

METHODS TO RESTORE NATIVE PLANT COMMUNITIES AFTER INVASIVE  
SPECIES REMOVAL: MARL PRAIRIE PONDS AND AN ABANDONED PHOSPHATE  
MINE IN FLORIDA

By

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To my sister, Daniela Dutra, science will never be the same again

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Abstract of Thesis Presented to the Graduate School  
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Because Florida's natural ecosystems are increasingly invaded by exotic and undesirable plant species, invasive species removal is a major part of ecosystem restoration, and revegetation efforts after invasive species clearing is often necessary. Invasive species removal can be achieved through mechanical, cultural, chemical, or biological means. Few studies have addressed methods for successful native plant recolonization after invasive species removal using revegetation strategies.

Different techniques for native species establishment were investigated in formerly invaded hydric and mesic-xeric ecosystems. The hydric site, consisting of two marl prairie ponds, and a mesic-xeric site, an abandoned phosphate mine, were both initially monotypic stands of *Salix caroliniana* Michx. (coastalplain willow) and *Imperata cylindrica* (L.) P. Beauv. (cogongrass), respectively. Following removal of the invasive species either by mechanical or chemical methods, we investigated different revegetation techniques.

At the hydric site, the effects of two planting densities, two elevations, and two different propagule sizes on plant survival and volume of installed native plants were

researched. Results conclude that planting at sparse densities (3 ft centers) is most desirable since it is cost-effective and survival rates were higher, although dense plantings initially increased biodiversity and species richness. Effect of planting elevation on survival and volume was species-specific. Using 5-inch potted plants is recommended to maximize survival and reduce the necessity for subsequent plantings.

At the mesic-xeric site, two planting regimes (grasses or grasses/forbs/shrub) and two herbicide applications for removal of *I. cylindrica* were studied to assess the effects of these factors on plant volume and survival of planted native species.

Generally, using grasses/forbs/shrub plantings increases species richness, initial establishment and survival. Herbicide application to encroaching *I. cylindrica* decreased cover of the invasive species, but did not increase native plant volume in low structural diversity plots, and actually decreased native volume in high structural diversity plots.

Results show that revegetating after invasive species removal initially increases biodiversity and species richness. Installing plants at sparse densities is both cost effective and promotes planted species growth, and using larger propagules maximizes survival. Planting native species alone deterred *I. cylindrica* spread, and application of herbicide after revegetating was not beneficial.

## CHAPTER 1 INTRODUCTION AND LITERATURE REVIEW

### **Introduction**

Florida's landscape consists of unique and highly valued natural conservation areas including: forests, flatwoods, prairies, swamps, marshes and waterways. Rapid population growth and natural processes make Florida susceptible to land disturbances. Disturbed land results naturally from hurricanes (Lugo 2000) and severe fire as well as from anthropogenic causes such as mining (Carrick & Kruger 2007) urbanization, and construction. Similarly, upon large-scale removal of invasive species, ecosystems may become disturbed and barren, susceptible to further reinvasion (Harper et al. 1961; Johnstone 1986), and may not adequately be recolonized with desirable native species. In efforts to restore degraded ecosystems on Florida's public land, over 33 million dollars were spent in 2007 controlling 122,000 hectares of both aquatic and terrestrial invasive species (DEP 2009). If revegetation strategies are not implemented, primary removal of invasive species can lead to the introduction of other undesirable species, potentially resulting in a secondary monotypic population (Ogden & Remanek 2005; Shilling et al. 1997). Methods for native species restoration after invasive species removal are needed, but little research has addressed this issue.

### **Invasive Species Effects on Ecosystems**

When invasion of an undesirable species occurs in a healthy ecosystem, several scenarios are possible: (1) the ecosystem remains unchanged, (2) after invasion, minor alterations to habitat or biodiversity occur, or (3) the invasive species becomes dominant in the ecosystem. Invasion can lead to extreme habitat degradation, loss of ecosystem function, and a decrease in biodiversity (Williams 2007). Of these three

scenarios, this research will address the third situation where invasive species removal is a critical component of restoration efforts.

Invasive species have negative ecosystem impacts in both hydric and mesic-xeric ecosystems. Where invasive species impacts to hydrology lead to extensive changes in native species distribution, removal is critical for ecosystem recovery. For instance, in Everglades National Park, *Melaleuca quinquenervia* (Cav.) S.F. Blake (punktree) alters hydrology through interception in rainfall (where precipitation is intercepted by the canopy and evaporated back into the atmosphere), raises evapotranspiration rates higher than native species (such as *Cladium mariscus* (L.) Pohl ssp. *jamaicense* (Crantz) Kük. (Jamaican swamp sawgrass)), and also reduces surface flow (Laroche 1998). Other aquatic invasive species, such as *Hydrilla verticillata* (L. f.) Royle (hydrilla), *Pistia stratiotes* (L.) (water lettuce) and *Eichhornia crassipes* (Mart.) Solms (water hyacinth), elevate evapotranspiration rates. Invasion of floating aquatic species also results in drastic loss of desired submerged vegetation because of interruption of sunlight (Deuver et al. 1986). Invasive species in more mesic sites affect the ecosystem in a variety of ways; for instance *Cyperus* spp. displaces native species through rapid reproduction and *Imperata cylindrica* (cogongrass) alters fire regimes and plant community composition. An invasive fern species, *Lygodium microphyllum* (Cav.) R. Br. (small-leaf climbing fern), acts as a fire ladder and, upon fire entry, decimates populations of native trees (Pemberton & Ferriter 1998).

These negative impacts to ecosystems caused by invasion motivate natural resource managers to develop strategies to reinstate self-sustaining diverse ecosystems by accelerating ecosystem recovery. Removal of invasive species

represents elimination of a barrier to ecosystem recovery, and is a major component of most restoration projects.

### **Removal of Invasive Species**

Methods for removing invasive species include mechanical, chemical, biological, and cultural methods of control. An example of mechanical control is harvesting for *E. crassipes* or excavation of *S. caroliniana*. The alligatorweed flea beetle is used for biocontrol of *Alternanthera philoxeroides* (Mart.) Griseb. (alligatorweed), just as *Ctenopharyngodon idella* (grass carp) is used on *H. verticillata*. Cultural control, such as crop rotation, can be effective in removing undesirable weedy species from agronomic crops. However, the use of chemical herbicides is the most widely-used means of controlling invasive species (Valenti 2002).

Repeated chemical herbicide applications to treat invasive species can alter soil properties (Pickart et al. 1998) and microbial communities (Buisson et al. 2006), as well as decrease plant cover. Similarly, mechanical control, such as scraping, removes plant communities and leaves modified soil conditions. These disturbances alter ecosystems on both spatial and temporal scales (White 1985). In some instances, moderate soil disturbance creates favorable conditions for the establishment of a new suite of species (Platt 1975; McIntyre et al. 1995). In contrast, other studies suggest severe soil disturbance provides little shelter for seedlings thereby creating a less tolerable environment for seedling growth and development (Rapp & Rabinowitz 1985). Regardless, recolonization of a high quality plant community on disturbed soil will take years for degraded ecosystems, and may never occur unassisted for others.

## **Revegetating after Removal**

Several factors affect plant community recovery after large scale invasive species removal: (1) distance from off-site propagule sources of the original invasive species, (2) factors affecting the natural dispersal of weed seeds, (3) recolonization from on-site propagule sources (seed bank and surviving individuals, and (4) reinvasion by secondary undesirable species on bare ground. Because the transition from invasive species control to native plant recolonization is uncertain, and planting after disturbance can prevent or slow invasion (Spieles 2005), revegetation is an important, logical subsequent step upon removal of invasive species (Gutrich et al. 2009). Little is known about maximizing the potential of revegetation success in a restoration context. Selecting appropriate plant material, evaluating water source-plant location, post-planting invasive species control, and monitoring newly establishing native species can increase the chances for revegetation success.

Revegetation can be accomplished through seeding or installing plants. Seeding is more common and appropriate when monetary resources are limited (Walker et al. 2004), land is easily sowable (Kiehl et al. 2006; Lindborg 2006), and seeds are not persistent in the seedbank (Milberg 1995; Bakker et al. 2002). If seeding fails to establish a native species, installing plug-plants which have been grown in a greenhouse may be another option since plug-plants have greater establishment success than seed (Wallin et al. 2009), though little species-specific information is available to maximize the establishment with this method.

Understanding factors that affect revegetation of disturbed sites could potentially maximize the impact of these efforts. Utilizing different plant propagule sizes (smaller plugs versus container plants), determining the correct planting location (shade versus

sun, wet versus dry, acidic versus alkaline soil), and planting at an appropriate planting density (dense or sparse) can collectively influence the success of restoration.

Planting density is important in both promoting the growth and spread of native species and reducing reinvasion. Kim et al. (2006) planted *Salix* spp. stakes of 90 cm tall x 1.3-2.5 cm in diameter at densities of 0.60 m and 0.91 m on center and concluded these are most effective for control of *Phalaris arundinacea* L. (reedcanary grass) compared to planting on 1.41 m centers. Conversely, a less dense planting provided greater plant response (growth and viability) with *Zostera marina* L. (eelgrass) at 5 plants m<sup>-2</sup> in an intertidal zone near Balgzand (Netherlands) (Bos & van Katwijk 2007). From the references above, it can be inferred that the optimal planting density required is a function of the ecosystem, the invader, and the native species being planted.

Propagule size is another important consideration to assure successful establishment of desired plants. Ratliff & Westfall (1992) found that 5.1 cm diameter plugs of *Carex exserta* had 52% greater survival over a 4 year study period than 1.9 cm diameter plugs of the same species because greater root development decreased transplanting stress. Restoring with larger plants is likely more costly than using small plugs due to associated labor and space requirements for plant production and handling. Little research has addressed the topic of propagule size as it relates to establishment success for other native plant communities.

A greater degree of species richness in restoration projects is also an important consideration for revegetation efforts. Ecosystems constantly evolve to become resilient to disturbance (Cropp & Gabric 2002) where changes in species composition are common (Walker et al. 1999). Species diversity increases the ability of an

ecosystem to become more resilient at the community level (Steiner et al., 2006) and resistant to nonnative plant invasion (Stachowicz et al. 2002; Hooper et al. 2005). However, some theorists have argued that ecosystems with high biodiversity are intrinsically unstable since species routinely appear and disappear. Regardless of the stability of diverse systems, high species richness is usually a common restoration goal since a large species pool is more likely to support a functioning ecosystem subjected to disturbance. Therefore, higher diversity revegetation efforts are more likely to result in ecosystems less prone to impact by potential future invasions and ecosystem degradation.

Plant growth and development in aquatic and wetland sites is dependent upon water availability and thus planting location with respect to water depths. Native wetland species (e.g. *Potamogeton pectinatus* (L.) Böerner (sago pondweed), *Najas flexilis* (Willd.) Rostk. & Schmidt (nodding waternymph), and *Lemna* spp. L. (duckweed)) are especially responsive to alterations in hydrology and hydroperiod, therefore, planting elevation should be a limiting factor (van der Valk 1981; Spence 1982). Budelsky & Galatowitsch (2000) showed that water level conditions are most crucial during the first year of plant establishment because this is the time frame in which both growth and mortality is greatest.

An understanding of the relative importance of factors that influence plant community establishment can only be confirmed experimentally. Few studies have addressed this research need using multiple species, so generalizations across species may or may not be appropriate. In their study of two forbs native to wooded hay meadows, Wallin et al. (2009) found that for both species studied, plants were better

established from plug-plants versus seed addition. However, for many ecosystems, particularly spatially heterogeneous wetlands, we expect species-specific differences with respect to optimum planting methods. Despite the benefits that active revegetation offers for ecosystem restoration, for many degraded ecosystems in which the restoration need is great, species-specific information on revegetation methods is lacking.

## **Model Systems**

### **Freshwater Depression Marshes within Everglades Marl Prairies**

Hydrologic alterations and fire suppression have led to major changes in the vast Everglades wetland ecosystem of south Florida, USA over the past century. Changes in plant community and structure result in degraded ecosystem health and a reduction of several species indigenous only to the Everglades. The Everglades are of considerable biodiversity value, containing a disproportionately large number of the world's endangered and threatened species such as the Florida panther (*Felis concolor coryi*), the Cape Sable seaside sparrow (*Ammodramus maritimus mirabilis*) and several native orchids (*Oncidium* spp., *Cyrtopodium* spp.). Therefore, removing invasive species would likely increase native species populations and could potentially prevent extinction.

Hydrologic alteration in the Everglades includes channelization of the Kissimmee River and construction of the Central and Southern Florida Project (McCoy et al. 2007) which protect agriculture and over 5 million people. Alteration of fire regimes has also been considerable; the National Park Service rapaciously suppressed wildfires in the Everglades starting in 1947. Prescribed fires were reinstated in 1958 (Segar 2009) but the lower frequency and intensity has dramatically altered marshes and other

ecosystems in the area by altering the plant populations which were not fire-adapted (Lodge 2005).

Areas which were historically freshwater depression marshes have become dominated by *Salix caroliniana* (a native facultative wetland species which has become opportunistic) which has excluded native species. Freshwater depression marshes within marl prairies occur only in south Florida (Whitney et al. 2004) and are characterized as shallow, rounded depressions in a sandy soil or subsurface hardpan and can either be permanent or temporary with hydrology ranging from 50—200 days per year (Florida Natural Areas Inventory and Florida Department of Natural Resources 1990). These marshes are composed of mesic species such as, asters (*Aster* spp.), beaksedges (*Carex* spp.), bluestems (*Andropogon* spp.), milkweeds (*Asclepias* spp.), and lovegrasses (*Eragrostis* spp.), as well as wetland species such as American white waterlily (*Nymphaea odorata* Aiton), cypress (*Taxodium* spp.), pickerelweed (*Pontederia cordata* L., *P. rotundifolia* L.), sawgrass (*Cladium* spp.), arrowhead (*Sagittaria latifolia* Willd., *Sagittaria lancifolia* L.), spikerush (*Juncus* spp.), and starrush whitetop (*Rhynchospora colorata* (L.) H. Pfeiffer), among other wetland species.

### **Pine Flatwoods**

Pine flatwoods are the most extensive terrestrial ecosystem in Florida with very few virgin pine flatwoods left in existence. Pine flatwoods act as refuges for many faunal and floral species including white-tailed deer (*Odocoileus virginianus*), the threatened Florida black bear (*Ursus americanus floridanus*), gopher tortoise (*Gopherus polyphemus*), the threatened red-cockaded woodpecker (*Picoides borealis*) and Chapman's rhododendron (*Rhododendron chapmanii*). Pine flatwoods possess unique characteristics such as low, flat topography, poorly drained, acidic soils, and frequent

fires (every 4-10 years). These ecosystems are comprised of an open pine (*Pinus* spp.) canopy, an extensive shrub layer, most commonly saw palmetto (*Serenoa repens* (Bartram) Small) but also gallberry (*Ilex glabra* (L.) A. Gray), fetterbush (*Lyonia lucida* (Lam.) K. Koch), wax myrtle (*Myrica cerifera* (L.) Small), blueberries (*Vaccinium* spp.), wiregrasses (*Aristida* spp.), broomsedges (*Andropogon* spp.), and sporadic forbs including, asters (*Aster* spp.) and Catesby's lily (*Lilium catesbaei* Walter).

Flatwoods are often degraded as a result of phosphate mining, and required reclamation may fall short of restoring the flatwoods plant community. Former phosphate mines are characterized by increased soil disturbance, decreased biodiversity, and altered plant community composition (Manner et al. 1984). Also, this ecosystem type has been compromised throughout the state of Florida because of extensive exotic plant invasions by *Imperata cylindrica*, *Lygodium* spp, *Sporobolus* spp. (smutgrass), *Cyperus* spp., and several other weedy taxa. Anthropogenic results of pine flatwoods degradation include poaching of endangered plants, reduced prescribed burns, and undesirable species invasion.

*Imperata cylindrica* was introduced accidentally as a packaging cushion for shipping cargo from Asia to America (1911) and later intentionally as a potential forage (1920s), and has become a noxious invasive exotic. This species invades rapidly, forming monotypic stands that exclude native species, especially during wildfire (Hubbard et al. 1944; Gaffney 1996). Effective methods for initial control of *I. cylindrica* have been developed (applying 1.5% imazapyr in fall in conjunction with tilling), but revegetation efforts in pine flatwoods have not been researched (MacDonald 2004).

## Research Goals

Determining optimum revegetation methods may be critical to restoration of invaded freshwater marshes and pine flatwoods. This research investigates several aspects of revegetation following invasive species removal in a mesic-xeric and hydric site.

The first objective of this research will be to evaluate the effects of planting density, planting elevation, and propagule size on native plant establishment and survival in depression marshes at the Florida Panther National Wildlife Refuge. Two planting densities (dense and sparse), elevations (high and low), and propagule sizes (plug and container) will be analyzed to determine the effects of planting method for several species on native plant re-establishment.

The second objective is to determine the effects of planting species composition and subsequent chemical control of *I. cylindrica* on native species re-establishment at the Tenoroc Fish Management Area in Lakeland, Florida. Two planting regimes (high and low structural diversity) and herbicide applications (treated or untreated) will be evaluated to determine if structural diversity and subsequent herbicide control of *I. cylindrica* will suppress re-invasion.

## CHAPTER 2 REVEGETATING MARL PRAIRIE PONDS AFTER INVASIVE SPECIES REMOVAL

### **Introduction**

Wetlands provide many functions in an ecosystem such as recycling nutrients, filtering pollutants, and providing habitats for endangered and protected flora and fauna. It follows that wetland degradation resulting from anthropogenic changes has been of major concern since the mid-twentieth century (Lodge 2005; McPherson & Halley 1996). The major effect of anthropogenic development is alteration in hydrology and fire regime (Carrick & Kruger 2007; McPherson 1974) with south Florida providing a prime example (Lodge 2005). Everglades National Park and its surrounding wetland ecosystems, including Big Cypress National Preserve and Fakahatchee Strand Preserve State Park, are restoration focal areas for both researchers and practitioners. In these systems, altered disturbance regimes have led to plant invasions that are significant in scope.

For restoration of invaded ecosystems, invasive species removal is often a key component of restoration goals and success. Invasive species alter ecosystem functions, values, and biodiversity by changing soil components, altering hydrology, outcompeting native species, and can act as catalysts to wildland fires (Lugo 2000; Carrick & Kruger 2007; Williams 2007). Upon large scale removal of invasive species, natural landscapes become disturbed resulting in “safe sites” for other undesirable invasive species (Johnstone 1986). Native species can act as a barrier to weed seed emergence if active revegetation strategies are implemented (Blumenthal et al. 2005)

Revegetating an area upon removal of invasive species encourages ecosystems to become more resilient to potential future alterations (Spieles 2005). Rapidly

establishing vegetation is often key to satisfying restoration contracts and mitigation requirements. Despite the critical nature of revegetation efforts, factors that should be taken into account before initiating a revegetation project are not well-known.

Possible considerations for revegetation include planting density, species selection, planting elevation, and propagule size.

Planting at greater densities may allow for greater biodiversity and faster recolonization (Bos & Katwijk 2007). Alternatively, sparser planting densities may allow for greater growth since plants are not as compact and competition is not as prevalent (Kim et al. 2006). In order to quickly establish a healthy, productive seedbank, using larger plant material (3-5 inch pot size) compared to smaller plant material (plugs from 72 count plug trays, approximately 2-inch diameter cells) may be more desirable (Ratliff & Westfall 1992). Plants installed at higher elevations may have lower survivorship than plants installed at lower elevations since soil moisture is limiting at higher elevations due to the fluctuating water table (van der Valk 1981).

To determine the effects of several factors on native plant establishment and revegetation efforts in a south Florida wetland, two experiments were initiated. The first experiment examined the effects of planting density and planting elevation on establishment of eight native wetland species. Experiment 2 examined the effects of propagule size and planting elevation on native plant establishment and growth. The ultimate goal of these studies is to provide information pertinent to the development of revegetation protocols for freshwater depression marshes.

## Materials and Methods

### Study Site

The Florida Panther National Wildlife Refuge [FPNWR] is located near Naples, Florida in the Big Cypress Basin. Anthropogenic alterations in ecosystem hydrology and fire suppression have led to a significant decrease in ecosystem function, value, and structure. As such, opportunistic species like *Salix caroliniana* Michx. (coastalplain willow) have colonized several freshwater depression marshes in marl prairies leading to a preclusion of native, desirable species. Two areas of focus at FPNWR have been documented through historic aerial photographs as formerly open-water depression marshes, or ponds, within the marl prairie. No published studies address restoration of *Salix caroliniana*-dominated wetlands, therefore no information on revegetating freshwater depression marsh species is available.

### Site Preparation

The two study ponds [(Pond 1 (lat 26°9.636 N, long 81°20.902 W) and Pond 2 (lat 26°9.773 N, long 81°21.077 W)] were surveyed in January 2006 before developing the experimental studies. The following parameters were assessed: extent of *S. caroliniana* invasion, soil nutrient status, current plant community composition, and seed bank composition (Adams et al. 2009). As suggested in the Comprehensive Conservation Plan for FPNWR (Krakowski et al. 1998), regrading the marshes is necessary to protect, restore, and manage for the following: (1) candidate, threatened, and endangered species, (2) migratory birds and their habitats, (3) wetlands and freshwater habitats, and (4) biodiversity. *Salix caroliniana* was removed from the site via excavation in January 2006 by FPNWR staff.

Two separate experiments were implemented in June 2007 to determine effectiveness of both planting density and species size at two different elevations. Plots were placed at high (mean elevation= 0.9 m above water level) and low (mean elevation= 1.2 m above water level) elevations with respect to the littoral zones of the ponds. In order to measure water table location, monitoring wells were installed according to Soil Conservation Service (1993) guidelines.

At time of planting (post-excavation and regrading), both study ponds contained no vegetation. From January 2008 to December 2008, *Typha* spp. (cattail) invaded Pond 1 and cover increased from 30% to >80% of the open water area (below the elevation of study plots). Because *Typha* spp. is opportunistic and grows densely in disturbed wetlands, it was critical to restoration goals to control this invasive species. Glyphosate (3% solution of aquatic-labeled herbicide) was applied on December 18, 2008 to invading *Typha* spp. Herbicide was applied when plants were entering dormancy, and translocation of carbohydrates in the downward direction was optimal for herbicide translocation to rhizomes.

