transylvania County, North Carolina.

**Discussion**

*Helonias* exhibited high germination (>50%) after all treatments, except dry conditions in the growth chamber. Germination significantly (p=0.0001) decreased for seeds exposed to dry conditions at 60% RH for eight days and no germination occurred following a 35-day dry treatment. The presence of mold on seeds exposed to dry conditions for 35 days indicated they were non-viable. Based on these results, *Helonias* seeds unlikely entered into dormancy and may be short-lived. In addition, *Helonias* seeds appeared to lack dormancy when shed because they germinated to high percentages after all treatments, except dry controlled conditions, within a small germination window (10-30 days). Tweedle et al. (2003) suggested that lack of seed dormancy may confer a fitness advantage for plants in environments with a limited "window of opportunity" (Eriksson and Froborg et al. 1996) by maximizing seedling development during favorable conditions. Rapid germination when conditions are appropriate may be important for *Helonias* since it occurs in forested wetlands where spatial and temporal heterogeneity can create a narrow window for regeneration. Lack of dormancy allows mass germination of viable seeds soon after shedding, but may result in the death of all seedlings if environmental conditions change (Brock et al. 1989). Since *Helonias* reproduces primarily by asexual reproduction, the benefits associated with rapid germination probably outweigh the potential costs of seedling mortality.

*Helonias* seeds dry in the field for 30 days did not germinate while in treatment. Conversely, after 30 days, 77% of seeds germinated in the saturated stream margin and developed a cotyledon and primary root axis approximately 3-5 cm long. Based on these results, moisture availability appears to be the major factor that determines the germination of *Helonias* seeds in the field. Since *Helonias* may have short-lived seeds

(U.S. Fish and Wildlife Service 1991), appropriate moisture conditions for germination at time of seed arrival may be important for recruitment. Chen and Xie (2007) demonstrated that soil moisture content at time of seed arrival was the main factor that determined the germination of *Myricaria laxiflora*, an endangered plant with short-lived seeds that occurs along Yangtze River flood zone. Chen and Xie (2007) concluded that the soil water content of soils during seed dispersal and germination likely restricted the distribution of this species along the river's flood zone. Although *Helonias* reproduces primarily by asexual reproduction, seeds appear heavily dependent on moisture availability for germination, which may explain the high occurrence of rosettes in and along stream channels (Laidig et al. 2009). In addition, these results partly explain why rosette density decreases when soil moisture is below 80% (Dodds 1996), and rosettes are absent when the water table is 35-45 cm below the ground surface (Laidig et al. 2009). Guilloy-Froget et al. (2002) demonstrated for the floodplain species *Populus nigra* L., that after seeds released, germination, seedling recruitment, and subsequent survival depended on a continuously moist, bare substrate for four weeks. Guilloy-Froget et al. (2002) concluded that the recruitment of *P. nigra* was likely restricted to post-spring flooding events when moisture was readily available.

The seed release of many wetland plants coincides with periods of appropriate moisture conditions for germination and establishment, such as post-flooding events (Pierce and King 2007). *Helonias* flowers early spring and fruit maturation occurs by the end of May/early June (Chafin 2007), thus, seed release may coincide with high moisture availability associated with receding water levels in early summer. In the Pink Beds, where *Helonias* rosettes occur, the water table generally stays near the surface with only slight seasonal water table fluctuations (Brady Dodd, personal communication). In southern New Jersey, where over half of the world's *Helonias* populations occur (U.S. Fish and Wildlife Service 1991), flooding can take place from mid-winter to mid-summer with seasonal high water levels in early spring (Ehrenfeld and Schneider 1991). Thus, the maturation and release of *Helonias* seeds in late spring/early summer may coincide with periods of high moisture availability.

Only a small percentage (3%) of *Helonias* seeds germinated while submerged in Pigeon Branch. In contrast, 77% of seeds germinated while submerged in controlled conditions. The presence/absence of soil between these two treatments likely caused the variability in germination responses; seeds removed from Pigeon Branch were covered in soil. These results support DeBell and Naylor's (1972) study on the germination ecology of *Nyssa sylvatica var. biflora* (Walt.) Sarg. (swamp tupelo) because seeds germinated while submerged without soil; however, no germination occurred for seeds submerged in conditions with soil. The difference between germination responses is likely due to a lower oxygen supply, the presence of microbes, and organic matter in flooded soil (Rudinger and Dounavi 2008). Although only a few *Helonias* seeds germinated while submerged, final germination percentages were high after submergence for 1-30 days in Pigeon Branch. In addition, germination onset occurred earlier for seeds submerged for 8-30 days. Based on these results, *Helonias* seeds submerged in hollows, seepage areas, or streams could maintain germinability for up to 30 days and germinate once water levels recede. The ability to retain viability while submersed is noted as an important trait for seeds dispersed via water (Rudinger and Dounavi 2008; Kestring et al. 2009), and for plants occurring in areas periodically flooded (Guo et al. 1998). Overall, since

*Helonias* inhabits swamps where flooded conditions can occur from mid-winter to mid-summer (Ehrenfeld and Schneider 1991), maintaining germinability while submerged may be important for seed survival. However, *Helonias* seeds submerged for prolonged periods of time that germinate by mid-summer or thereafter, may not establish by the end of the growing season because seedlings reportedly have slow growth rates (Dodds 1996).