### **Plant Material Acquisition and Propagation**

The Guide to the Natural Communities of Florida, a comprehensive guide which classifies each natural community in Florida based on hydrology and vegetation, was used to determine species selection for revegetation in depression marshes.

Depression marsh species for both experiments (8 species for experiment 1, 5 species for experiment 2, Table 2-1) were selected from a related seedbank assay (Adams et al. 2009) based on their wildlife value, likelihood of recolonization, wetland indicator status (facultative (usually occur (67-99%) in wetlands) or obligate (occurs almost always (99%) in wetlands) wetland species (United States Fish and Wildlife Service 1998) and

potential for propagation. After species selection, in February 2007, species were propagated via cuttings or division, placed in a fine-textured planting media (Fafard® 2P Mix), moved to a misthouse for 3 weeks at mist intervals every 10 minutes, then hardened off in a temperature monitored greenhouse for 3 months prior to installation (June 2007) in Gainesville, FL.

## **Experimental Design**

### **Experiment 1: Effects of density and elevation on plant establishment**

Two parameters for Experiment 1 were tested: effects of planting density and planting elevation on survival and growth. Three density treatments were tested: 1) dense [0.46 m between plants (1.5 ft centers), 48 plants per plot], 2) sparse [0.91 m between plants (3.0 ft centers), 24 plants per plot], and 3) control (unplanted). All density treatments were installed at two levels of elevation: high (1.2 m above water level) and low (0.9 m above water level). Plot sizes varied to accommodate plant material and spatial arrangement of treatments, such that dense plots were 9.5 ft x 12.5 ft (2.9 m x 3.81 m) and sparse plots were 11 ft x 17 ft (3.35 m x 5.18 m). Six and three specimens of each of the eight species were planted for dense and sparse treatment plots, respectively. Treatments were replicated five times for a total of 30 plots per pond and installed at both study ponds.

### **Experiment 2: Effects of propagule size and elevation on plant establishment**

Two parameters for Experiment 2 were tested: the effects of planting elevation and propagule size on survival and plant volume. Propagule size treatments consisted of: 1) 72-count plug tray size (2-inch diameter cells), 2) 5-inch diameter pot size plants, or 3) unplanted (control) plots. These treatments were applied at two levels of elevation: high (1.2 m above water level) and low (0.9 m above water level). Plot dimensions were

10 ft x 12 ft (3.04 m x 3.66 m) with density consistent across treatments (plants installed at 1.5 ft centers, 0.457 m). Five individuals of five species were installed in each plot for a total of 25 plants per plot. Treatments were replicated three times for a total of 12 plots and installed at Pond 2 only, as Pond 1 size would not also accommodate space for this experiment.

### **Data Collection**

Data were collected from June 2007 through June 2008. Plant volume and percent survival data were collected at time of planting, as well as 6 and 12 months after planting. Plant volume (m<sup>3</sup>) was measured using a three-dimensional measuring device constructed from PVC. Percent survival was measured by locating planted individuals and observing them as alive (green shoots present) or dead. Species richness (quantity of species) data were collected at zero, three, six, and 12 months after planting by visually counting the number of species present in each treatment plot.

Water table depth data were collected from the installed wells 6 and 12 months after planting. Soil samples were obtained after excavation, at time of planting, and 18 months after planting using a tulip bulb planter to extract the top 5 cm of soil, placed in plastic zip bags, and transported on ice to prevent fungi or mold from accumulating. Soils were analyzed at the Analytical Research Laboratory (ARL) at the University of Florida for phosphorus, total Kjeldahl nitrogen (TKN), NO<sub>3</sub>-N, organic matter, and pH.

### **Statistical Analyses**

Data were analyzed using Statistical Analysis Software (SAS®) version 9.1.3 (SAS Institute Inc. 2004). For Experiments 1 and 2, analysis of variance (ANOVA) was used to assess main effects and interactions, and means were compared using the Least Significance Difference (LSD) procedure at  $p \leq 0.05$ .

## Results

All species planted in both experiments had greater than 20% survival, and some had near 100% survival. The effects of all treatments (elevation, planting density, propagule size) significantly affected plant volume and survival. However, there were species-specific exceptions to the general trends. For both experiments, plant volume generally increased while survival generally decreased over the 12 month study period.

At the time of planting (June 2007), water levels were excessively low due to a regional drought. Throughout the study period, water availability (depth to water at planting elevations) in both ponds varied with the seasonal pattern of precipitation (Figure 2-1). Although elevation treatments in each pond were initially set at hydrologically equivalent levels with respect to water table height, depth to water measurements were not equivalent across ponds, such that depth to water table differed significantly between parallel treatments (ANOVA  $F_{\text{pond}}=3.56$ ,  $p=0.04$ ).

Soils data reveal that soil quality was low in both ponds. Over time, pH levels did not vary. However, total nitrogen levels decreased by almost 6 times over an 18 month period. Organic matter levels also decreased over 30 month period by almost 9 percent (Table 2-2).

### **Experiment 1 – Effects of Planting Elevation and Planting Density on Percent Survival and Plant Volume**

Overall, plant volume (Table 2-3) was greater at sparse planting densities ( $p \leq 0.01$ ) across species. Overall percent survival (Table 2-4) was not affected by planting density ( $p=0.14$ ). Plant volume was greater at lower planting elevations ( $p=0.01$ ), but again did not affect overall percent survival ( $p=0.41$ ) for all species. Species-specific responses to treatments are summarized by species in Figures 2-2

through 2-9. Below, we detail the overall effect of each factor tested, focusing on 12 months after planting.

### **Effects of elevation**

At 12 months after planting, both *Cyperus haspan* (Figure 2-2) and *Fuirena breviseta* (Figure 2-4) had greater plant volume at the lower elevation ( $p \leq 0.01$ ), but *Pluchea rosea* (Figure 2-6) had greater volume at the sparse density at higher elevation ( $p=0.03$ ); whereas *Proserpinaca palustris* (Figure 2-7) had greater volume at both sparse density and low elevation ( $p_{\text{density}}=0.01$ ,  $p_{\text{elevation}}=0.44$ ). For all other species, changes in volume over time differed with respect to a combination of planting treatments, but in most cases, elevation had little impact on plant volume.

Overall survival was higher at the high elevation for *Proserpinaca palustris* (Figure 2-7) and *Rhynchospora colorata* (Figure 2-8). For all other species, planting elevation had little effect on survival. Survival decreased over time at high elevations for *C. haspan* (Figure 2-2), *F. breviseta* (Figure 2-4), and *V. hastata* (Figure 2-9). At lower elevations, survival decreased over time for *F. breviseta*, but either remained the same or increased for most other species.

### **Effect of planting density**

At 12 months after planting, volume was greater at sparse densities for all species except *Eleocharis atropurpurea* ( $p=0.17$ ), *C. haspan* ( $p=0.91$ ), and *F. breviseta* ( $p=0.76$ ) (Figures 2-3, 2-2, and 2-4, respectively). Survival was also greater at sparse densities for *P. palustris* ( $p=0.03$ ) and *V. hastata* ( $p=0.01$ ), but greater at higher densities for *R. colorata* ( $p=0.03$ ). For the other five species, survival was similar at both planting densities.

*Proserpinaca palustris* and *E. atropurpurea* volume decreased over time in dense planting treatments. *Eleocharis atropurpurea* volume also decreased over time in sparse planting treatments. On the contrary, volume increased at sparse planting densities over time for *P. rosea*, *P. palustris*, *R. colorata*, and *V. hastata*. Survival in sparse planting densities increased or remained the same over time for *P. rosea*, *J. polycephalos*, *P. palustris*, *R. colorata*, and *V. hastata*. Survival of all other species either remained the same or decreased over time at sparse planting densities.

### **Effects of elevation and planting density on species richness**

Species richness was not affected by elevation ( $p=0.54$ ) or density ( $p=0.56$ ) at either pond (Table 2-5 and Figure 2-10), however, pond was significant ( $p<0.01$ ). All unplanted plots had no species at time of planting, where planted plots contained the 8 planted study species at time of planting. More species were discovered at Pond 2 for unplanted and dense treatments during the study period but only for 6 through 12 months after planting for sparse treatments. Species richness increased rapidly in all treatments in the first three months after planting (at this point, control plots contained at least 3 or more species compared to planted plots). After this increase, species richness gradually leveled off for the rest of the study period. Considering only densely planted plots, Pond 2 had 8 more species per plot than Pond 1 six months after planting. The highest levels of species richness (18 species) observed were in unplanted plots 3 months after planting. At 12 months after planting, species richness ranged from 8-15 species (Table 2-11).

## **Experiment 2 – Effects of Planting Elevation and Plant Size on Percent Survival and Volume**

Overall, volume ( $p=0.68$ ) and survival ( $p=0.11$ ) did not differ with elevation, but did differ with propagule size (Tables 2-6 and 2-7). Volume ( $p\leq 0.01$ ) and survival ( $p=0.01$ ) were greater for 5-inch potted plants for all species except *V. hastata* (regardless of planting elevation). Species-specific responses to treatments are summarized by species in Figures 2-11 through 2-15. Below, we detail the overall effect of each factor tested.

### **Effects of elevation**

At 12 months after planting, planting elevation did not affect volume for all species except *P. rosea* ( $p=0.0453$ ) where lower elevations provided for greater growth (Figure 2-13). The volume of *P. palustris* decreased over time at high elevations for 5-inch potted plants (Figure 2-14). Conversely, volume increased at low elevations for the same species and plant size. All other species increased in volume over time regardless of elevation or plant size. Survival was greater for *V. hastata* ( $p=0.0353$ ) at higher elevations both 6 and 12 months after planting (Figure 2-15). For all other species, survival varied with time and plant size.

### **Effects of propagule size**

For all species except *P. palustris* and *V. hastata*, volume ( $p\leq 0.01$ ) was greater for 5-inch potted plants. At higher elevations, volume was greater for *P. palustris* and *V. hastata* as plug-plants, but at lower elevations, volume was greater as 5-inch potted plants. Survival was greater than 70% for all species except *P. palustris* and *V. hastata* both 6 and 12 months after planting at either elevation and planting size.

## Effects of elevation and propagule size on species richness

Species richness was not affected by any factor tested except propagule size (Table 2-8). Regardless of propagule size, species richness increased in planted plots past 3 months after planting. Unplanted plots increased in species richness from initial time of planting to 3 months after planting by almost 20 species, but over time, species richness decreased in unplanted plots and eventually leveled off at or below 12 species per plot (Figure 2-16; Table 2-12).

## Discussion

Establishment of native species following invasive species removal can be accomplished with a multi-species planting. However, results also show that following species-specific guidelines will maximize establishment, and that consideration of several factors in planting methods can significantly increase revegetation success. As an example, for 5-inch potted plants, *P. palustris* and *V. hastata* had 80% greater plant volume at low elevation than high elevation during the final data collection period.

Because conditions following invasive species removal present many barriers for native species establishment, selecting the appropriate plants with respect to water table and other environmental criteria is particularly important to establishing a successful plant community. In this study, planting at the correct elevation can increase plant survival significantly. For instance, planting an obligate wetland species like *P. palustris* at low elevations (with increased water availability) resulted in 1.5 times the survival than at high elevations for this species. With greater disparity in elevation treatments, more species-specific preferences for high or low elevation planting would have been observed given the differences in plant response found at this relatively

small variation in elevation. Also, several of the species selected have a fairly broad elevation range.

Revegetation experiments such as the two performed in this project may be useful for land managers and restoration practitioners who execute large scale wetland restoration projects. To communicate species-specific planting recommendations, perhaps categorizing species based on treatments that resulted in the most successful establishment is optimal. As an example, see Tables 2-8 and 2-9 that reflect the relative importance for each factor to establishment of species tested in this study.

### **Hydrology and Elevation Drive Establishment**

Hydrology is a key determinant for plant community development and patterns of plant zonation (Finlayson & Mitchell 1999). This is particularly true for emergent wetland plants, which are best suited for anoxic soil conditions while foliage remains above water (van der Valk 1994). Due to this adaptation, insufficient soil moisture may be the most common reason for wetland revegetation failure. Experiments with newly establishing seedlings have shown that drought stress was an important cause of decreased seedling survival rates (Rood et al. 2008). From this body of research, it was expected that hydrology would be a critical factor to plant establishment and success.

Water levels in the study ponds varied within season (according to expected seasonal precipitation patterns) but also were highly variable during the study period. At time of planting (June 2007) water levels were 90 cm and 120 cm to water table at low and high elevations, respectively, indicative of drought conditions. Despite setting high elevation treatments at equivalent elevations initially, high elevations in Pond 2 were drier than those in Pond 1. Abiotic characteristics related to water retention (e.g. soils, basin morphology) may have differed in the ponds, such that although ponds had

equivalent hydrology initially, Pond 2 retained more water over time. During the fall and winter months (September-February) over the entire study period, however, ponds experienced somewhat similar hydrologic conditions, as the entire study area of both ponds was inundated.

Species' hydrologic response was associated with typical patterns of occurrence in wetlands: plant volume was greater for obligate wetland species at low elevations and for facultative wetland species at higher elevations (Figures 2-2 through 2-9). This is to be expected since obligate wetland species rely more on a semi-permanent – permanent water source whereas facultative wetland species thrive in a more transitional water gradient (United States Fish and Wildlife Service 1988). There were some surprising results, e.g. survival was greater for some facultative wetland species at low elevations, but at high elevation obligate wetland species survival was greater. This suggests there is an elevation gradient in which hydrologic niches for both facultative and obligate wetland species overlap. A relatively small difference in elevation was tested, perhaps encompassing this niche, and therefore it is cautioned that if a greater range in elevation was chosen, more species-specific preferences may have been detected.

Revegetation was generally successful (despite drought conditions at time of planting) in that plant volume increased throughout the study period for all species. *Eleocharis atropurpurea* was one exception. One reason *E. atropurpurea* volume decreased is because this species is an annual (other species used were perennials) and senescence of planted individuals may have occurred between the 6 and 12 months after planting observation periods.

Despite the increasing trend in volume, percent survival showed a more complicated response. Survival fluctuated over time, but consistently declined in the first 6 months after planting for all species. Ponds were severely dry as a result of the summer 2007 drought during the initial establishment period, likely causing the decrease in survival at 6 months after planting. It is important to note, however, that over time precipitation generally increased, inundating both ponds and resulting in complete submergence of all planted species which caused some specimens to die. In some instances, specimens were recorded as dead in response to receding water levels, but eventually emerged with new growth. Therefore higher levels of survival were observed at 12 months than at 6 months after planting.

### **The Role of Planting Density and Propagule Size**

It is assumed that revegetating with larger plants results in greater likelihood of establishment because larger plants are hardier and more resistant to environmental stress since the root structure is more developed (Steed & Dewald 2003), whereas smaller-sized plants require more ideal conditions. Similarly, planting at high densities is thought to be optimal because of the nurse-plant concept: a member of one species facilitates the growth of another species by ameliorating the stressors of the local environment (decreasing soil evaporation rates, decreasing microsite temperatures causing less evapotranspiration in foliage) (Niering et al. 1963; Raffaele & Veblen 1998). Revegetation projects are primarily constrained by budget. Reducing cost, either by planting in lower densities, or by using smaller plug plants, would be an important increase in cost efficiency, if either of these strategies resulted in successful establishment.

Results showed plant volume was generally higher at sparse densities, possibly because of a lack of competition (fewer individuals competing for scarce resources). Budelsky et al. (2000) also found that at low planting densities, sedges obtained greater volumes due to reduced competition. Not only is planting at lower densities more cost-effective than higher densities, but this lower-cost option actually results in greater volume of establishment.

As expected, most species utilized in this experiment grew larger installed as 5-inch potted plants. This difference is not likely to be related only to the larger initial size of the propagules; rather, the 12 month study period is sufficiently long enough to afford plug plants the opportunity to reach volumes equivalent to that of 5-inch potted plants. The intense water stress experienced by study plants during the initial planting may have provided the 5-inch potted plants with an advantage, because the more developed root system in these plants allowed for increased water absorption capacity.

*Proserpinaca palustris* and *V. hastata* were exceptions to this trend in that 5-inch potted plants did not consistently outperform plugs. Complicating this result is that initial *P. palustris* volume was equivalent in both plug-plants and 5-inch potted plants, likely a result of its prostrate growth habit, therefore it might be expected that there was no difference in performance due to initial propagule size. Regarding *V. hastata*, overall survival rates were so low (0-65%) that few plants were measurable, resulting in considerable variability in plant volume, and difficulty detecting preferences for this species. Although the experiment did not provide a generalized recommendation for propagule size across all species, results show that when developing revegetation

plans, for certain species, establishment from plug plants may be as cost-effective as or better than establishment from large plants.

### **Comparing Planting to Natural Recolonization**

High plant community species richness is often an important restoration goal. This parameter provided insight into the utility of the planting methods tested, and also revealed some interesting site-to-site differences in natural recolonization.

Species richness varied over time and between ponds. Surprisingly, species richness did not differ within density or elevation treatments, despite the fact that establishment is highly dependent upon hydrologic regime (Casanova & Brock 2000). Pond 2 had higher species richness than Pond 1 across treatments. One key difference in post-planting pond species composition is the cover of *B. caroliniana*, which was 10% or less in Pond 2, compared to 60% in Pond 1. Dense mats formed by this herbaceous perennial (Villazon 2009) are likely to preclude other species and therefore may contribute to the low species richness observed in Pond 1.

Results point out that site differences offer another potential explanation for differences in species composition between ponds. Site-to-site variability (e.g. in extant adjacent vegetation) may have a strong influence on revegetation patterns, even when restoration locations are semi-contiguous. Pond 1 was surrounded by a high pineland (e.g. *Serenoa repens*, *Pinus* spp., various herbaceous perennials, Lodge 2005), whereas Pond 2 was surrounded by sawgrass marsh (e.g. *Crinum americanum*, *Eleocharis cellulosa*, *Sagittaria lancifolia*, *Cladium jamaicense*). The high pineland may have limited anemochorous dispersal into Pond 1 since lower cover of several wind dispersed species (e.g. *Eupatorium capillifolium*, *Typha* sp., *Echinochloa crusgalli*) was noted.

Percent cover of species that naturally recolonized was generally similar in planted and unplanted plots (Tables 2-11 and 2-12). Research has shown that active revegetation using propagules larger than seeds (rhizomes, stakes, plants) increase the likelihood of greater species richness (De Steven & Sharitz 2007). While the plantings in this study did not increase species richness, it also did not limit recruitment from unplanted species.

### **Restoration Trajectories**

Advancement of restoration from an unrestored, sometimes disturbed, ecosystem to the desired recovery state can be viewed as a restoration trajectory (Baer et al. 2004). Recent reviews suggest that these trajectories are rarely born out of observations of restored wetlands over time (Zedler & Callaway 1999). Although many outcomes are possible for these depression marshes, the previous observations provide some insight into potential development of these ecosystems. For instance, the data suggest that over time, dense colonization of *B. caroliniana* in Pond 1 may exclude colonization of other species, and limit species richness. In contrast, in Pond 2, limited *B. caroliniana* cover may result in overall greater species richness. Despite the emergence of several trends, for many response variables (survival, volume) there were no consistent trends in development, supporting the contention that trajectories may not exist, or may not be observable under a typical monitoring period for any project (1-3 years).

Soils, particularly pH, NO<sub>3</sub>, and organic matter, are an important component to any restoration project involving active revegetation, and there were degraded soil conditions at time of planting. Continual decreases in organic matter levels from time of regrading to 12 months after initial planting may be a result of exposure of the lower soil

horizons due to the invasive species removal and removal technique (Ugen & Wortmann 2001). Since TKN decreased by almost 75%, plant growth may be limited by nitrogen availability in these systems. Unfortunately, slow growing vegetation in these restored marshes means that there may be a considerable lag before the organic matter layer results in humus creation.

The potential exists for invasive species to limit plant community development at these sites. Native plant arrival and establishment in prairie pothole wetland restorations is limited over the long term by the invasion and persistence of *Phalaris arundinacea* (Aronson & Galatowitsch 2008). Similarly, invasion of *Typha* spp. (likely *angustifolia*) may limit further colonization or persistence of existing native species in these restorations. Control of this invasive species was necessary during the experimental period to maintain the restoration goal of an open water system (Holm et al. 1997; Mitich 2000) and may be necessary if and when future reinvasion occurs.

### **Conclusions: Implications for Practice**

Since propagule size had significant impact on survival for all species except *L. alatum*, revegetating areas using 5-inch potted plants is recommended to reduce the necessity for a second planting, increase initial ground cover and biodiversity. Use of *V. hastata* is not recommended since survival of this species was low. Three species recommended for revegetating a marl-prairie pond are *L. alatum*, *E. atrypourpurea* and *P. rosea* because they generally establish well in all circumstances. If planting at sparse densities, using *J. polycephalos* and *P. rosea*, *C. haspan*, *E. atropurpurea*, *F. breviseta* is recommended since survival of these species was unaffected by density.

Invasion of *Typha* spp. could be a potential problem while attempting to restore an open water pond and removal the species is likely critical to maintaining an open water pond. Because significant changes in plant community composition and cover were observed in the 1 year study period, it is likely that further development of the plant community will occur in these restorations over the next decade or longer, and therefore monitoring and subsequent invasive species management is recommended.

Table 2-1. Species used for revegetation in both experiments.

Experiment 1	Experiment 2	Wetland status*
-	<i>Bacopa caroliniana</i> (Walter) B.L. Rob. (waterhyssop)	OBL
<i>Cyperus haspan</i> L. (haspan flatsedge)	-	OBL
<i>Eleocharis atropurpurea</i> (Retz.) J. & K. Presl. (purple spikerush)	-	OBL
<i>Fuirena breviseta</i> Coville (saltmarsh umbrella-sedge)	-	OBL
<i>Juncus polycephalos</i> Michx. (manyhead rush)	-	OBL
-	<i>Lythrum alatum</i> Pursh. variety <i>lanceolatum</i> (Ell.) T. & G. Rothr. (winged loosestrife)	OBL
<i>Pluchea rosea</i> Godfrey (rosy camphorweed)	<i>Pluchea rosea</i> Godfrey (rosy camphorweed)	FACW
<i>Proserpinaca palustris</i> L. (marsh mermaidweed)	<i>Proserpinaca palustris</i> L. (marsh mermaidweed)	OBL
<i>Rhynchospora colorata</i> (L.) H. Pfeiffer (starrush whitetop)	-	FACW
<i>Verbena hastata</i> L. (swamp verbena)	<i>Verbena hastata</i> L. (swamp verbena)	FACW

\* OBL= obligate, FACW= facultative wetland.

Table 2-2. Soil analysis for both ponds after regrading, at time of planting, and 18 months after planting.

Soil collection date	P (mg/kg)	pH	TKN (mg/kg)	NO <sub>3</sub> -N (mg/kg)	OM(%)
After regrading (January 2006)	0.24	7.7	-	17.54	13.10
Planting date (June 2007)	-	8.0	2846	6.59	7.95
December 2008	0.29	7.9	561	2.66	4.61

Table 2-3. Analysis of variance for effects of planting density, elevation, months after initial planting, and pond location on plant volume and for each of the eight species planted.

Source	Overall volume		<i>Cyperus</i>		<i>Eleocharis</i>		<i>Fuirena</i>		<i>Juncus</i>		<i>Pluchea</i>		<i>Proserpinaca</i>		<i>Rhynchospora</i>		<i>Verbena</i>	
	F	P	F	P	F	P	F	P	F	P	F	P	F	P	F	P	F	P
Density	28.8	<0.01	0.0	0.90	2.7	0.10	0.1	0.76	8.2	0.01	4.9	0.02	8.5	0.01	22.2	<0.01	6.7	0.01
Elevation	8.4	0.01	15.2	0.01	1.8	0.17	41.4	<0.01	1.0	0.31	4.7	0.03	0.6	0.44	1.3	0.24	0.0	0.93
Density*elevation	0.8	0.36	0.0	0.94	0.3	0.57	0.2	<0.01	1.6	0.20	4.3	0.03	3.1	0.07	0.1	0.72	0.0	0.92
Pond	4.0	0.04	13.1	0.01	22.3	<0.01	49.2	0.69	0.5	0.46	0.0	0.87	1.1	0.27	1.9	0.16	3.7	0.05
Density*pond	0.1	0.70	0.0	0.97	0.2	0.67	0.2	<0.01	1.3	0.26	4.6	0.03	8.3	0.01	0.0	0.86	1.1	0.29
Elevation*pond	9.4	0.01	1.2	0.27	2.7	0.10	8.3	0.01	14.9	0.01	0.0	0.86	1.2	0.25	0.0	0.90	2.2	0.13
MAP	957.5	<0.01	84.7	<0.01	17.4	<0.01	193.3	<0.01	222.2	<0.01	29.9	<0.01	1.4	0.23	453.9	<0.01	5.8	0.01
Density*MAP	25.2	<0.01	0.1	0.80	0.3	0.62	0.8	0.35	6.3	0.01	3.9	0.04	6.3	0.01	18.2	<0.01	5.6	0.01
Elevation*MAP	4.3	0.03	8.9	0.01	0.7	0.39	37.3	<0.01	0.1	0.75	1.7	0.18	1.2	0.25	5.7	0.01	0.2	0.63
Pond*MAP	8.6	0.01	7.8	0.01	48.7	<0.01	46.3	<0.01	1.1	0.29	5.7	0.01	13.4	0.01	1.6	0.20	4.7	0.03

Table 2-4. Analysis of variance for effects of planting density, elevation, months after initial planting, and pond location on percent survival overall and for each of the eight species planted.

Source	Overall survival		<i>Cyperus</i>		<i>Eleocharis</i>		<i>Fuirena</i>		<i>Juncus</i>		<i>Pluchea</i>		<i>Proserpinaca</i>		<i>Rhynchospora</i>		<i>Verbena</i>	
	F	P	F	P	F	P	F	P	F	P	F	P	F	P	F	P	F	P
Density	2.1	0.14	0.1	0.78	0.1	0.77	0.0	0.98	1.8	0.18	1.0	0.31	4.8	0.03	4.6	0.03	12.3	0.01
Elevation	1.1	0.28	4.8	0.02	0.3	0.58	3.1	0.07	2.8	0.09	0.0	0.92	7.0	0.01	12.7	0.01	1.8	0.17
Density*elevation	30.7	<.01	0.1	0.78	17.5	<0.01	5.9	0.01	3.1	0.07	4.6	0.03	4.6	0.03	3.9	0.05	2.5	0.11
Pond	39.9	<.01	0.8	0.36	0.3	0.58	0.4	0.54	2.0	0.15	0.0	0.88	0.1	0.78	7.4	0.01	187.3	<0.01
Density*pond	2.4	0.12	1.9	0.16	5.0	0.02	5.2	0.02	0.4	0.50	0.3	0.56	0.0	0.94	0.2	0.65	1.4	0.24
Elevation*pond	2.0	0.15	6.6	0.01	2.7	0.09	8.3	0.01	1.1	0.28	6.5	0.01	7.6	0.01	0.3	0.56	0.2	0.65
MAP	5.6	0.01	0.7	0.38	0.3	0.58	1.3	0.25	5.3	0.02	10.8	0.01	1.0	0.32	15.2	0.01	14.1	0.01
Density*MAP	4.1	0.04	0.5	0.46	1.1	0.28	8.4	0.01	0	0.98	0.0	0.95	12.7	0.01	0.1	0.70	10.9	0.01
Elevation*MAP	0.3	0.56	1.6	0.20	10.9	0.01	12.0	0.25	2.8	0.09	0.2	0.67	4.7	0.03	9.0	0.01	1.8	0.17
Pond*MAP	0.1	0.85	10.4	0.01	1.2	0.27	0.0	0.98	8.6	0.01	0.2	0.67	2.9	0.09	0.0	0.87	58.8	<0.01

Table 2-5. Analysis of variance for effects of planting density and planting elevation on species richness.

Source	F	P
Elevation	0.38	0.5385
Pond	17.53	<0.0001
Elevation*pond	0.02	0.8904
Density	0.58	0.5597
Elevation*Density	0.86	0.4269
Pond*Density	1.96	0.6182
MAP	19.59	<0.0001
Elevation*MAP	1.50	0.2266
Pond*MAP	5.25	0.0063
Density*MAP	3.26	0.0137

Table 2-6. Analysis of variance for effects of propagule size, elevation, and months after initial planting, on overall plant volume and for each of the five species planted.

Source	Overall volume		<i>Bacopa</i>		<i>Lythrum</i>		<i>Pluchea</i>		<i>Proserpinaca</i>		<i>Verbena</i>	
	F	P	F	P	F	P	F	P	F	P	F	P
Propagule size	22.62	<.0001	57.97	<0.0001	53.54	<0.0001	43.41	<0.0001	2.98	0.0903	0.04	0.8533
Elevation	0.16	0.6867	0.12	0.7309	0.02	0.8976	4.10	0.0453	1.24	0.2697	1.76	0.2023
Propagule size*elevation	0.01	0.9413	1.27	0.2619	0.19	0.6664	0.44	0.5109	0.23	0.6367	1.10	0.3093
MAP	34.71	<.0001	72.11	<0.0001	74.77	<0.0001	88.75	<0.0001	0.17	0.6789	0.93	0.3490
Propagule size*MAP	15.14	0.0001	22.88	<0.0001	44.97	<0.0001	43.16	<0.0001	0.65	0.4226	0.00	0.9638
Elevation*MAP	0.14	0.7087	.049	0.4867	0.00	0.9875	4.78	0.0310	1.26	0.2673	0.42	0.5271

Table 2-7. Analysis of variance for effects of propagule size, elevation, and months after initial planting on overall percent survival and for each of the five species planted.

Source	Overall survival		<i>Bacopa</i>		<i>Lythrum</i>		<i>Pluchea</i>		<i>Proserpinaca</i>		<i>Verbena</i>	
	F	P	F	P	F	P	F	P	F	P	F	P
Propagule size	14.22	0.01	9.95	0.01	1.05	0.31	6.75	0.01	4.16	0.04	13.13	0.01
Elevation	2.61	0.10	0.12	0.72	3.01	0.08	2.95	0.08	0.27	0.60	4.54	0.03
Propagule size*elevation	0.81	0.36	0.12	0.72	6.03	0.01	3.00	0.08	0.02	0.87	0.25	0.61
MAP	0.00	1.00	14.86	0.01	0.14	0.70	0.74	0.39	0.34	0.56	22.58	<0.01
Propagule size*MAP	3.22	0.07	9.95	0.01	0.14	0.70	0.75	0.38	0.05	0.82	9.48	0.01
Elevation*MAP	0.13	0.71	0.12	0.72	0.10	0.74	0.00	1.00	2.64	0.10	2.06	0.15

Table 2-8. Analysis of variance for effects of planting elevation and propagule size on species richness.

Source	F	P
Elevation	1.84	0.1768
Size	16.65	<0.0001
Elevation*Size	0.82	0.4442
MAP	12.17	<0.0001
Elevation*MAP	4.96	0.0083
Size*MAP	19.48	<0.0001

Table 2-9. Species-specific plant volume response to planting treatments.

Density	Elevation		
	High	Low	No preference in elevation
Dense	-	-	-
Sparse	<i>Pluchea rosea</i>	-	<i>Juncus polycephalos</i> <i>Proserpinaca palustris</i> <i>Rhynchospora colorata</i> <i>Verbena hastata</i>
No preference in density	-	<i>Cyperus haspan</i> <i>Fuirena breviseta</i>	<i>Eleocharis atropurpurea</i>

Table 2-10. Species-specific percent survival response to planting treatments.

Density	Elevation		
	High	Low	No preference
Dense	<i>Rhynchospora colorata</i>	-	-
Sparse	<i>Proserpinaca palustris</i>	-	<i>Verbena hastata</i> <i>Eleocharis atropurpurea</i>
No preference	-	<i>Cyperus haspan</i>	<i>Fuirena breviseta</i> <i>Juncus polycephalos</i> <i>Pluchea rosea</i>

Table 2-11. Percent cover and species composition of naturally recolonizing species and their frequency of occurrence 12 months after planting for effects of planting density and planting elevation on species response.

Species	Percent cover (%)			
	Planted plots*		Unplanted plots**	
	Pond 1	Pond 2	Pond 1	Pond 2
<i>Bacopa caroliniana</i>	50	10	70	10
<i>Pluchea rosea</i>	20	25	5	30
<i>Proserpinaca palustris</i>	10	<5	10	<5
<i>Rhynchospora colorata</i>	20	45	5	10
<i>Cyperus haspan</i>	10	15	5	<5
<i>Fuirena breviseta</i>	10	15	10	<5
<i>Juncus polycephalos</i>	5	15	<5	<5
<i>Eleocharis atropurpurea</i>	20	10	20	<5
<i>Ludwigia repens</i>	65	30	65	45
<i>Lythrum alatum</i>	30	20	35	20
<i>Acmella spp.</i>	10	0	<5	0
<i>Mikania scandense</i>	20	<5	20	<5
<i>Ludwigia microcarpa</i>	45	10	40	5
<i>Eupatorium capillifolium</i>	<5	25	<5	30
<i>Saigattaria lancifolia</i>	10	<5	5	<5
<i>Typha spp.</i>	30	0	30	0
<i>Echinochloa crus-galli</i>	<5	0	<5	0
<i>Salix caroliniana</i>	0	<5	0	<5
<i>Solidago spp.</i>	0	<5	0	<5
<i>Mitreola sp.</i>	0	<5	0	5
<i>Lobelia feayana</i>	0	<5	0	<5

\* Mean of 40 plots

\*\* Mean of 19 plots

Table 2-12. Percent cover and species composition of naturally recolonizing species and their frequency of occurrence 12 months after planting for effects of planting elevation and propagule size on plant response.

Species	Percent cover (%)		
	5-inch potted plant*	Plug-plant	Unplanted**
<i>Acmeilla</i> spp.	<5	0	0
<i>Bacopa caroliniana</i>	10	<5	10
<i>Eupatorium capillifolium</i>	<5	30	30
<i>Juncus polycephalos</i>	<5	<5	<5
<i>Ludwigia microcarpa</i>	45	<5	5
<i>Ludwigia repens</i>	<5	65	45
<i>Lythrum alatum</i>	70	30	20
<i>Mikania scandens</i>	<5	0	<5
<i>Pluchea rosea</i>	35	10	30
<i>Proserpinaca palustris</i>	<5	<5	<5
<i>Verbena hastata</i>	15	<5	<5
Bare soil	75	>95	55

\* Mean of 6 plots

\*\* Mean of 10 plots

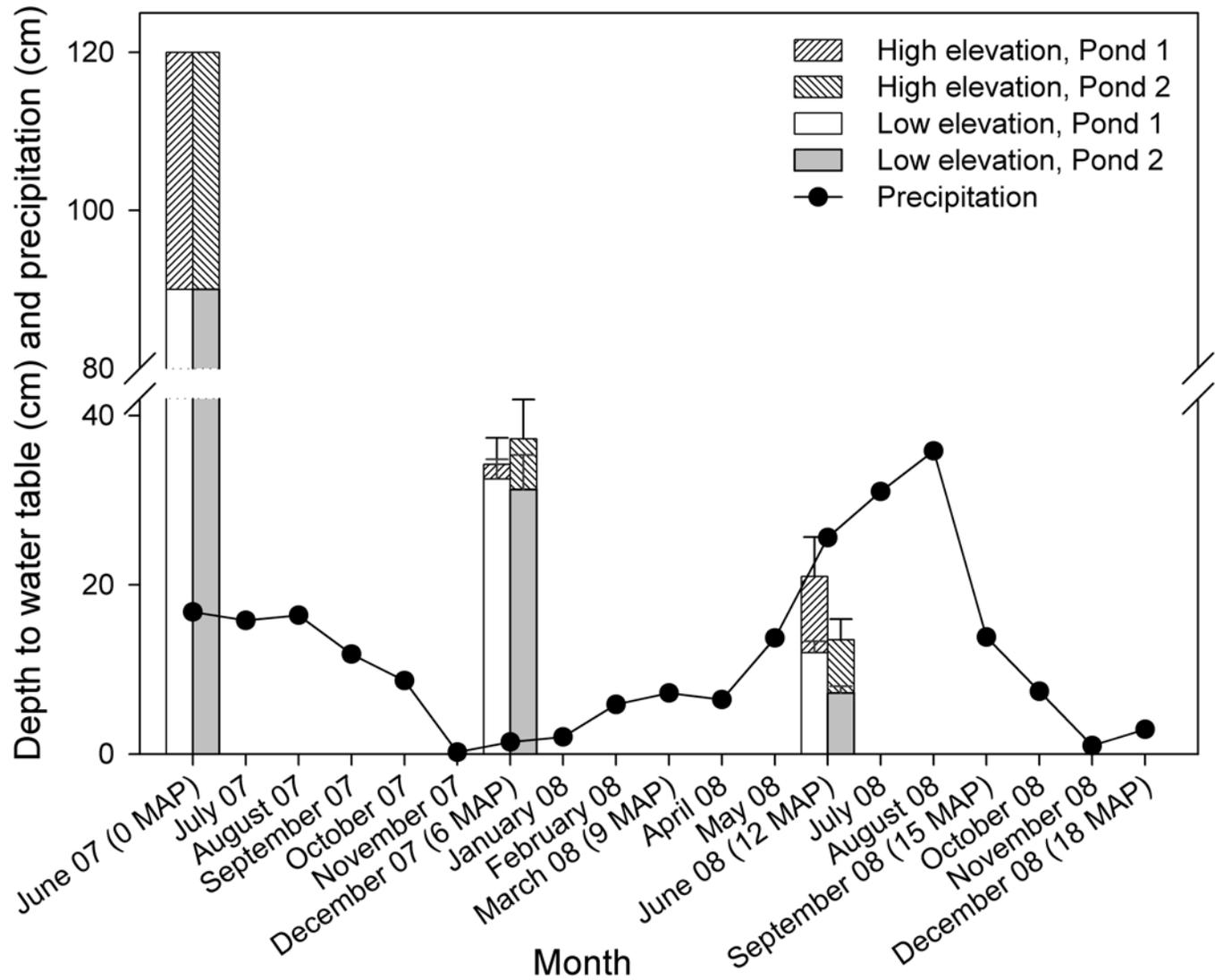


Figure 2-1. Depth to water table was measured using a laser level at time of planting and installed wells thereafter at either planting elevation. Precipitation data also shown over data collection period (for elevation data, mean of water table height at 3 wells and standard error are shown).

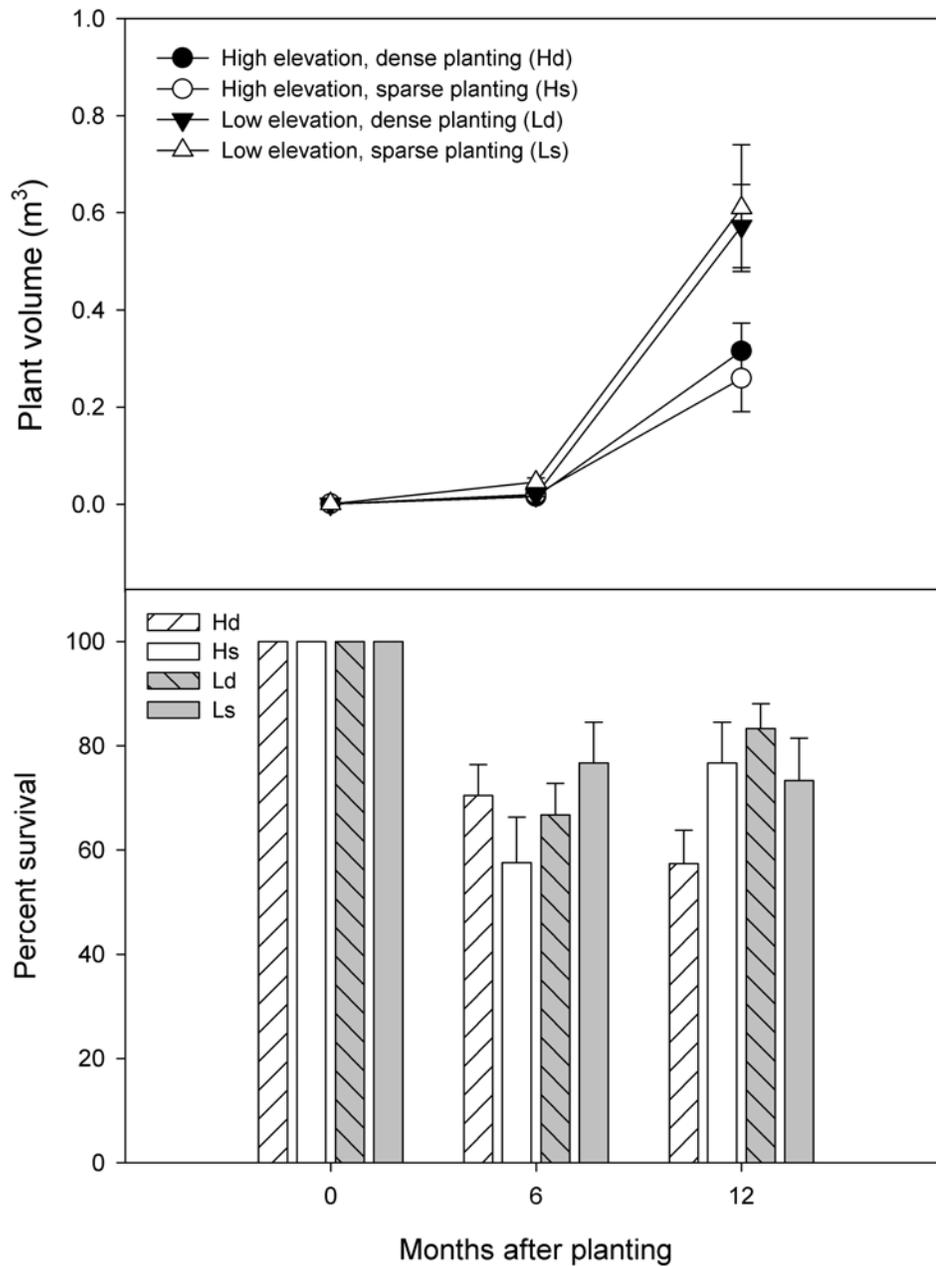


Figure 2-2. *Cyperus haspan* – the effects of planting elevation and planting density on plant volume and percent survival. Mean of 10 replications followed by standard error; n=30 for sparse planting, n=60 for dense planting.