*Helonias* exhibited high floating capability; 94% of seeds remained buoyant for the length of the experiment (35 days) in controlled conditions. Since only a small percentage of seeds germinated while submerged in Pigeon Branch, the high floating capability of *Helonias* seeds may increase the likelihood they deposit on 'safe sites' for germination. Schneider and Sharitz (1988) concluded that because germination was inhibited for the seeds of *Taxodium distichum* (bald cypress) and *Nyssa aquatica* (water tupelo) while submerged in flooded conditions (DuBarry 1963), the high floating capability of seeds (2-3 mths) increased the probability they found elevated microsites for germination and establishment. In addition to high floating capability, germination was enhanced for *Helonias* seeds in contact with water in the field. For instance, final germination percentages for *Helonias* seeds floating in Pigeon Branch were significantly ($p=0.0004$) higher in comparison with seeds kept dry in the field. In addition, germination onset occurred faster for *Helonias* seeds floating in the Pigeon Branch after 8-30 days and for seeds in controlled conditions after 3-8 days. For instance, 90% of seeds floating in controlled conditions and 85% of seeds floating in Pigeon Branch germinated while in treatment after 13 and 30 days, respectively. Water may serve as an important dispersal mechanism for *Helonias* seeds because they exhibited two key traits that determine water dispersal success: high floating capability and germinability relative to the amount of time spent in the water (Schneider and Sharitz 1988). In the Pink Beds, Sutter (1984) reported that wind unlikely disperses seeds greater distances than 40 cm. As *Helonias* rosettes typically occur along and within poorly defined stream channels (Laidig et al. 2009), water may serve as a more effective dispersal agent than wind or gravity by dispersing *Helonias* seeds further distances.

*Helonias* germinated and formed a cotyledon and primary root 3-5 cm long while floating on distilled water after 21-35 days and while floating in the Pigeon Branch after 30 days. *Helonias* appears to form floating "seedling banks" (Scarano 1998). *Orontium aquaticum* L. (goldenclub) forms floating seedling banks, which gives them ample time for development before anchoring into substrate during summer drawdown (Conti and Gunther 1984). *Hottonia palustris* L. (water violet) an emergent perennial that inhabits forested wetlands in the Netherlands, forms floating seedling banks, which may be an adaptation to occur in areas with shallow water bodies that frequently recede (Brock et al. 1989). In southern New Jersey, *Helonias* occurs in areas where water levels can range from 20-30 cm above hollow bottoms in early spring to 20-30 cm below hollow bottoms late summer and fall (Ehrenfeld and Schneider 1991). *Helonias* seeds that germinate after making contact with standing water in hollows or lateral flow in seepage areas could establish faster once water levels recede during summer months. Overall, the ability to germinate while floating and form seedlings, provided roots and shoots stay alive, may be an adaptation to rapidly establish once water levels recede (Brock et al. 1989); to avoid seedling submersion and anoxic stress (Wittmann et al. 2007); and to inhabit areas with frequent flooding with a short terrestrial phase (Scarano et al. 2003).

*Conservation Efforts*

*Helonias* appears to have a "regeneration niche" (Grubb 1977), that is dependent on water availability not only for dispersal, but also for germination. In the field, *Helonias* seeds appear to have a short germination window (3-4 wks.) heavily dependent on moisture availability for rapid germination. Water likely serves as an important dispersal mechanism for *Helonias* seeds since they have high floating capability and germinability relative to the length of time spent in the water. Thus, recovery efforts for declining *Helonias* populations should focus on hydrological restoration (ground water levels and surface flow). To increase groundwater levels, restoration efforts could include restoring the natural sinuosity of channelized streams, removing overburden, and/or restoring microtopographic relief (Rossell et al. 2009). In addition, the installation of log sills (hardwood log placed perpendicular to a stream) can increase ground water levels.

*Helonias* populations in small, isolated or disjunct habitat patches may be at a higher risk of extinction or extirpation due to the equilibrium theory of island biogeography postulated by MacArthur and Wilson (1967). *Helonias* populations occurring in highly disjunct wetland habitat or "islands in a terrestrial landscape" (Edwards and Sharitz 2000) may be more threatened due to a (1) distance effect (islands closer to the mainland are more likely colonized by new individuals than islands further away) and (2) an area effect (extinction rates will be higher for small islands than on larger islands) (Batzer et al. 2006). Recovery efforts, including habitat restoration and reintroduction may be necessary to reduce the “area effect” and “distance effect” for small, isolated *Helonias* populations. Direct seeding or planting may be needed at restored sites. To facilitate the natural dispersal process, direct seeding efforts are preferred over planting individuals because multiple genotypes are utilized (Primack and Miao 1992). Direct seeding should be attempted over multiple years because recruitment may depend on the unique combination of a particular genotype, in a certain location, during a certain year with favorable conditions (Primack and Miao 1992). Based on the results of this study, direct seeding should be done in areas with high moisture availability such as stream margins, seepage areas, and/or *Sphagnum*-covered hummocks.

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