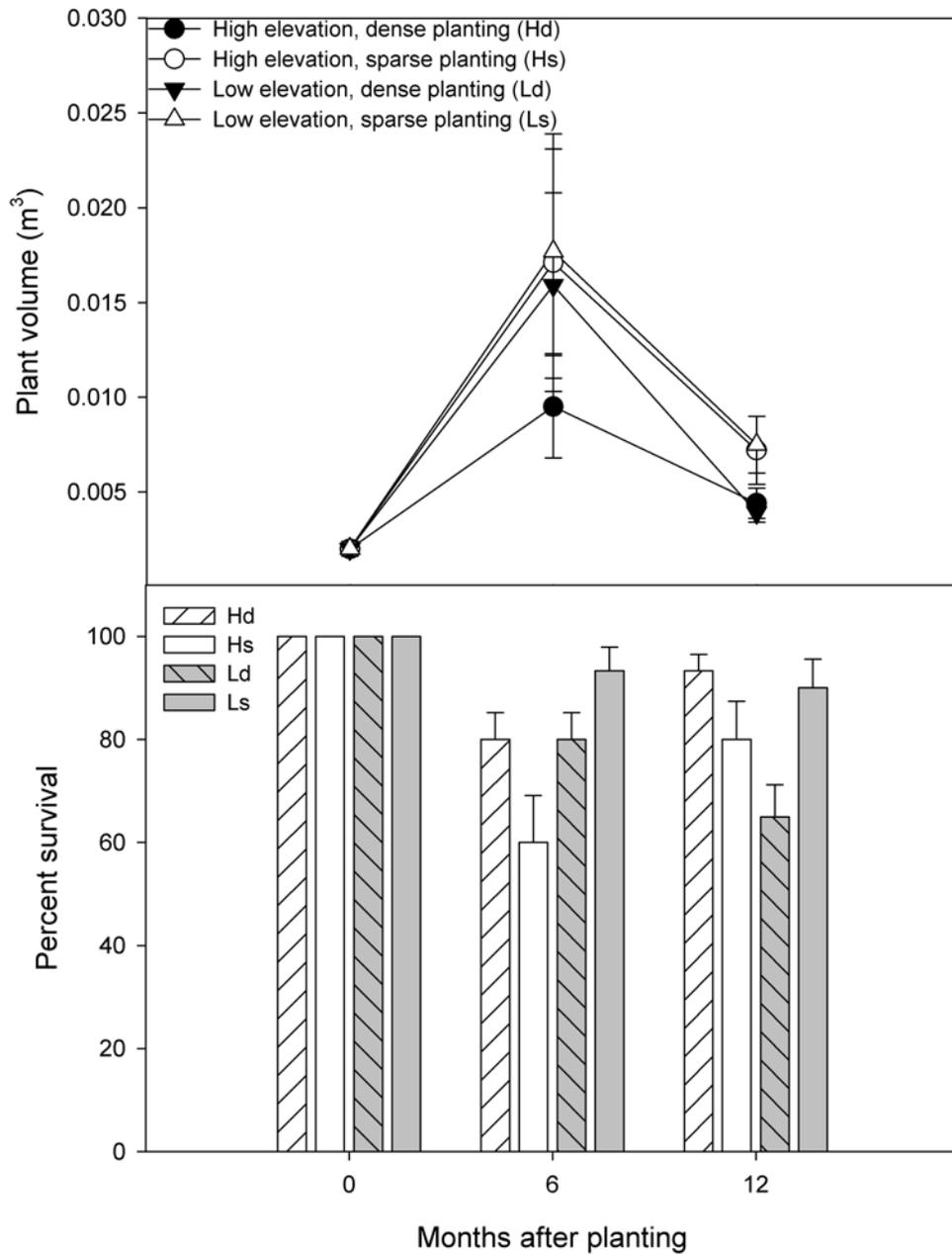


Figure 2-3. *Eleocharis atropurpurea* – the effects of planting elevation and planting density on plant volume and percent survival. Mean of 10 replications followed by standard error; n=30 for sparse planting, n=60 for dense planting.

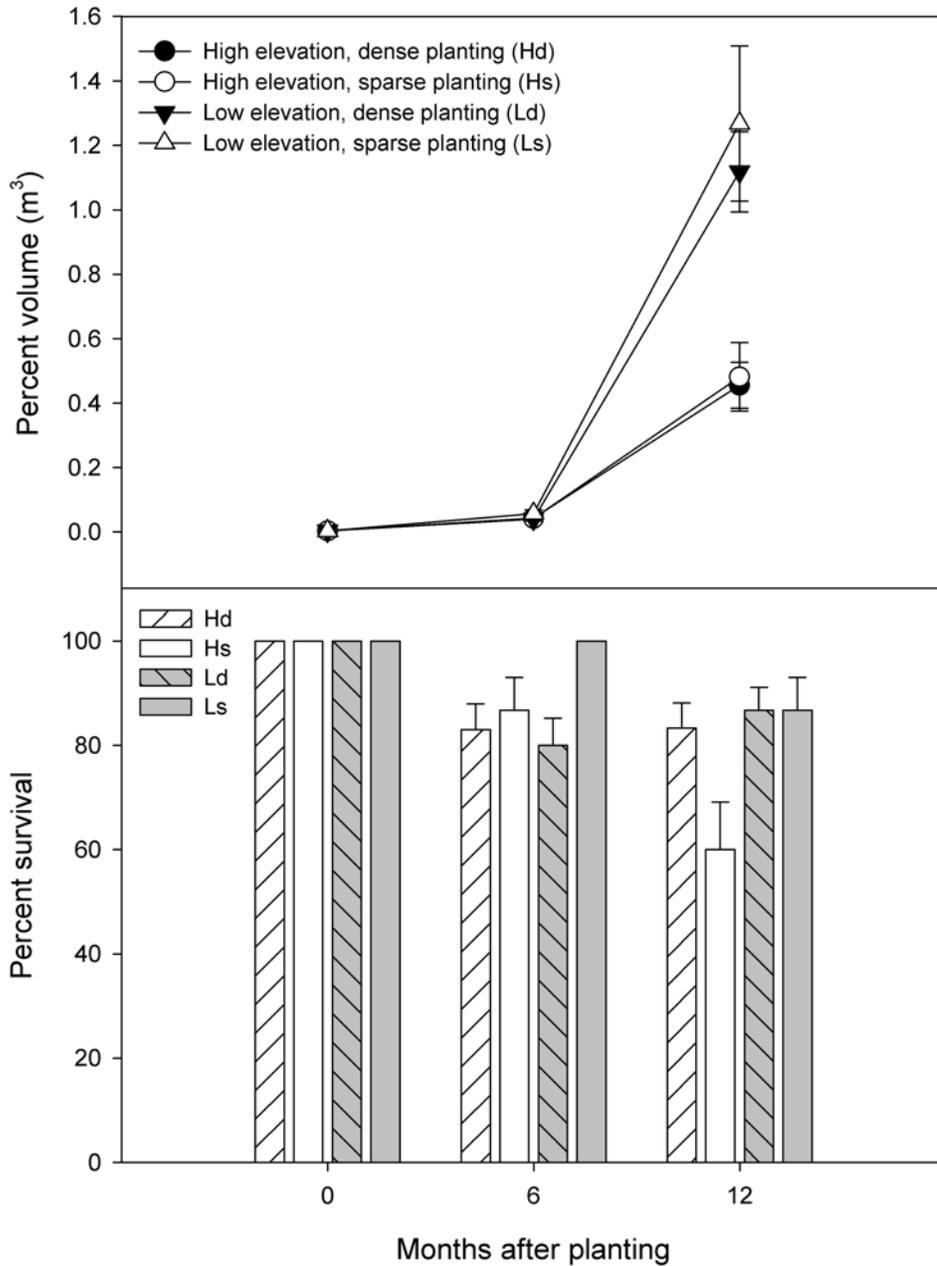


Figure 2-4. *Fuirena breviseta* – the effects of planting elevation and planting density on plant volume and percent survival. Mean of 10 replications followed by standard error; n=30 for sparse planting, n=60 for dense planting.

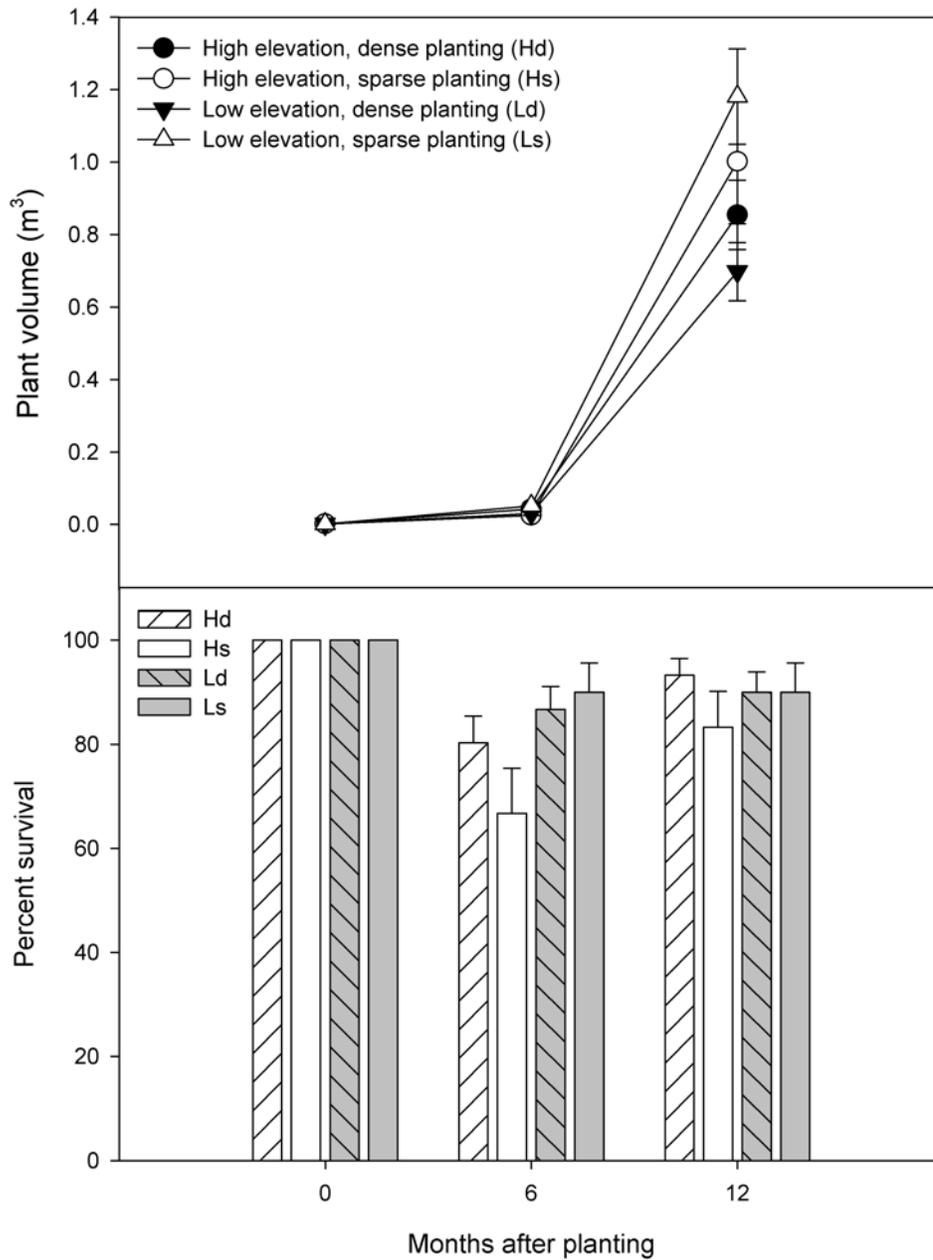


Figure 2-5. *Juncus polycephalos* – the effects of planting elevation and planting density on plant volume and percent survival. Mean of 10 replications followed by standard error; n=30 for sparse planting, n=60 for dense planting.

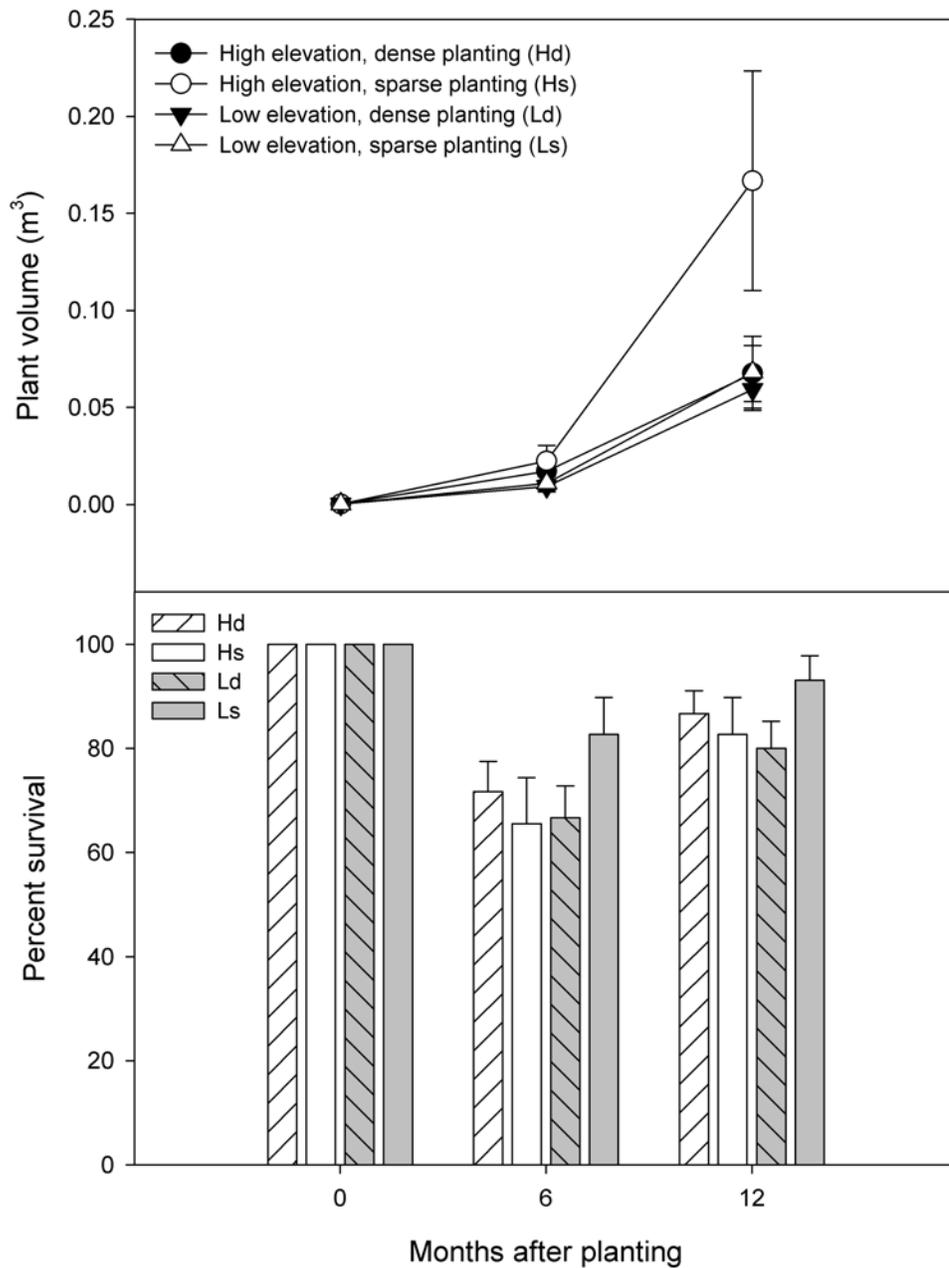


Figure 2-6. *Pluche rosea* – the effects of planting elevation and planting density on plant volume and percent survival. Mean of 10 replications followed by standard error; n=30 for sparse planting, n=60 for dense planting.

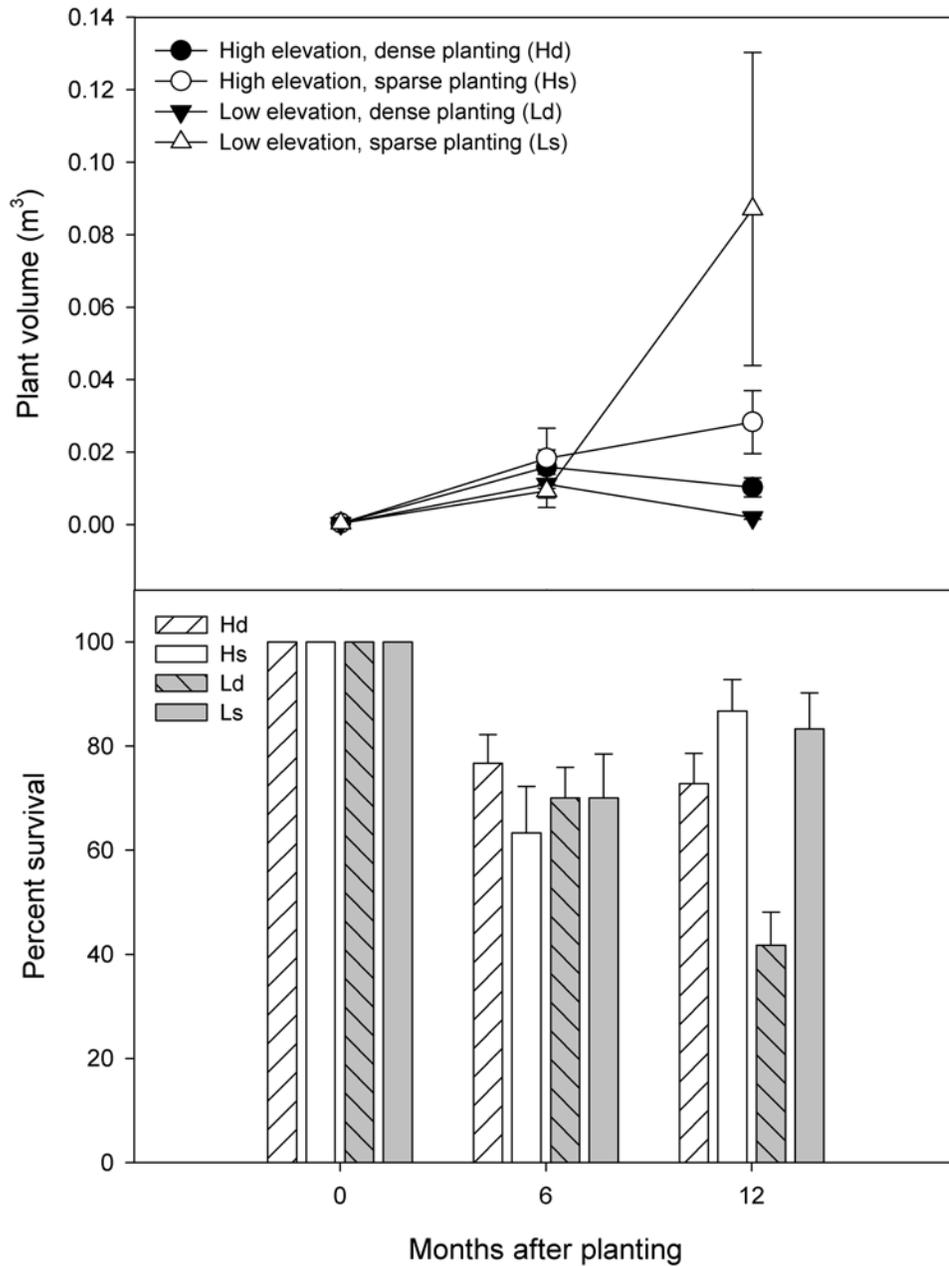


Figure 2-7. *Proserpinaca palustris* – the effects of planting elevation and planting density on plant volume and percent survival. Mean of 10 replications followed by standard error; n=30 for sparse planting, n=60 for dense planting.

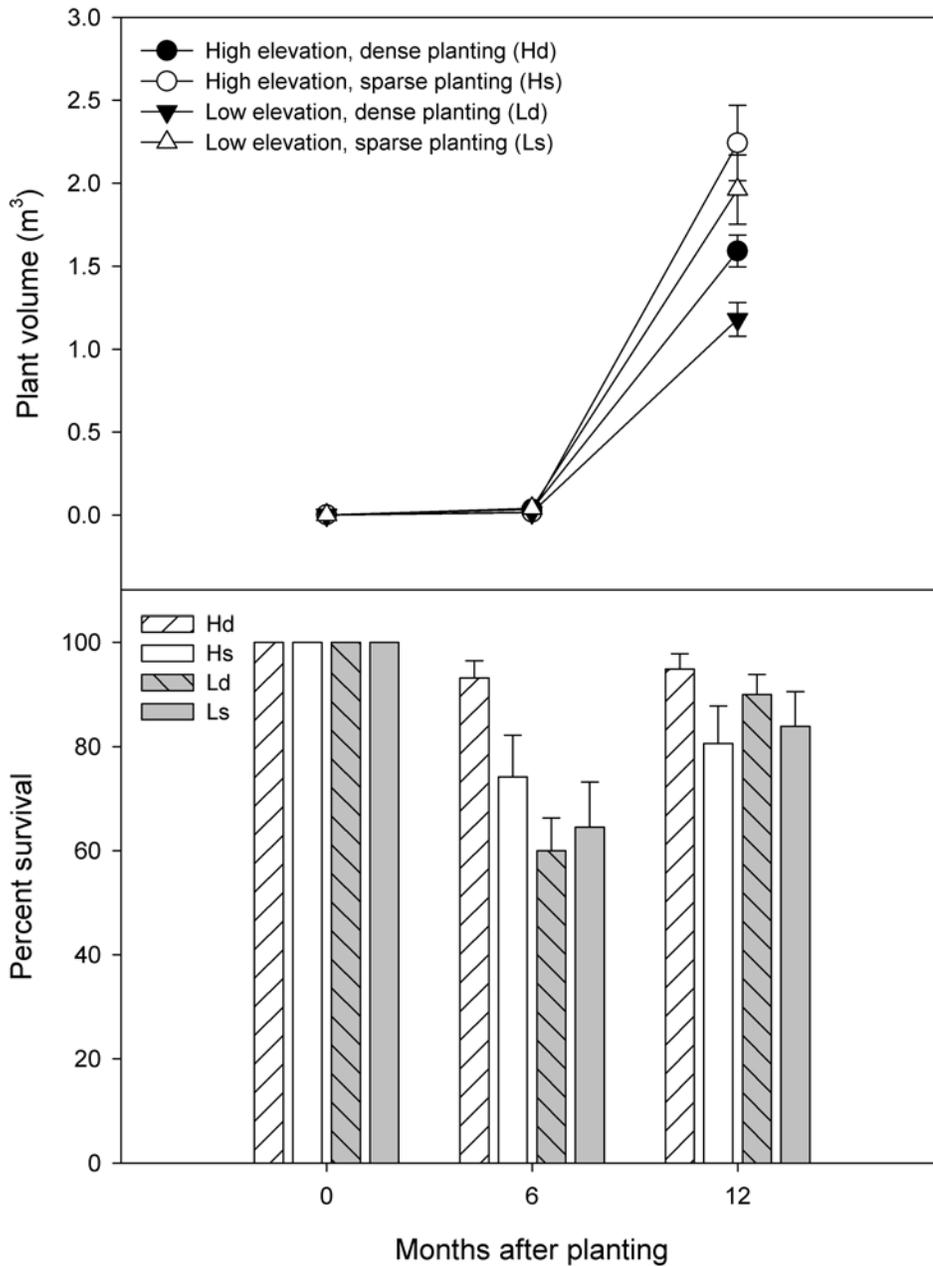


Figure 2-8. *Rhynchospora colorata* – the effects of planting elevation and planting density on plant volume and percent survival. Mean of 10 replications followed by standard error; n=30 for sparse planting, n=60 for dense planting.

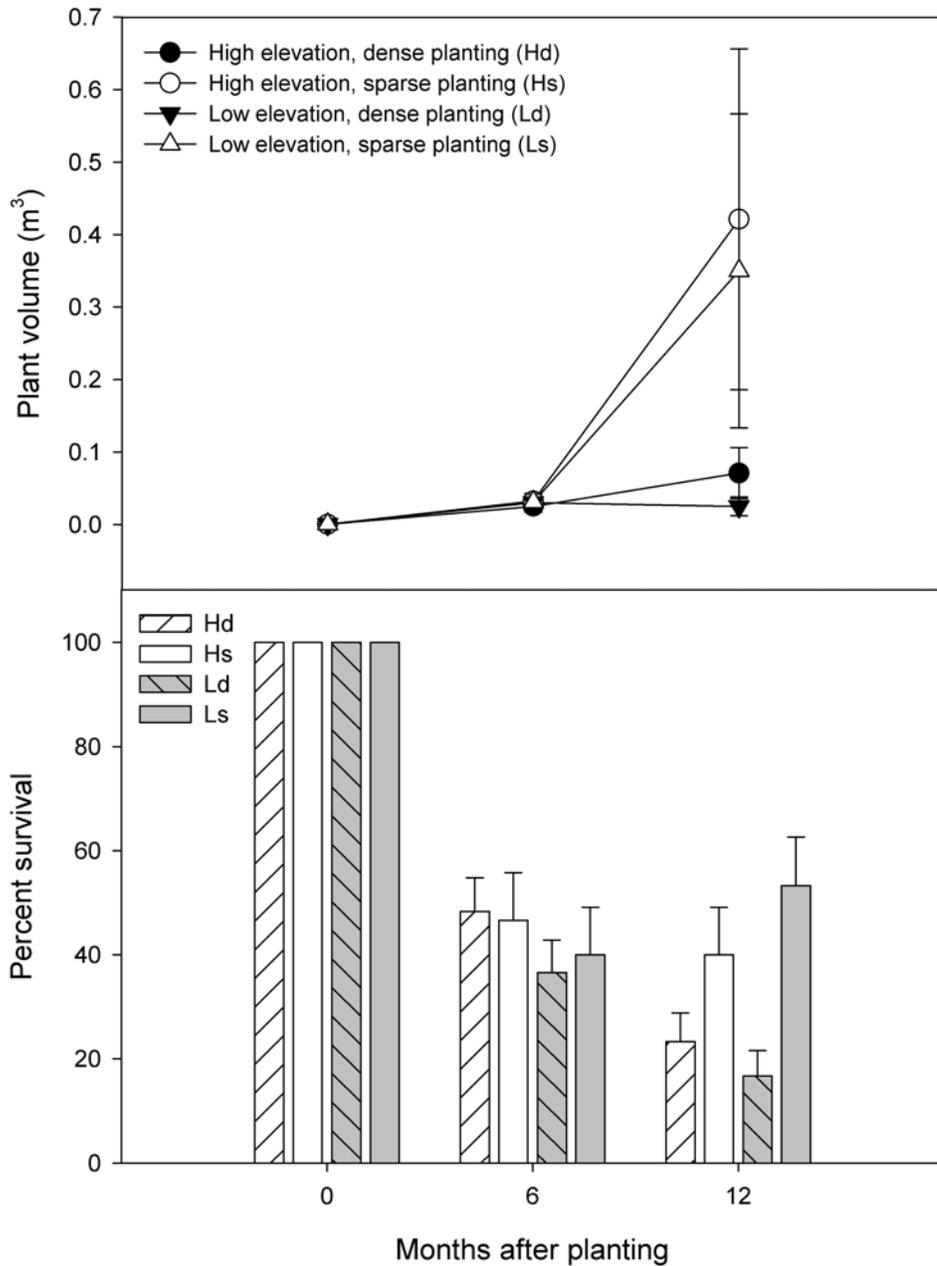


Figure 2-9. *Verbena hastata* – the effects of planting elevation and planting density on plant volume and percent survival. Mean of 10 replications followed by standard error; n=30 for sparse planting, n=60 for dense planting.

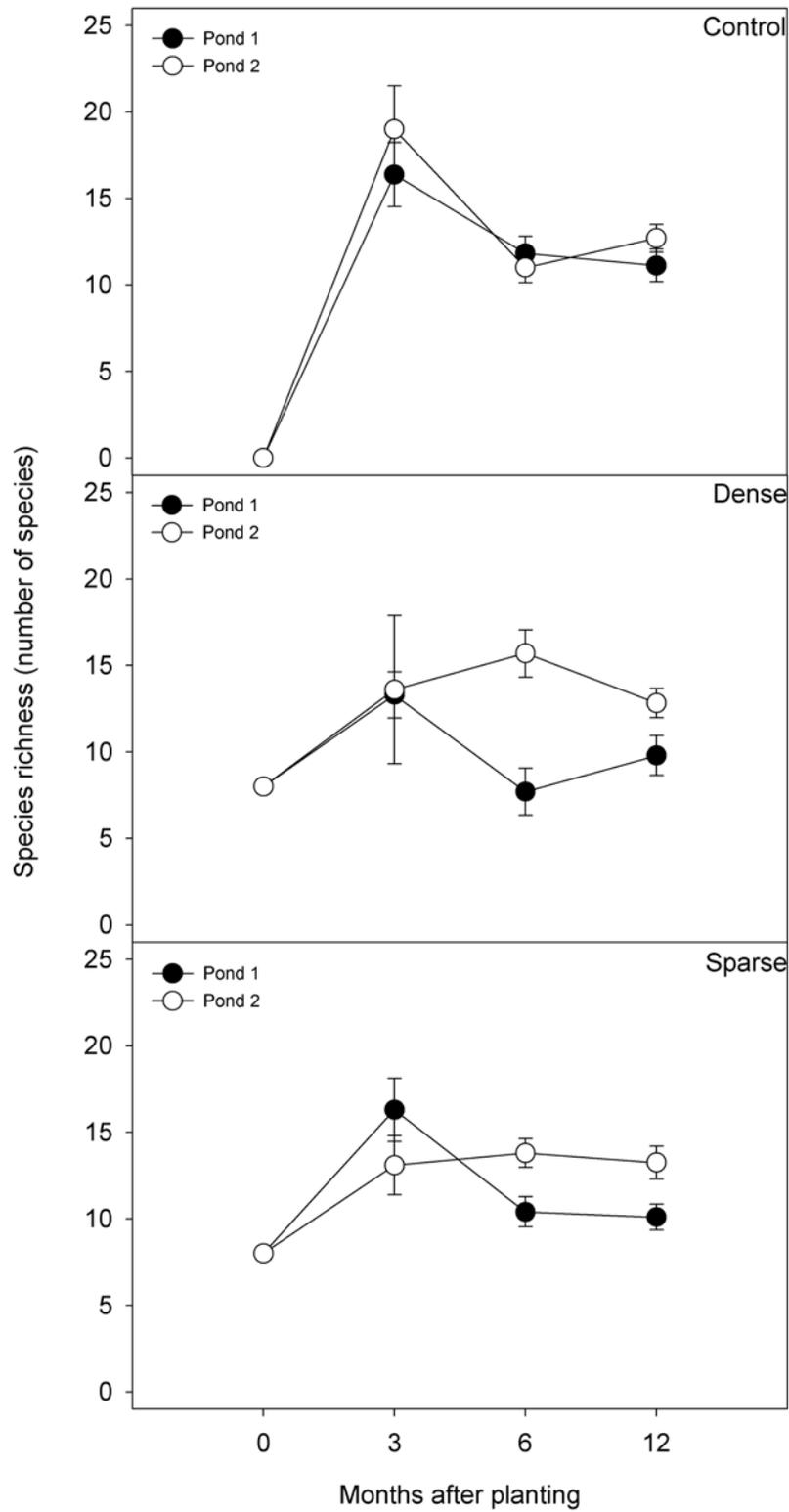


Figure 2-10. Effects of planting density on species richness at either study pond for Experiment 1. Mean of 10 replications followed by standard error.

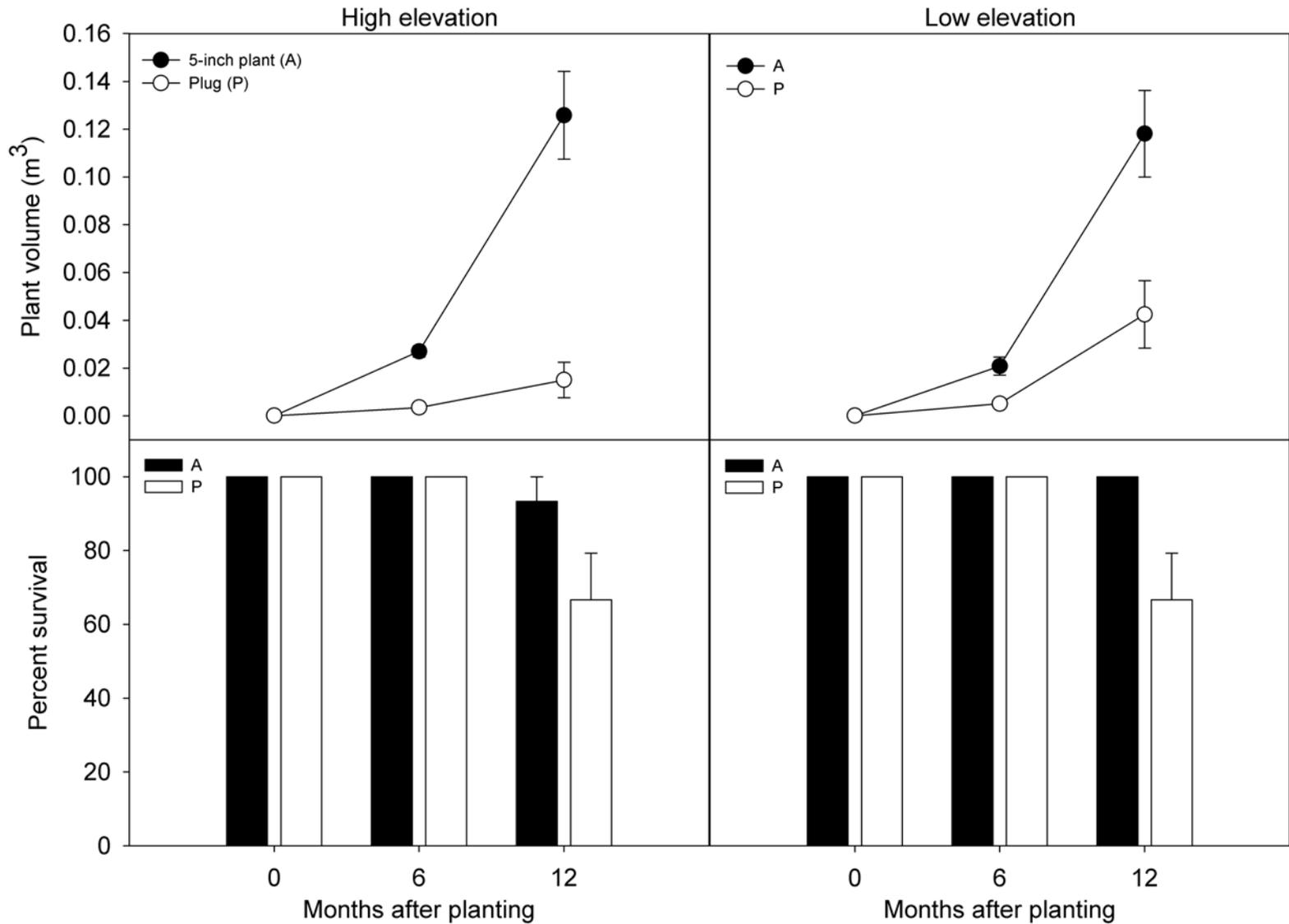


Figure 2-11. *Bacopa caroliniana* – the effects of planting elevation and propagule size on plant volume and percent survival.

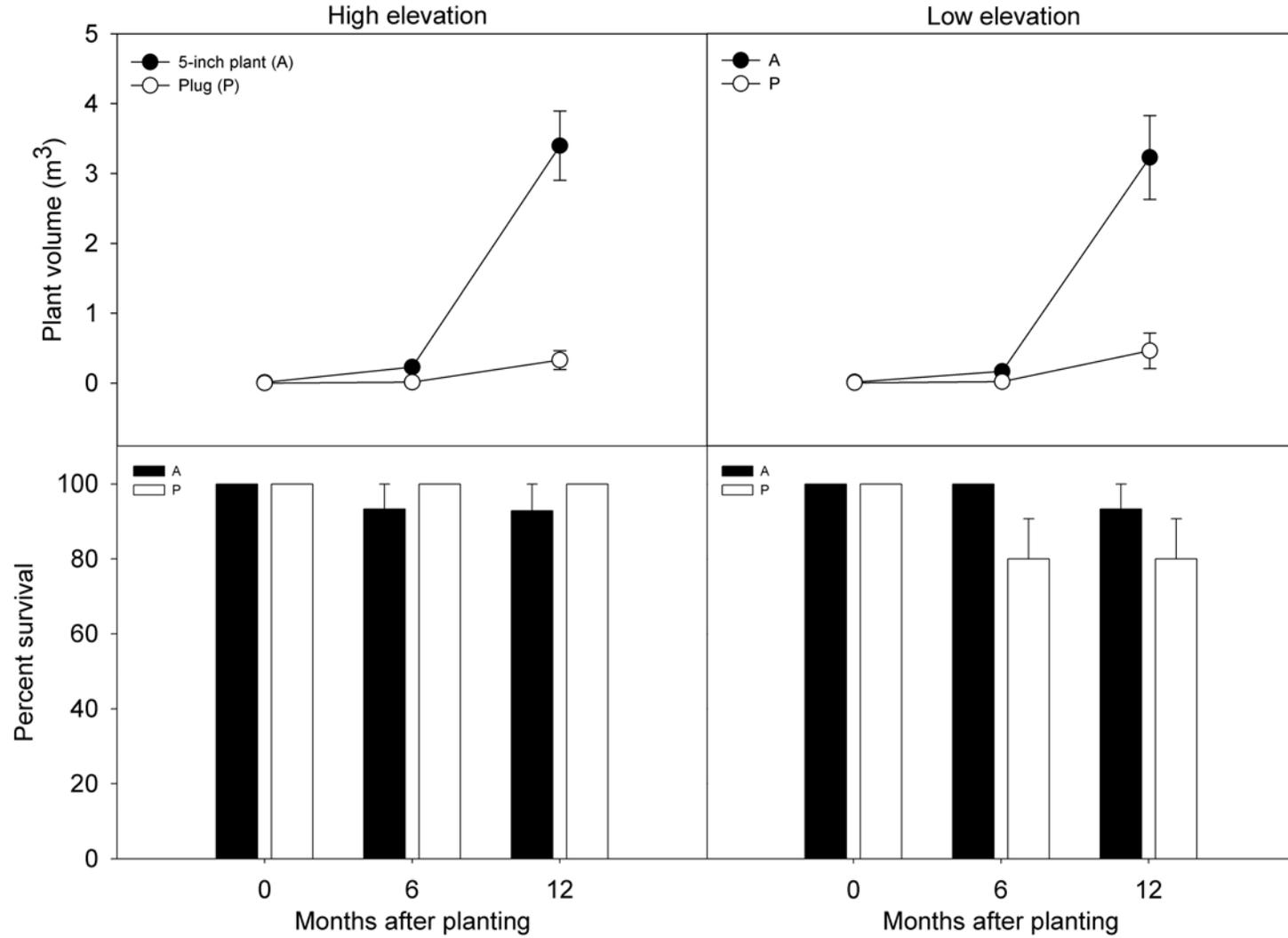


Figure 2-12. *Lythrum alatum* – the effects of planting elevation and propagule size on plant volume and percent survival. Mean of 3 replications followed by standard error, n=5.

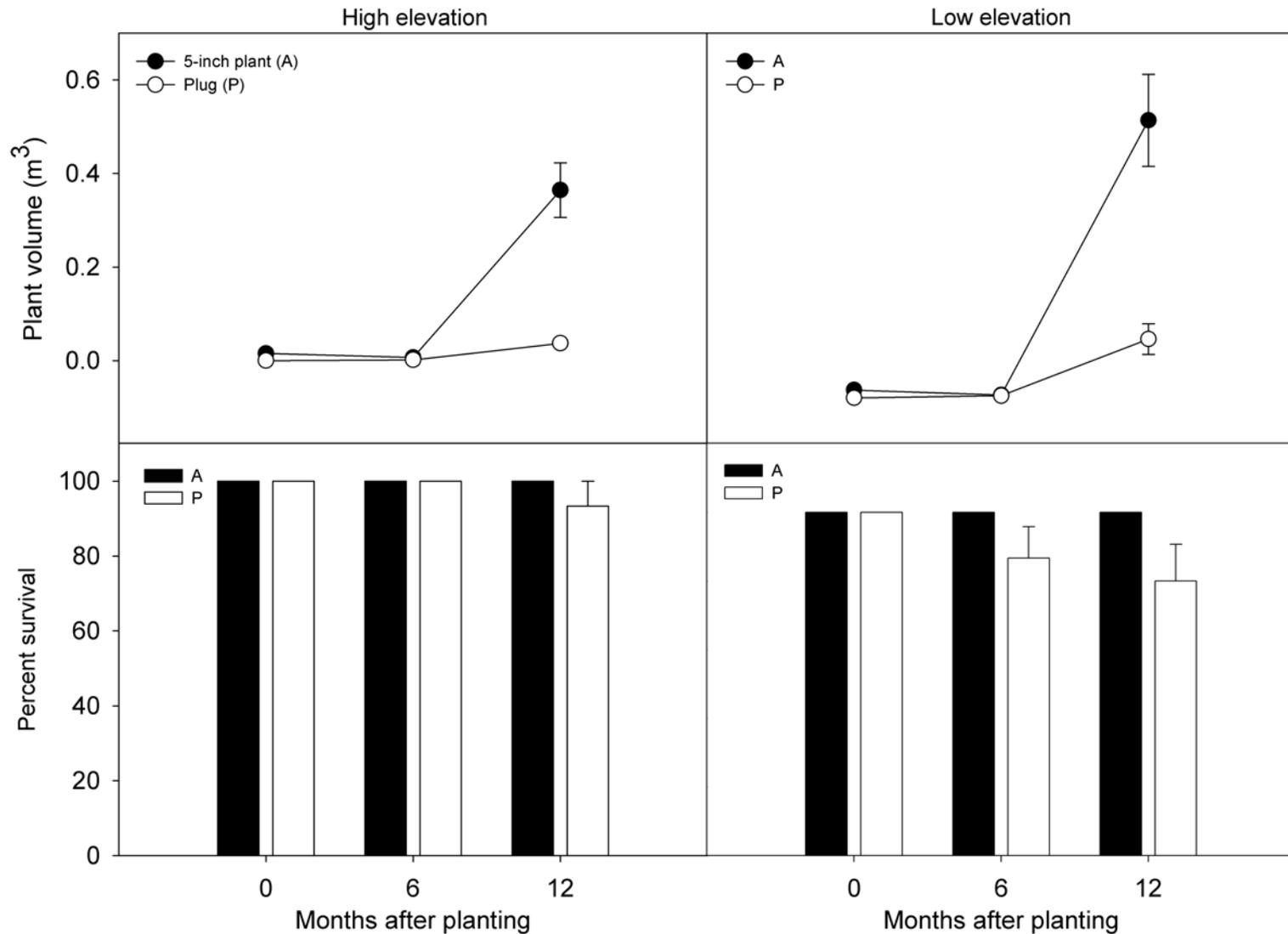


Figure 2-13. *Pluchea rosea* – the effects of planting elevation and propagule size on plant volume and percent survival. Mean of 3 replications followed by standard error, n=5.

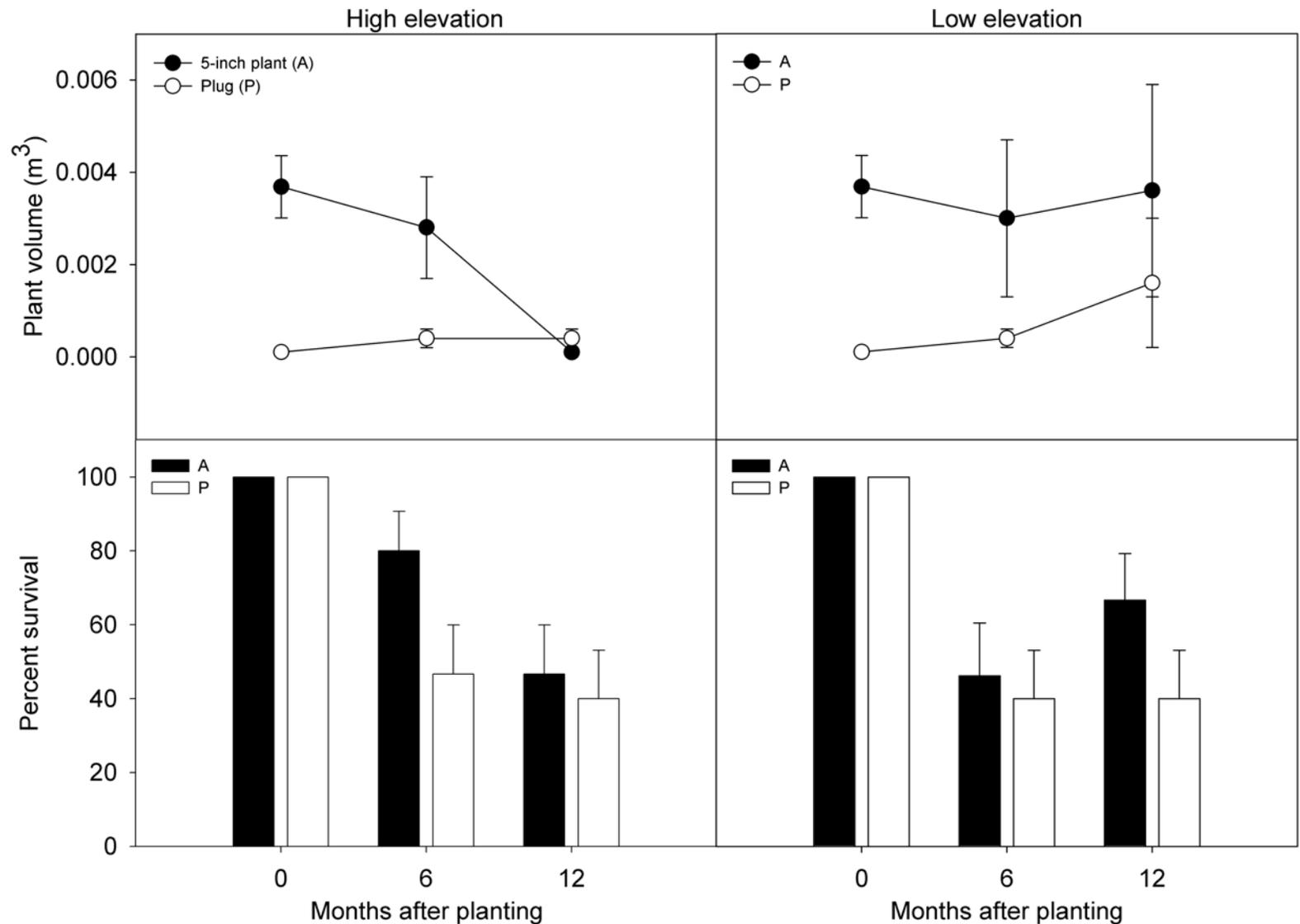


Figure 2-14. *Proserpinaca palustris* – the effects of planting elevation and propagule size on plant volume and percent survival. Mean of 3 replications followed by standard error, n=5.

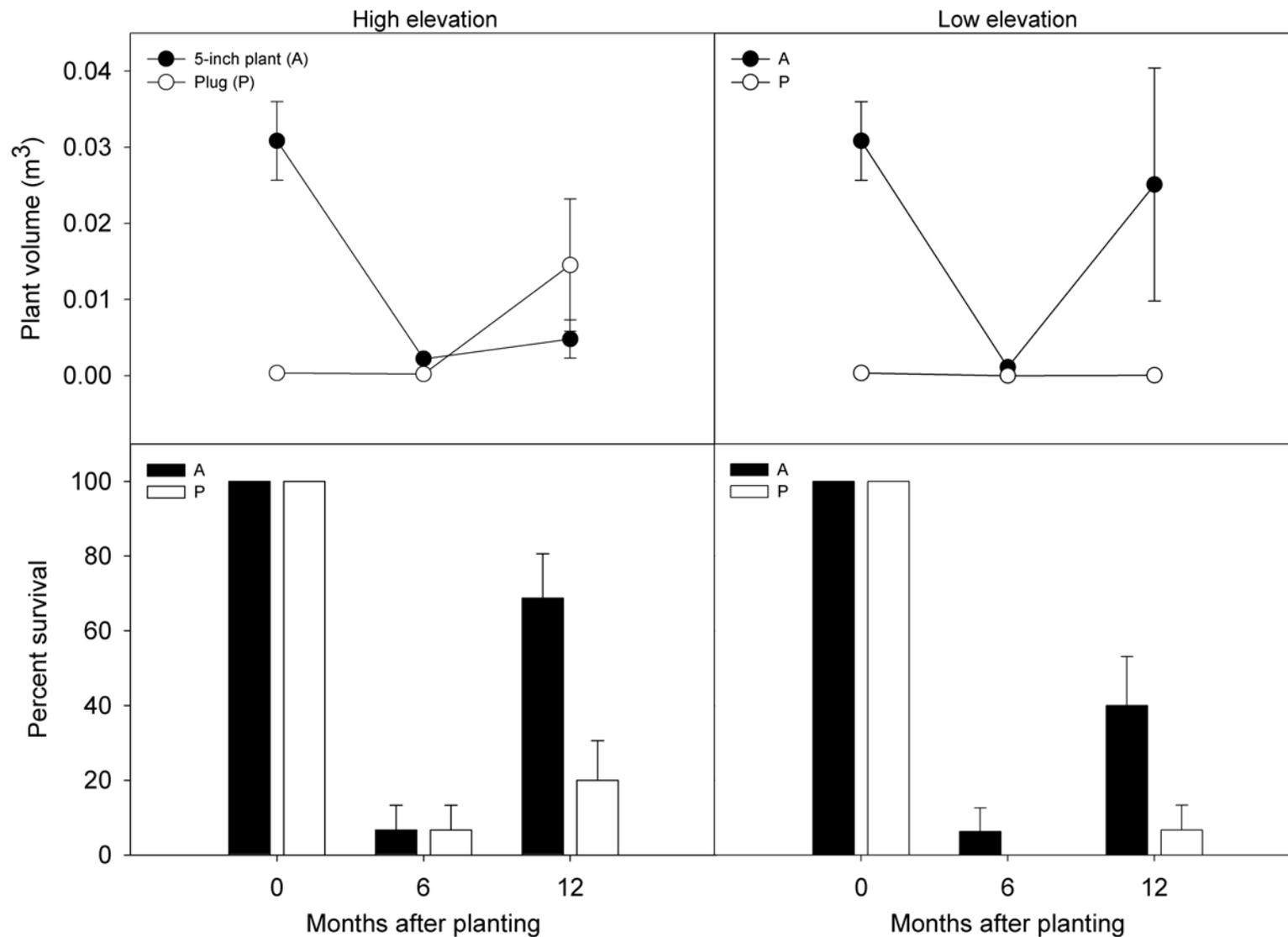


Figure 2-15. *Verbena hastata* – the effects of planting elevation and propagule size on plant volume and percent survival. Mean of 3 replications followed by standard error, n=5.

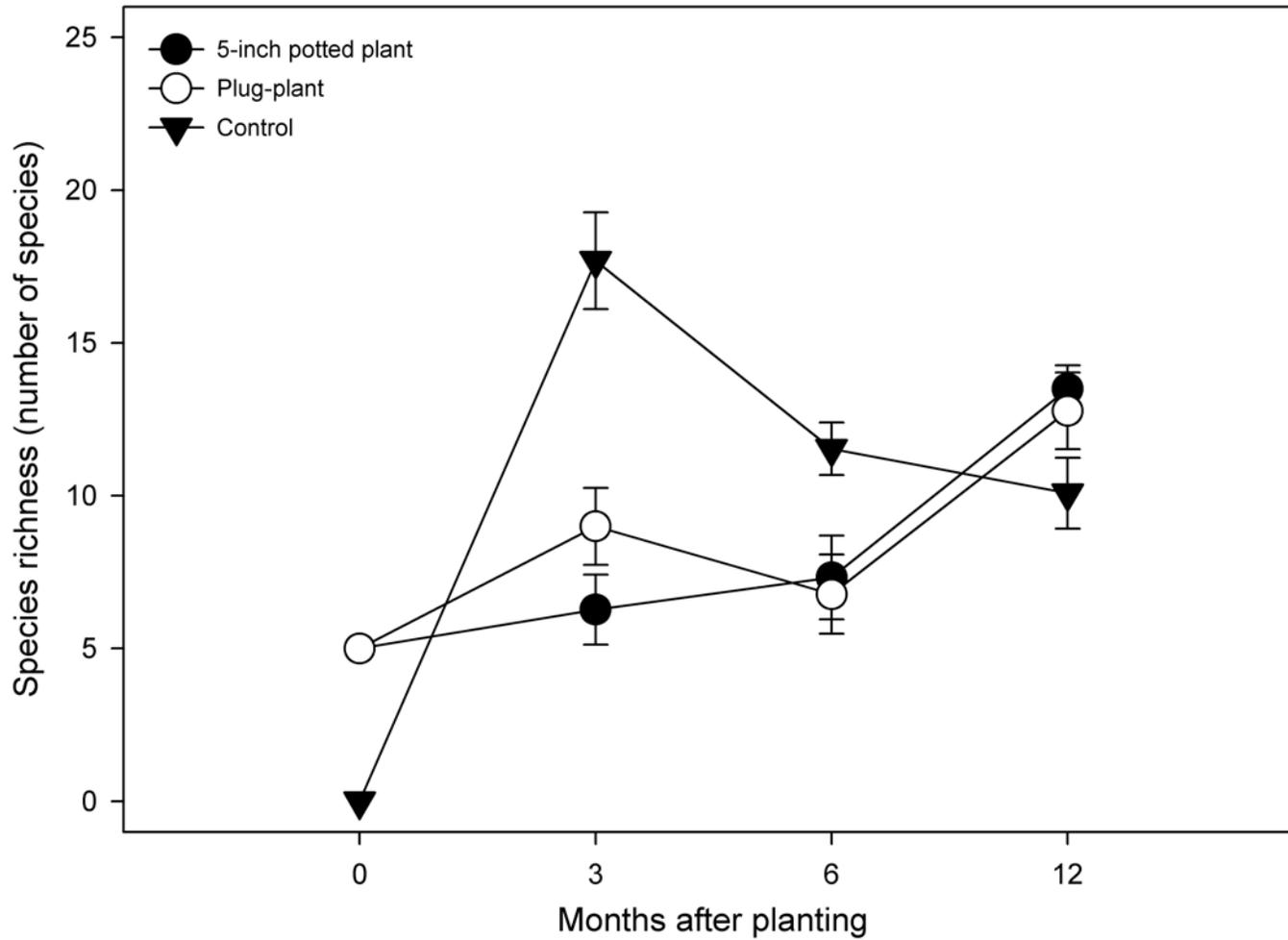


Figure 2-16. Effects of propagule size on species richness for Experiment 2. Mean of 3 replications followed by standard error.

CHAPTER 3  
RESTORATION OF PINE FLATWOODS IN FLORIDA UPON REMOVAL OF  
COGONGRASS (*IMPERATA CYLINDRICA*)

**Introduction**

Florida is one of the world's largest producers of phosphate because of its rich and easily accessible deposits (Jasinski 1999). Phosphate is actively mined in Polk, Hamilton, Hillsborough, Hardee, and Manatee counties in Florida (Tamang et al. 2008), where removal of the top 15 to 50 feet of the soil surface results in significant soil disturbance. This alteration in soil properties generally supports mainly opportunistic plant species, such as *Schinus terebinthifolius* Raddi. (Brazilian pepper), *Sporobolus indicus* (smutgrass), and *Ludwigia peruviana* (L.) H. Hara (primrose willow), which become problematic. Large-scale removal of these invasive species often leads to further disturbed and barren soils, which may result in "safe sites" for invasive species establishment (Johnstone 1986). Such removal of target invasive species from an ecosystem can lead to either a "moonscape" or introduction of another undesirable invasive species which can result in monotypic stands if revegetation strategies are not implemented (Ogden & Remanek 2005; Shilling et al. 1997). A major invasive species concern in post-mining sites is *Imperata cylindrica* (L.) Beauv. (cogongrass) (Coile & Shilling 1993), where broad-scale control methods are often implemented for this species, but these efforts rarely result in return of the native flatwoods plant community (MacDonald 2004).

***Imperata cylindrica*: Biology and Morphology**

*Imperata cylindrica* is an example of a federally listed noxious weed which requires extensive control efforts especially in post-mining areas (Ramsey et al.

2003). This rhizomatous, pyrophilous, invasive exotic is listed as one of the world's top ten worst weeds (Lum et al. 2005), and is problematic on every continent of the world except Antarctica (Holm et al. 1977). *Imperata cylindrica* was introduced accidentally as a packaging cushion for shipping cargo from Asia to America (1911) and later intentionally as a potential forage (1920s), and has become a noxious invasive exotic. This species rapidly invades both disturbed and undamaged flatwoods (Collins 2005), forms monotypic stands, and excludes native species (Lippincott 1997), especially during wildfire (Hubbard et al. 1944; Gaffney 1996). Holm et al. (1977) and Brook (1989) speculate that *I. cylindrica* spread is positively correlated with degree of site disturbance.

Growing in compacted tufts, *I. cylindrica* arises from underground rhizomes which can extend up to 10 feet (Bryson & Carter 1993). Since more than 60% of the plant biomass consists of cataphyllous rhizomes (Ayeni 1985), management and control proves difficult. *Imperata cylindrica* rhizomes can be found in fine textured or coarse textured soils at depths of 15 cm and 40 cm, respectively and optimally grow at pH of 4.7 (Holm et al. 1977; Gaffney 1996; MacDonald 2004). Ayeni & Duke (1985) reported that regenerative capacity of these rhizomes increased with rhizome age, but showed very little correlation between rhizome length and weight. Research suggests that, after apex removal, auxin-imposed apical dominance allows axillary buds to maintain dormancy after applications of IAA (indole-3-acetic acid) (Gaffney & Shilling 1995). Although *I. cylindrica* can produce approximately 3000 seeds per plant, spread results primarily from rhizome production (Willard et al. 1990). These

factors make control of *I. cylindrica* complex. *Imperata cylindrica* control methods include herbicide use, tillage, hand-pulling, slashing, and use of cover crops (Lum et al. 2005).

Revegetating with native plants after control of *I. cylindrica* has been futile and little is known about the potential for re-establishing plant communities to resist further invasion of *I. cylindrica* (MacDonald 2004). Projects to restore heavily disturbed *I. cylindrica*-dominated sites may be better served by an understanding of the factors that contribute to the success of native species revegetation after invasive species control.

### **Invasive Species and Revegetation**

Introduction of invasive species rapidly causes a loss of native species, reduces biodiversity, and extirpates populations and communities (Williams 2007); therefore invasive species removal is a critical component of restoration efforts. Revegetation is an important, logical subsequent step upon removal of invasive species, but little is known about maximizing revegetation success in a restoration context. Despite this, revegetating an area rapidly that has been disturbed is important in preventing the introduction of invasive species (Spieles 2005), or the reinvasion of former weeds.

Effective revegetation methods following *I. cylindrica* control have not been established; however several studies have identified potential post *I. cylindrica* control planting strategies. Plant selection and plant structure are likely important factors to consider in order to maximize the competitive ability of a revegetated native plant community, but it is unclear if *I. cylindrica* reinvasion can be constrained by a specific plant community composition. Mesocosm

experiments show decreased *I. cylindrica* spread and establishment when species such as *Andropogon* spp. was used in revegetation attempts (Jose 2007). However, a survey of existing native species composition at logged and unlogged sites found that species composition was independent of *I. cylindrica* spread (Collins 2005).

Often, post-planting herbicide treatments for invasive species control promote the growth and survival of desirable species (Valenti 2002). Though many studies have addressed controlling *I. cylindrica* with herbicides prior to planting native species, there is no information on follow-up herbicide control for revegetation efforts in initially *I. cylindrica*-dominated sites.

Revegetation efforts only occasionally follow invasive species clearing projects (Galatowitsch & Richardson 2005). Even when native plant communities are not assumed to readily return after control and revegetation is needed, limited funds for restoration may prohibit purchase of plant material. The cost of plant material can be part of the reason for high cost of restoration, though a lack of cost-reporting in revegetation studies makes this difficult to assess. Plants can be purchased from local native plant nurseries in plug size or larger, or as seeds in the order of decreasing cost.

The primary goal of this research was to establish techniques for the restoration of flatwoods groundcover plant communities in former phosphate mines following *I. cylindrica* removal. More specifically, this research addresses which plant species and follow-up herbicide treatment provides efficient revegetation and optimal ecosystem function in a restoration context. Costs of

restoration research were closely monitored so methods can be applicable in a practical context. The final goal was to create a management plan for the restored areas to encourage further native species establishment and address potential re-invasion.

## **Materials and Methods**

### **Study Site**

Tenoroc Fish Management Area [TFMA] is the former site of the Coronet Phosphate Company that actively mined phosphate during the 1960s and 1970s. After mining operations ceased, the land was abandoned, and the area became heavily invaded by *I. cylindrica*. Soils at TFMA are overburden (sand and clay mixture removed from the soil surface to uppermost part of the ore moved away from the site). The soil type is 80% sand, 8% silt, and 12% clay which enables the soil to more readily retain water, phosphorus, and potassium than Floridan soils (Richardson et al. 2003)

Previous research on *I. cylindrica* control at the study site found that treatments varied in effectiveness, with application of glyphosate (1.68 kg-ai/ha) resulting in 95% control, and imazapyr (alone or with glyphosate) resulting in less than 50% control (Ketterer 2007). Collectively, these treatments resulted in patchy distribution across the experimental study site, consisting of *I. cylindrica* and other weedy species [e.g. *Passiflora incarnata* L. (maypop passionflower), *Indigofera hirsuta* L. (hairy indigo), *Eupatorium capillifolium* (Lam.) Small (dogfennel), etc.]. Preparation of the study site for this experiment involved application of Round-up (6.18 kg-ai per hectare or 4 qt/A glyphosate), Pasturegard (0.843 kg-ai per hectare triclopyr+0.281 kg-ai per hectare fluroxypyr

or 2 qt/A fluroxypyr, triclopyr), and addition of a non-ionic surfactant (0.25%), followed by mowing. A grid map of existing plant species and distribution at the study site was developed noting patches of *I. cylindrica*. From this grid, areas with limited plant cover were identified. In these bare areas, native plant species were installed after mowing in January 2008 when *I. cylindrica* was dormant.

### **Experimental Design**

The experiment evaluated the effect of planting regime and follow-up herbicide treatments on establishment of planted native species. Three planting regime treatments were evaluated: no planting (control), high structural diversity (HSD: grasses, forbs, and a shrub) and low structural diversity (LSD: grasses only). Two follow up herbicide treatments were evaluated: no treatment (control), and glyphosate-treated plots. Treatments were applied in a 3x2 randomized complete factorial design with 6 replications, for a total of 36 plots. Experimental plots consisted of 12 ft diameter circles (area = 10.5 m<sup>2</sup>, 113 ft<sup>2</sup>) in which plants were installed. These circular plots were surrounded by a 2 ft buffer (area= 1.2 m<sup>2</sup> or 12.6 ft<sup>2</sup>) reserved for the herbicide follow up treatment (Figure 3-1).

Plants were installed in March 2008 at equal planting densities (42 plants per plot). Species composition and cost of HSD and LSD planting regimes is detailed in Table 3-1. All native plant species were obtained from The Natives, Inc., which were grown less than 20 miles away from the study site in a flatwoods ecosystem and selected based on reference ecosystem plant community composition and potential to establish (Nancy Bissett, The Natives, Inc., personal communication, January 18, 2007).

Follow up herbicide treatments were applied 9 months after planting, in December 2008 (optimal timing for controlling *I. cylindrica*, (Ketterer 2007). Glyphosate (2% solution) was applied to encroaching *I. cylindrica* at the edge of the plots using a backpack sprayer to spot spray. All efforts were taken to reduce potential impact to the native species installed, but achieve sufficient coverage of *I. cylindrica*.

### **Data Collection**

Plant volume (m<sup>3</sup>) was measured at time of installation and at 6, 9, 12, and 18 months after planting. Species survival and species richness of each experimental unit were assessed 6, 9, 12 and 18 months after planting. Percent cover of all unplanted species, including *I. cylindrica*, per plot was evaluated from 0-100% (rounded to the nearest 5%).

Seed bank assays were performed prior to planting, and 3, 6, and 9 months after planting to assess the potential contribution of the seed bank to vegetation dynamics. Seed bank samples were attained from planted patches, *I. cylindrica* patches, and in the 2ft (1.17 m<sup>2</sup>) diameter surrounding planted patches. Density of *I. cylindrica*, other undesirable species, and desirable species seed was represented by emergence from soil spread in the greenhouse.

### **Statistical Analysis**

Analysis of variance (Statistical Analysis Software (SAS®) version 9.1.3, SAS Institute Inc., 2004) was used to determine the effect of planting regime and use of herbicide on plant response (response variables= plant volume and survival rates for each species planted and for overall native species). Means

comparisons were performed using the Least Significant Difference (LSD) procedure. Alpha values were set equal to 0.05.

## Results

Over the study period, survival, growth, and response to treatments were species-specific. All species planted persisted throughout the 18 month observation period except *Ilex glabra*, for which no live individuals were observed after the first data collection period (therefore no figures display information on this species), and *Dyschoriste oblongifolia*, which did not persist beyond 9 months after planting. Precipitation was higher during time of planting compared with the ten year normal, but generally lower than average during the study period (Figure 3-2).

The seedbank assay results (see Appendix A) indicate that no *I. cylindrica* seed was present in the seed bank during the study period, nor was seed of native species used for revegetation detected. Unplanted native species were, however, found within the seedbank, including: *Gnaphalium purpureum*, *Oenothera laciniata*, *Indigofera hirsuta*, *Eupatorium capillifolium*, and *Sida rhombifolia*. Non-native species detected included: *Oxalis* spp., *Cyperus* spp., *Aeschynomene* spp., and *Wahlenbergia marginata*.

Costs incurred throughout the duration of this restoration project totaled less than \$1900.00. Total plant cost was approximately \$787.04, and herbicide for site preparation and the follow up post-planting application costs were approximately \$65.00 and \$15.00 respectively. Labor costs associated with this restoration project totaled approximately \$1000.00.

## Plant Survival and Volume Over Time

Overall (all native plant species combined) percent survival was not affected by planting treatment or herbicide application. Overall plant volume was greater in high structural diversity (HSD) planting treatments compared with low structural diversity planting treatments (LSD), particularly at 9 months after planting (Figures 3-3 through 3-11; note that each figure has a unique y-axis scale, depending on plant volume for the individual species). For all grasses, individual species plant volume was greater in HSD treatments in conjunction with no herbicide applied. An exception was *Andropogon brachystachyus*, for which volume was similar in both planting treatments ( $p=0.11$ ).

Survival of planted native species declined over the 18 month study period to less than 35% for all species except *Muhlenbergia capillaris* (85% survival) and *Aristida beyrichiana* (55% survival). No *Ilex glabra* survived. Overall native plant survival did not differ between planting regime (Table 3-2). Survival was greater in untreated plots after herbicide application for *E. spectabilis* (30%), *P. anceps* (15%), and *A. glomeratus* (15%) regardless of planting regime.

Plant volume increased over time beyond initial measurements for all species before herbicide application, but varied between planting regimes after herbicide application (Table 3-3). Although plant volume increased for all grasses in the LSD treatment, plant volume decreased for all grasses in the HSD treatment over time. Plant volume of *Panicum anceps*, *Muhlenbergia capillaris*, *Eragrostis spectabilis*, *Aristida beyrichiana*, and *Andropogon brachystachyus* was almost 3 times greater in HSD treatments than LSD treatments 9 months after planting. Plant volume in HSD treatments, however, declined for most species,

whereas plant volume continually increased over time for all species planted in LSD treatments. No live *Dyschoriste oblongifolia* individuals were observed beyond 9 months after planting (no further data on this species for 12 and 18 MAP).

### **Effects of Herbicide Application on *I. cylindrica* and Planted Species**

*Imperata cylindrica* cover increased rapidly from 0 to 6 months after planting, and then continued to persist at 45-50% cover in control plots (untreated, unplanted plots), but did not exceed 35% in planted plots (Figure 3-12). Herbicide application decreased *I. cylindrica* cover to less than 20% immediately following application, but *I. cylindrica* increased in cover 9 months after application (Figure 3-12). Percent survival and plant volume of planted native species was similar in herbicide treated and untreated plots before herbicide application, but varied thereafter (Figures 3-3 through 3-11).

Overall native species plant volume was lower in HSD plots where *I. cylindrica* was controlled with the follow up herbicide treatment ( $p \leq 0.01$ ). In contrast, individual species plant volumes in treated, LSD plots were greater with the exception of *P. anceps*. Percent survival for *P. anceps*, *E. spectabilis*, and *A. glomeratus* was greater than or equal in both planting treatments 12 and 18 months after planting where herbicide was not applied.

Plant volume and percent survival for *Liatris spicata* was not affected by herbicide application, but a minor difference in growth was detected both 12 and 18 months after planting. *Pityopsis graminifolia* volume was also not affected by herbicide application, however, greater survival was detected both 12 and 18 months after planting in treatments without herbicide application.

## **Effects of Structural Diversity on Species Richness and Percent Cover of *I. cylindrica***

Species richness generally increased through 9 months after planting for both planting regimes. After herbicide treatment (12 months after planting) species richness decreased in treated plots by almost 20%, however, HSD plots consistently had more species than LSD plots. As percent *I. cylindrica* cover decreased, species richness increased. Unplanted plots contained significantly less (up to 11) species than planted plots throughout the study period (Figure 3-12).

Overall percent cover of *I. cylindrica* was greater in experimental units not treated with herbicide regardless of planting regime. Without follow up herbicide treatment HSD plots had greater *I. cylindrica* percent cover compared with LSD plots at the end of the study. After herbicide application, *I. cylindrica* cover was 18% in treated plots compared to 40% in untreated plots (averaged over planting regime). This reduction in cover was accompanied by an increase in species richness for treated plots. Percent cover of *I. cylindrica* also decreased slightly (from 40 to 30%) in untreated plots, species richness also increased in this situation.

### **Discussion**

*Imperata cylindrica* invades in heavily disturbed areas, which by nature are difficult-to-revegetate. Revegetation efforts in these altered environments are further complicated by post-clearing reinvasion by *I. cylindrica*. Results show that planting a species mixture of high structural diversity offers the best approach to establishment of native species and defense against further *I.*

*cylindrica* reinvasion. Surprisingly, in this study, follow up herbicide did not promote species richness, and even had some negative impacts on newly establishing natives (especially when planted in high structural diversity plantings). Conversely, there was evidence that follow up herbicide promoted greater plant volume in LSD plots where grasses were planted alone.

Rates of establishment in this study were clearly impacted by drought conditions. During the critical establishment period (0 to 6 months after planting), many species did not achieve significant growth since precipitation was minimal. Adequate water is particularly critical during this phase, and dry conditions cause stress to plants, resulting in slow or reduced establishment (Villalobos & de Pelaez 2001; Villagra & Cavagnaro 2006; Sosebee & Wan 1987).

### **Structural Diversity Influenced Planted Species Establishment**

One of the most interesting observations throughout this study is that grasses in HSD plots attained greater volumes than LSD plots. It was hypothesized that grasses would perform better when planted alone (LSD plots) because competition would be limited by other species. One possible explanation for improved grass performance in the HSD planting is the nurse-plant concept: a member of one species facilitates the growth of another species by ameliorating the stressors of the local environment (decreasing soil evaporation rates, decreasing microsite temperatures, and causing less evapotranspiration from plant tissue) (Niering et al. 1963; Raffaele & Veblen 1998). Since the site was relatively dry during initial planting, reducing water losses from the soil was likely critical. As such, using higher structural diversity

planting treatments may have allowed for greater plant volume of grasses as well as greater plant survival of most species planted.

### **Follow-up Herbicide Provided Mixed Results**

Although it was anticipated that native plant volume would increase in response to *I. cylindrica* suppression as a result of the follow up herbicide, the outcome of this treatment on native plants was contradictory. For LSD plots, the follow up herbicide application resulted in higher native plant volumes, presumably a result of the reduction in *I. cylindrica* provided by the selectively-applied herbicide. However in HSD plots, plant volumes were greater where herbicide was not applied. A possible reason for decreased plant volumes in response to the herbicide application in HSD plots is that during application to *I. cylindrica*, some solution may have drifted onto the installed native plants. It could be that native species were more susceptible to these non-target herbicide effects in HSD plantings, where increased structure provided greater surface area to intercept drifting herbicide.

Survival, however, was generally greater in plots where herbicide was applied to treat surrounding *I. cylindrica* regardless of planting regime where herbicide drift may have caused plant stunting, but not mortality. Control of the invasive generally allows for greater spread and survival of desirable species (Shilling et al. 1997; Miller 2000).

### **Post-planting Community Dynamics**

There was also an abundance of undesirable forbs which recolonized throughout the study. Perhaps planting grasses initially, controlling for undesirable forbs and *I. cylindrica*, then establishing native, desirable forbs after

control is complete would increase the likelihood of restoring certain ecosystem functions and values.

It is interesting to note that species richness was always greater in planted plots regardless of herbicide application. After herbicide application, however, species richness initially declined in all planting treatments (when measured during winter months at 12 months after planting), then increased 18 months after planting. Unplanted plots, however, never reached a richness quantity greater than six. Possibly because species were planted at consistent densities, species richness was not as variable as expected since there was a lack of interspecific competition where members of a different species vie for the same environmental resources such as water and soil nutrients (Budelsky & Galatowitsch 2000). Another possible reason for the lack of increase in species richness could be the minimal vegetation surrounding the site (monotypic stands of *Sporobolus indicus* (smutgrass)) was not diverse.

### **Relative Costs of Treatments**

Restoration project costs vary from \$2000 for less than 10 hectares to greater than \$51,000 per hectare for restoration where exotic invasive species removal is part of the restoration goal (Henri et al. 2004; Macmillan et al. 1998; Rashford & Adams 2007). In comparison, the cost of this restoration project was at the lesser end of other restoration projects.

### **Conclusions: Implications for Practice**

Initial *I. cylindrica* control efforts should result in relatively bare ground. In addition to reinvading *I. cylindrica*, ruderal weedy species may compete with any revegetation efforts. Therefore, identifying undesirable recolonizing species prior

to designing a revegetation plan has some benefit. For example, if recolonizing undesirable species are mainly forbs, planting grasses only at this initial stage would afford the use of a forb-specific herbicide that will not threaten planted grass establishment. Similarly, if grasses are primarily problematic, planting forbs and shrubs only, and using a grass specific herbicide would preserve planted native species and control undesirable grasses, including *I. cylindrica*. Note that a two-stage planting effort may be compatible with this approach; e.g. 1) plant native grasses, 2) selectively control undesirable forbs, and 3) plant native forbs. Because undesirable species may persist past an initial selective control herbicide application, plan for potentially multiple selective control efforts (these may take a year or more) prior to planting native species.

Selecting appropriate species for revegetation efforts will increase chances of establishment. Plant species with a high likelihood of establishment and ability to survive harsh environmental extremes, such as *Muhlenbergia capillaris*. *Aristida stricta* and *Liatrix spicata* may also be suitable for heavily-degraded and drought-prone areas.

Initial invasive species reduction and native species establishment may be resource intensive. However, after years of native species survival and reproduction, along with follow-up control of undesirable and invasive species via chemical application, management needs may eventually be reduced.

Table 3-1. List of species, quantity, total number of plants, cost per plant, and overall cost for species used for revegetation.

Species	Growth form	Quantity		Total number of plants	Cost per plant (\$)	Amount (\$)
		LSD*	HSD**			
<i>Andropogon brachystachyus</i> Chapm.	Grass	7	3	120	0.48	56.60
<i>Eragrostis spectabilis</i> (Pursh) Steud.	Grass	7	3	120	0.48	56.60
<i>Aristida beyrichiana</i> Trin. & Rupr.	Grass	7	4	132	0.51	67.32
<i>Panicum anceps</i> Michx.	Grass	7	3	120	0.48	56.60
<i>Andropogon virginicus</i> L.	Grass	7	3	120	0.48	56.60
<i>Muhlenbergia capillaris</i> (Lam.) Trin.	Grass	7	4	132	0.51	67.32
<i>Dyschoriste oblongifolia</i> (Michx.) Kuntzel	Forb	-	5	60	2.25	135.00
<i>Liatris spicata</i> (L.) Willd	Forb	-	6	72	1.25	90.00
<i>Pityopsis graminifolia</i> (Michx.) Nutt.	Forb	-	8	96	1.25	120.00
<i>Ilex glabra</i> (L.) A. Gray	Shrub	-	3	36	2.25	81.00
Total		42	42			787.04

\* LSD = low structural diversity

\*\* HSD = high structural diversity

Table 3-2. Analysis of variance for the effects of planting regime (Reg.), herbicide application (Herb.), and 0, 6, 9, 12, and 18 months after initial planting (MAP) on percent survival of all surviving species.

Source	Overall survival		Grasses												Forbs*															
			A. <i>brachystachyus</i>				Aristida				Eragrostis				Muhlenbergia				Panicum				A. <i>glomeratus</i>				Pityopsis		Liatris	
	F	P	F	P	F	P	F	P	F	P	F	P	F	P	F	P	F	P	F	P	F	P	F	P	F	P				
Reg.	0.39	0.53	0.23	0.63	0.28	0.59	1.76	0.18	0.08	0.77	1.03	0.31	0.31	0.57	-	-	-	-	-	-	-	-	-	-	-	-	-			
Herb.	3.38	0.06	0.00	0.95	2.15	0.14	8.11	0.01	2.48	0.11	4.70	0.03	3.91	0.04	3.09	0.08	0.32	0.57												
Reg*herb.	3.63	0.06	0.50	0.48	0.45	0.50	0.03	0.86	4.54	0.03	0.44	0.50	0.94	0.33	-	-	-	-												
MAP	14.58	0.01	7.46	0.01	0.00	1.00	0.40	0.52	0.06	0.80	31.62	<0.01	0.53	0.46	3.12	0.07	1.76	0.18												
Reg*MAP	0.00	0.98	0.10	0.75	0.06	0.79	0.91	0.34	0.44	0.50	0.00	0.96	0.23	0.62	-	-	-	-												
Herb*MAP	0.00	0.95	1.10	0.42	0.00	1.00	0.10	0.75	1.26	0.26	0.45	0.50	0.20	0.65	1.22	0.27	0.32	0.57												

\*Results are not shown for *I. glabra* or *D. oblongifolia* as these species did not survive past 9 months after planting.

Table 3-3. Analysis of variance for the effects of planting regime (Reg), herbicide application (Herb), and 0, 6, 9, 12, and 18 months after initial planting (MAP) on total plant volume and species richness of all surviving species.

Source	Overall volume		Species richness		Grasses												Forbs*			
					A.				<i>brachystachyus</i>		<i>Aristida</i>		<i>Eragrostis</i>		<i>Muhlenbergia</i>		<i>Panicum</i>		<i>A. glomeratus</i>	
	F	P	F	P	F	P	F	P	F	P	F	P	F	P	F	P	F	P	F	P
Reg.	226.73	<0.01	950.06	<0.01	2.65	0.11	31.41	<0.01	12.1	0.01	268.2	<0.01	28.33	<0.01	3.89	0.05	-	-	-	-
Herb.	3.35	0.06	42.74	<0.01	0.29	0.59	0.74	0.39	0.76	0.38	1.58	0.21	1.4	0.24	0	0.98	0.02	0.88	2.7	0.11
Reg*herb.	30.75	<0.01	0.25	0.77	7.0	0.01	9.26	0.01	4.58	0.04	4.15	0.04	0	0.97	9.25	0.01	-	-	-	-
MAP	68.99	<0.01	9.45	<0.01	21.03	<0.01	30.7	<0.01	3.59	0.06	0.02	0.88	56.76	<0.01	22.55	<0.01	0.07	0.79	24.94	<0.01
Reg*MAP	224.78	<0.01	5.47	<0.01	4.56	0.04	29.0	<0.01	4.43	0.04	266.6	<0.01	1.06	0.31	6.08	0.02	-	-	-	-
Herb*MAP	6.36	0.01	6.41	<0.01	6.0	0.02	1.12	0.29	0.6	0.44	1.63	0.20	0.82	0.37	3.36	0.07	0.18	0.68	0.65	0.43

\*Results are not shown for *I. glabra* or *D. oblongifolia* as these species did not survive past 9 months after planting.

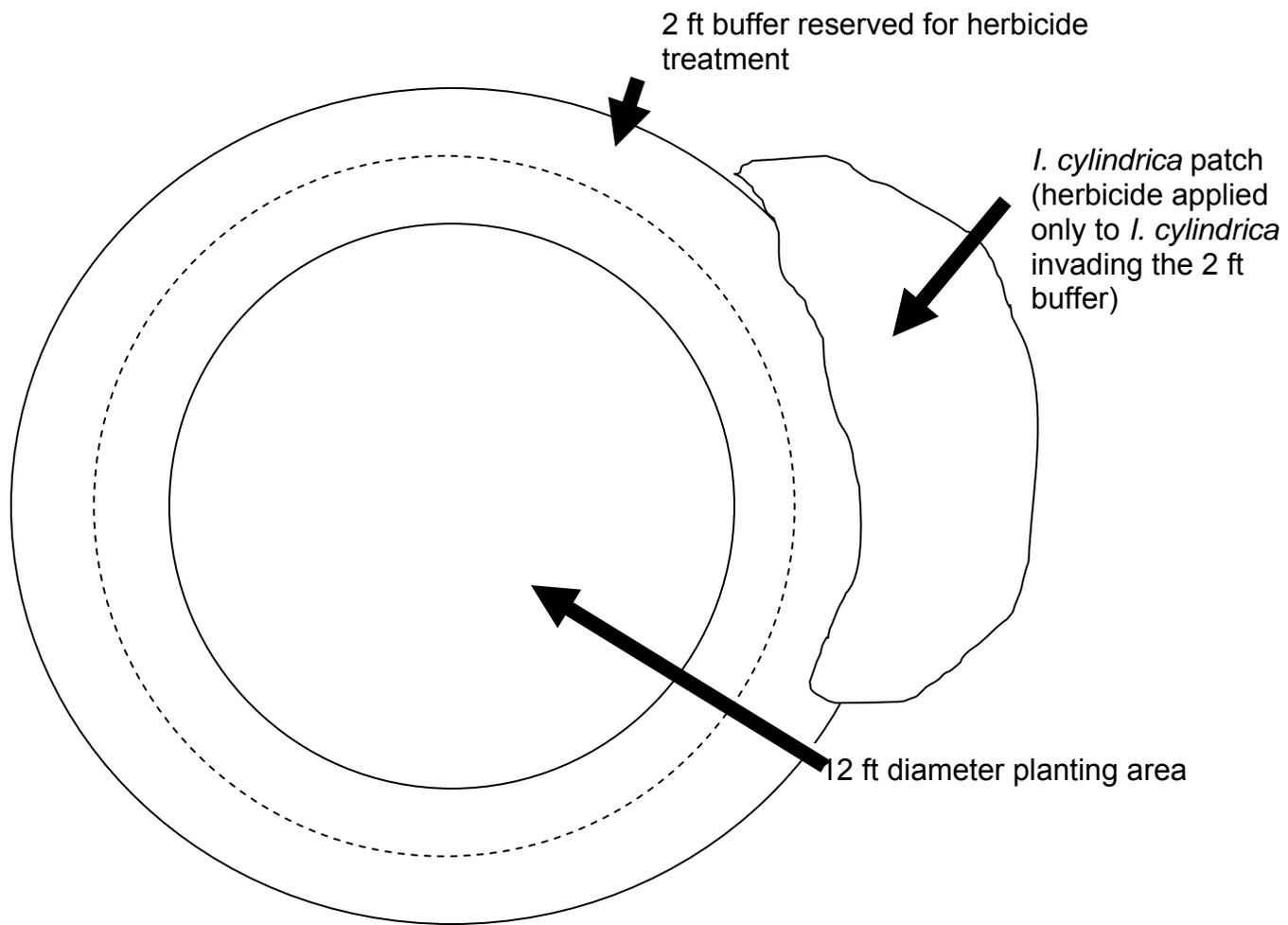


Figure 3-1. Schematic of a circular study plot with 12 ft diameter planting area and 2 ft buffer reserved for the follow up herbicide application. Half of all experimental units were assigned an herbicide treatment.

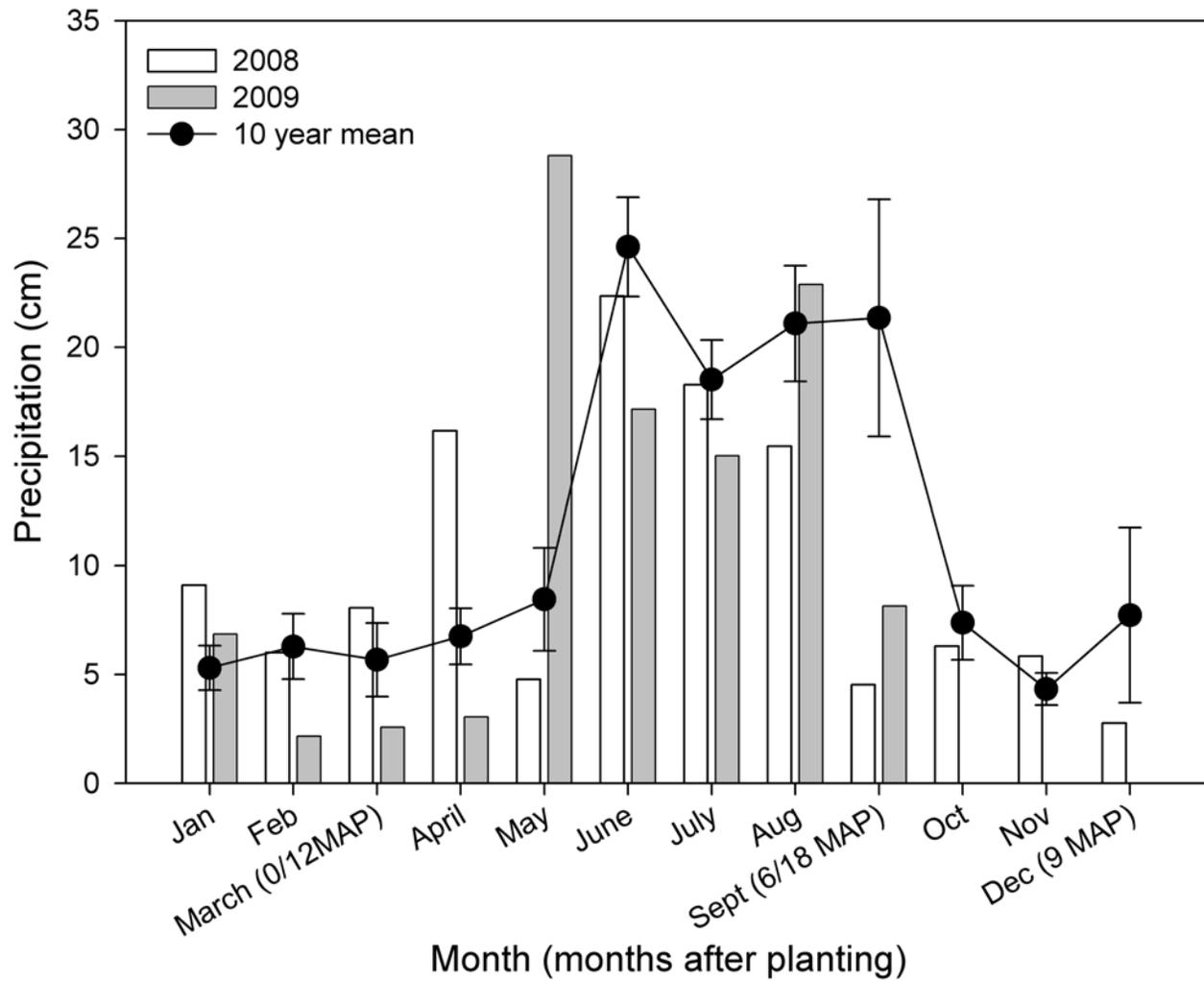


Figure 3-2. Precipitation patterns during the study period (March 2008 (initial time of planting and 12 months after planting)-September 2009 (6 months after planting and 18 months after planting)) and ten year average.

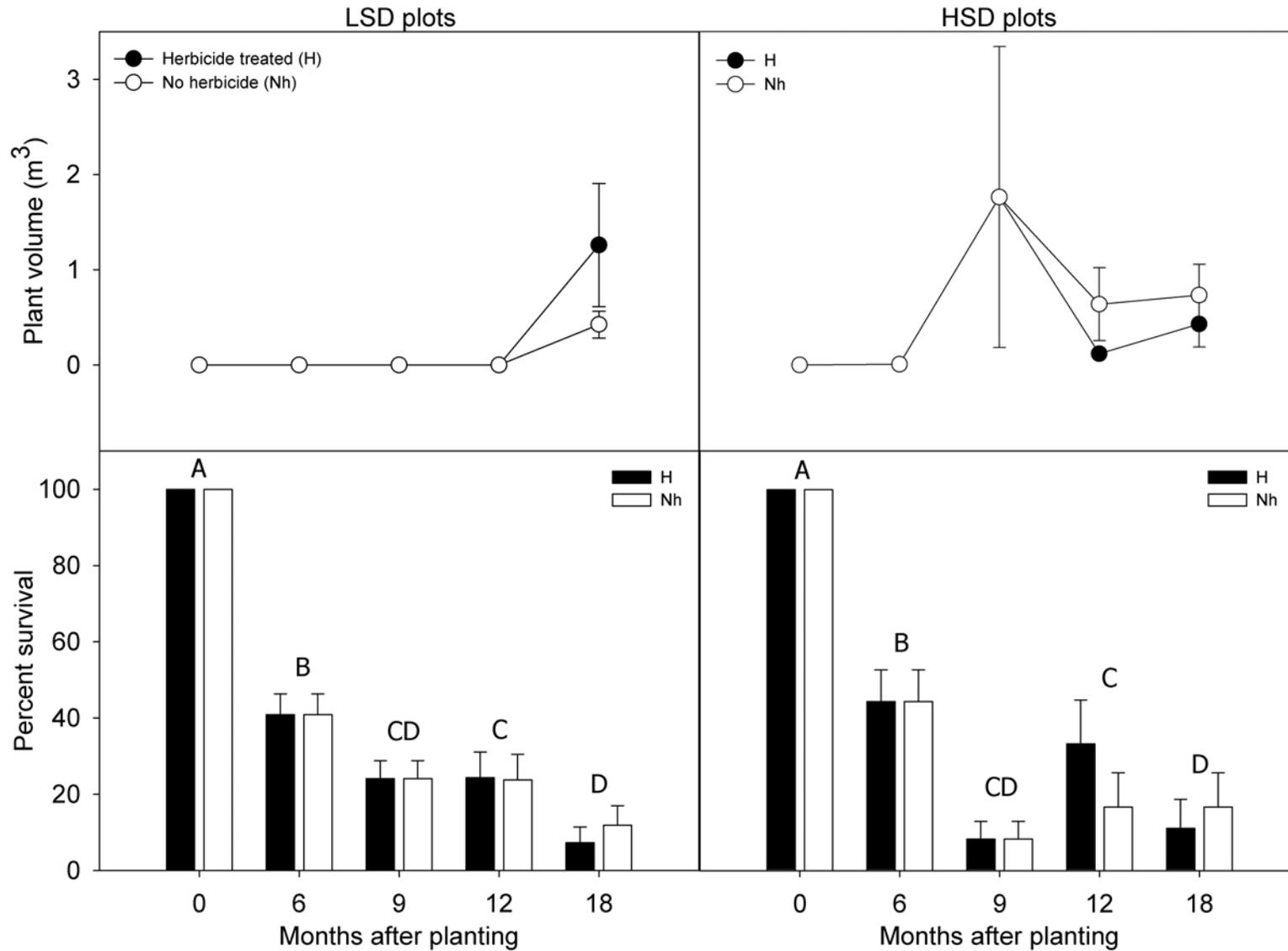


Figure 3-3. *Andropogon brachystachyus* – the effects of planting regime and herbicide application on plant volume and survival. Mean of 6 replications followed by standard error.

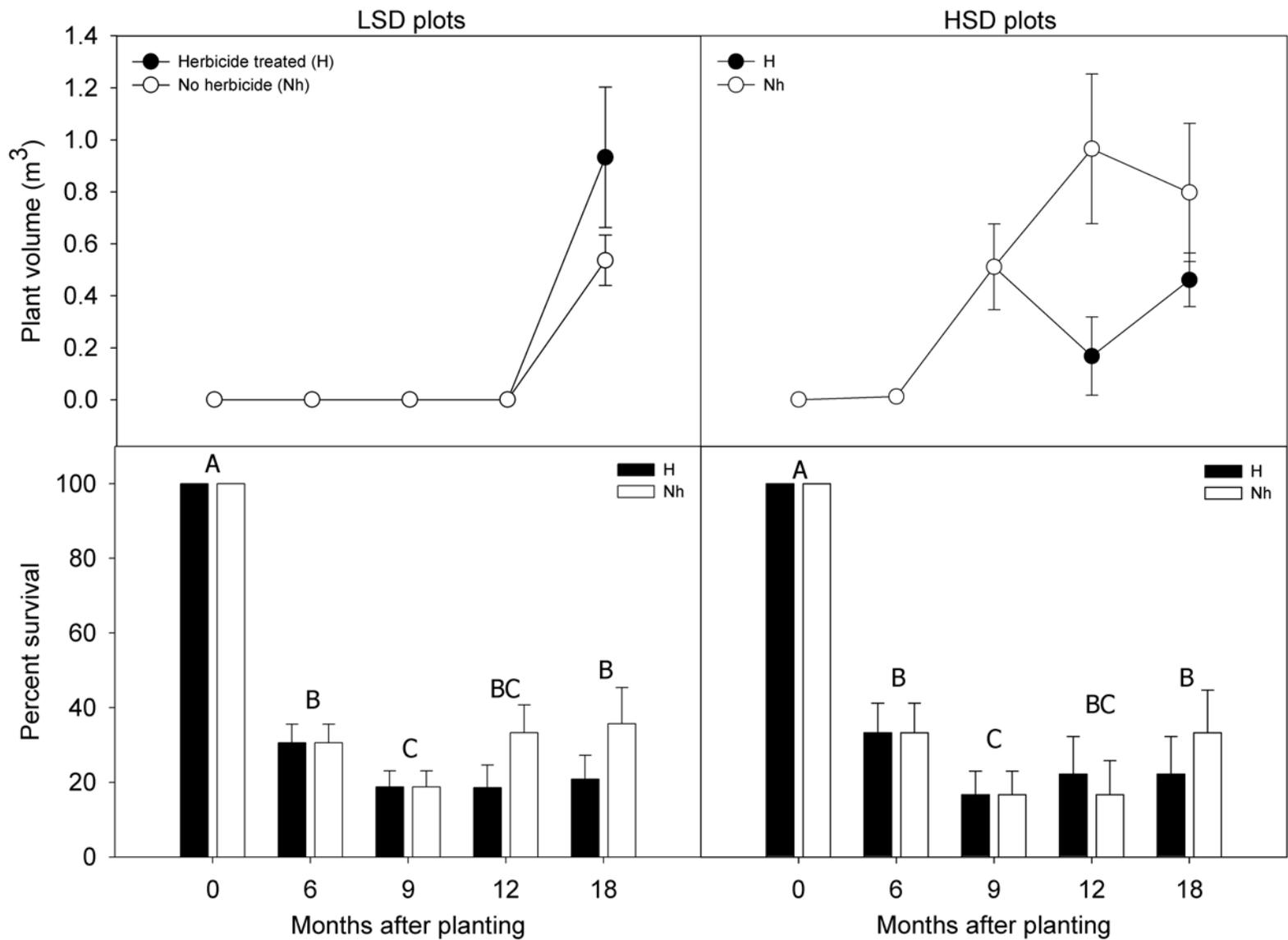


Figure 3-4. *Andropogon glomeratus* – the effects of planting regime and herbicide application on plant volume and survival. Mean of 6 replications followed by standard error.

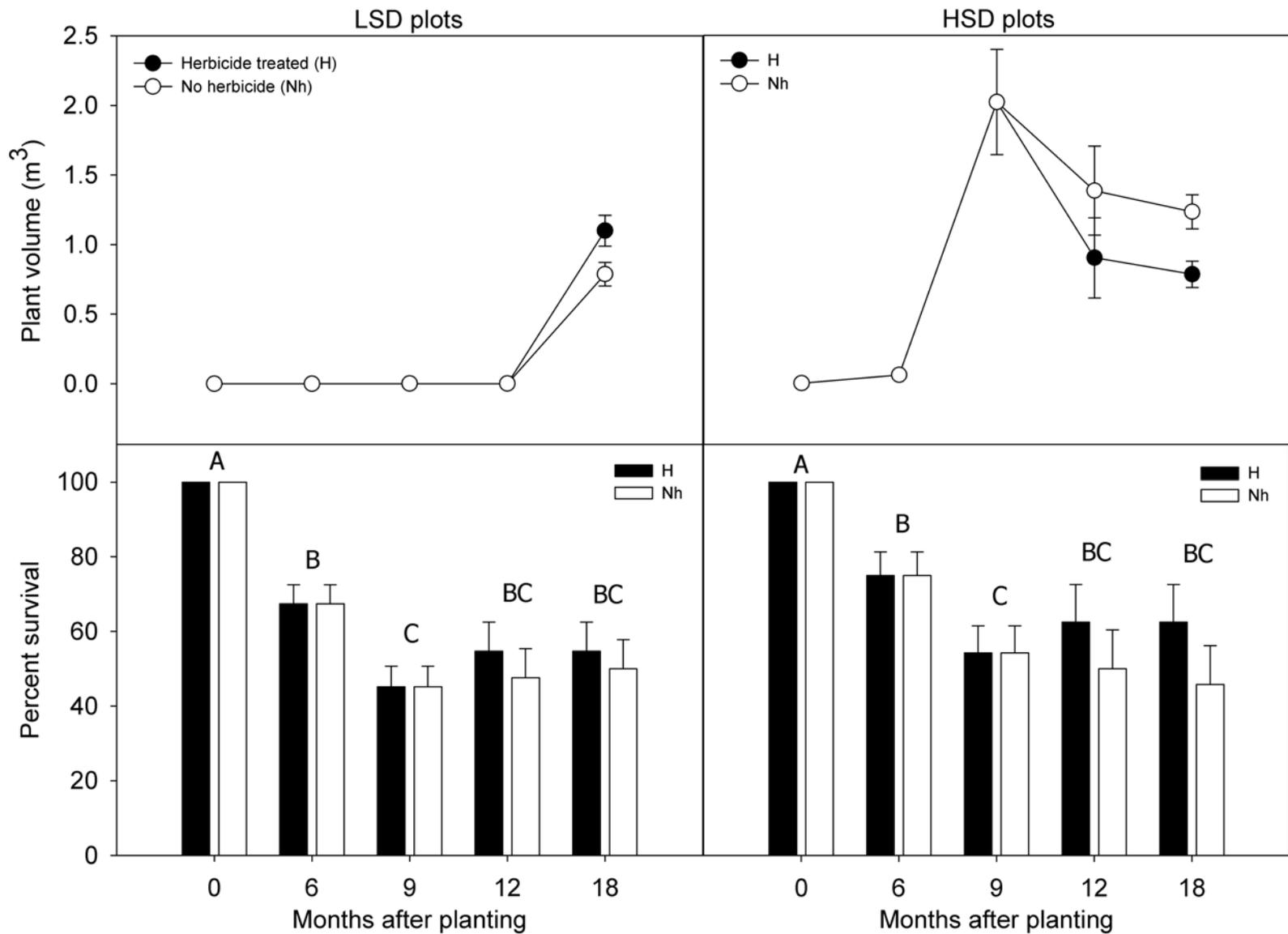


Figure 3-5. *Aristida stricta* – the effects of planting regime and herbicide application on plant volume and survival. Mean of 6 replications followed by standard error.

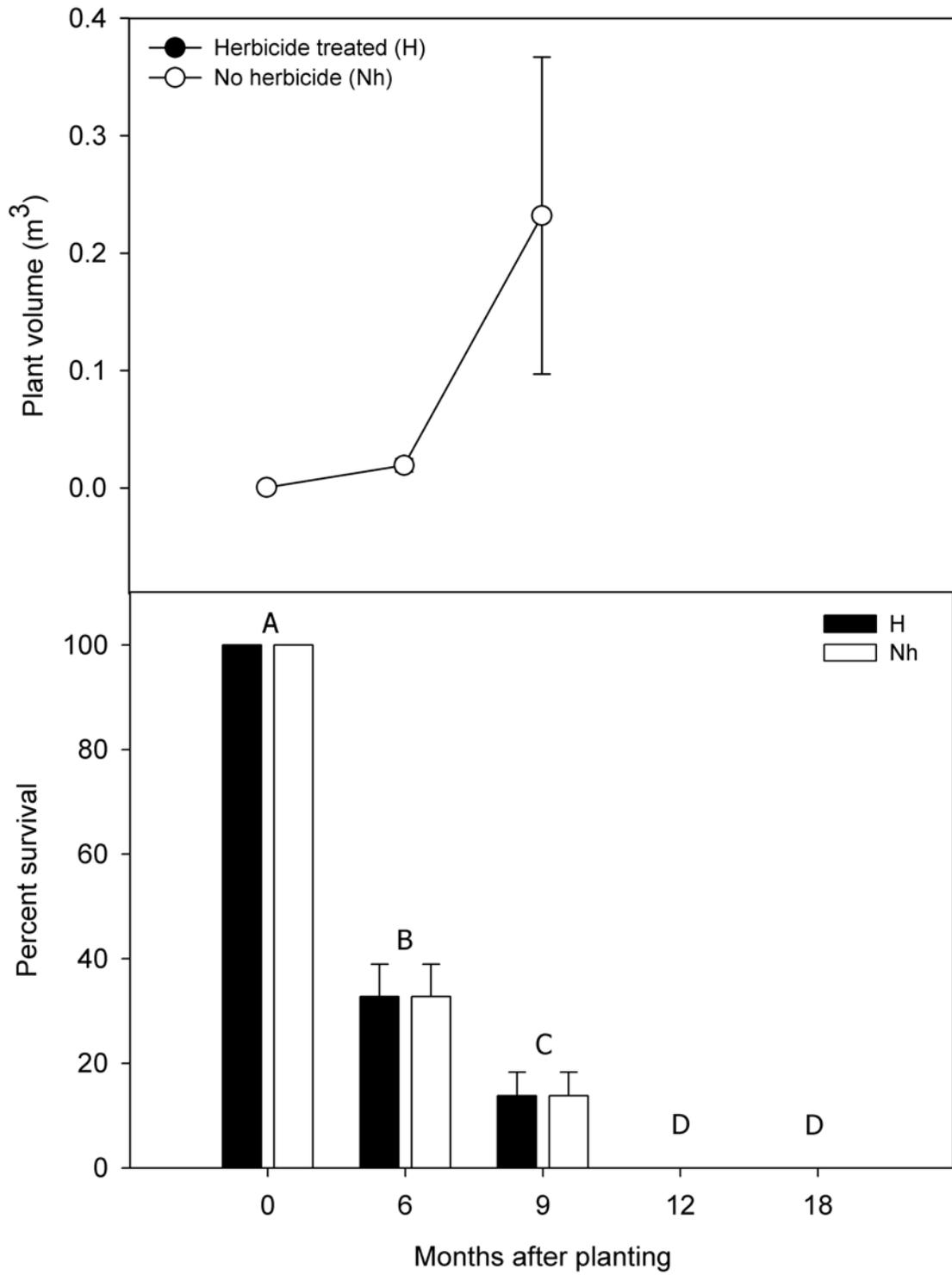


Figure 3-6. *Dyschoriste oblongifolia* – the effects of herbicide on plant volume and survival. Mean of 6 replications followed by standard error.

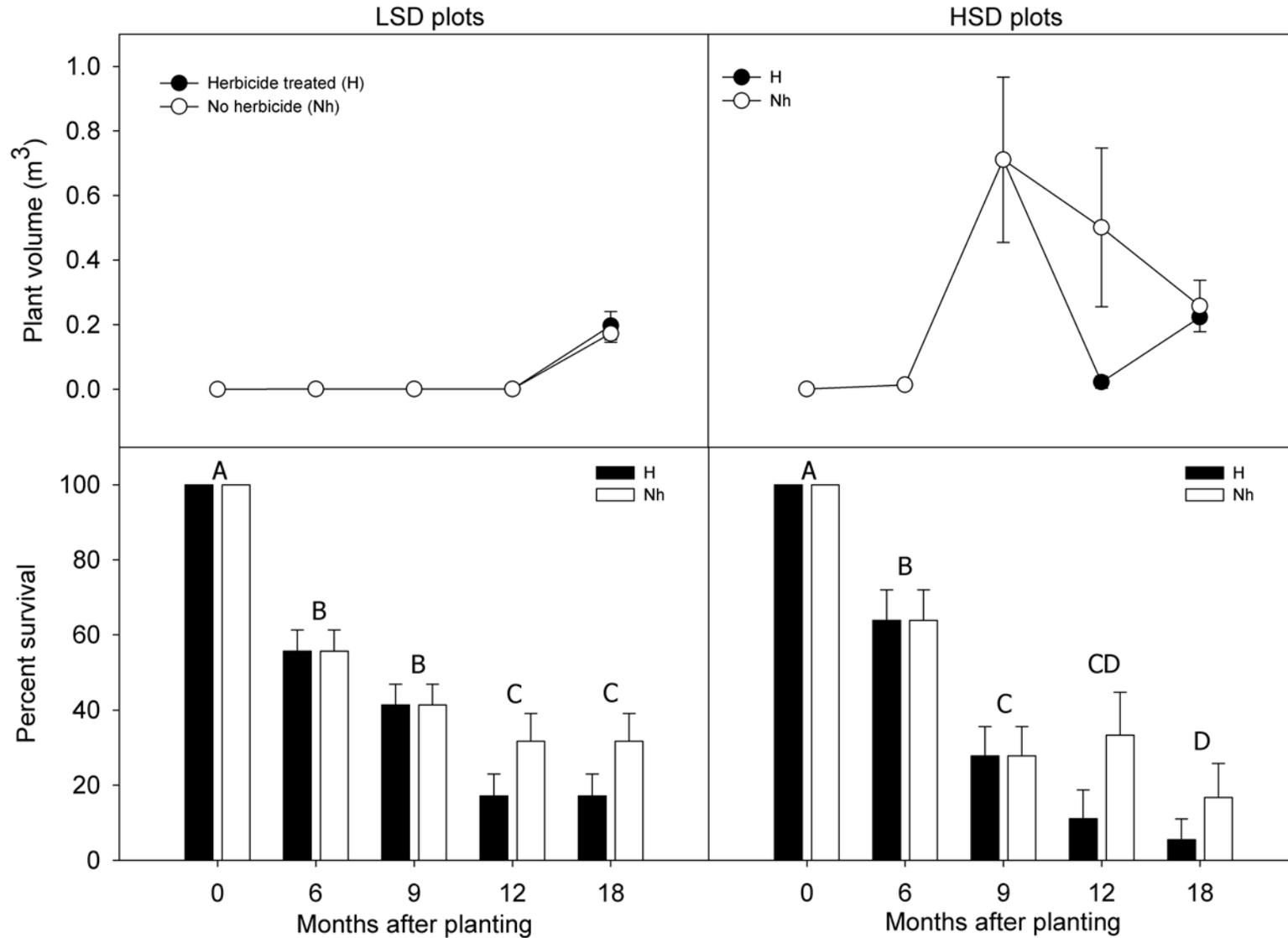


Figure 3-7. *Eragrostis spectabilis* – the effects of planting regime and herbicide application on plant volume and survival. Mean of 6 replications followed by standard error.

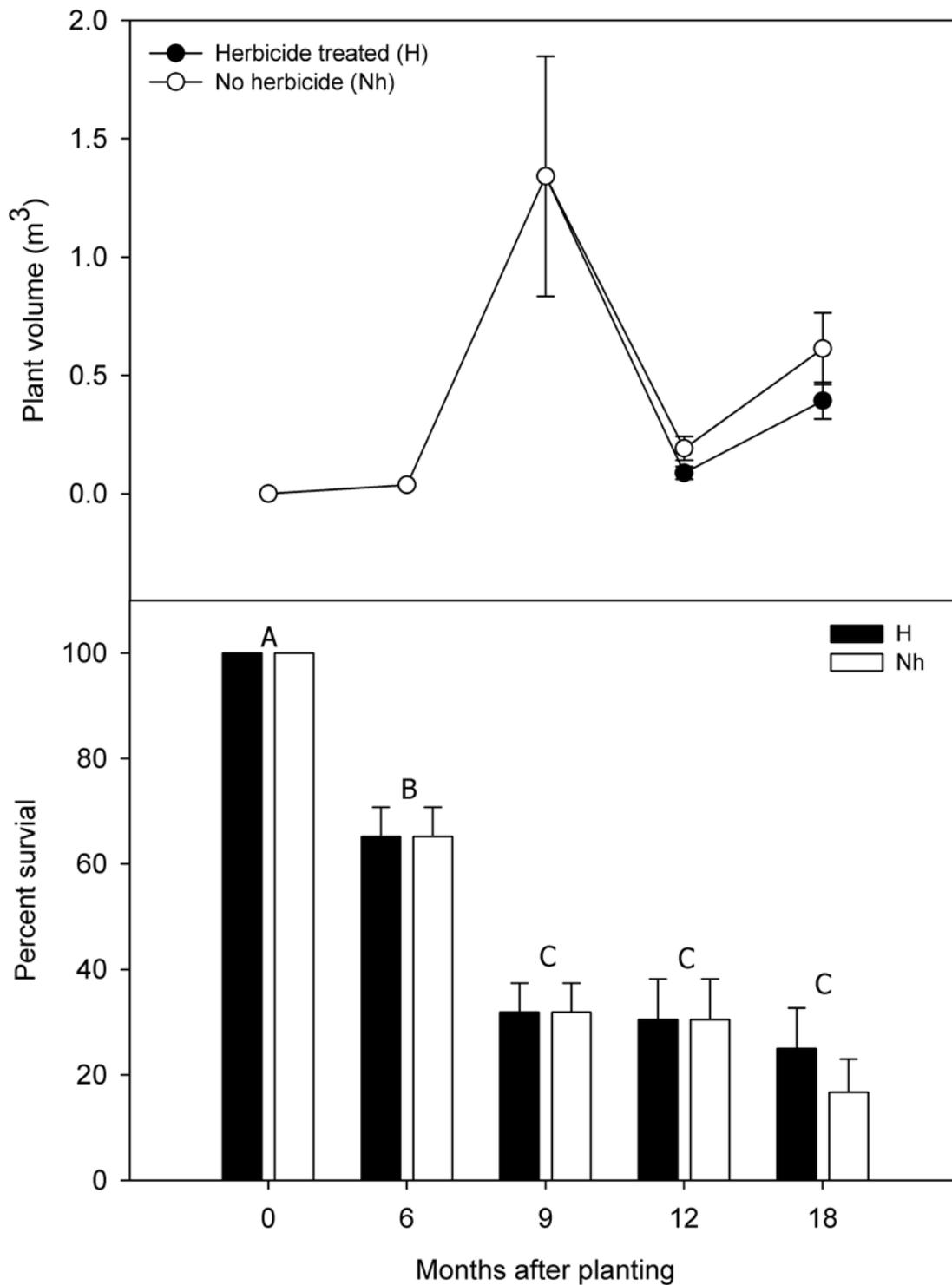


Figure 3-8. *Liatris spicata* – the effects of herbicide application on plant volume and survival. Mean of 6 replications followed by standard error.

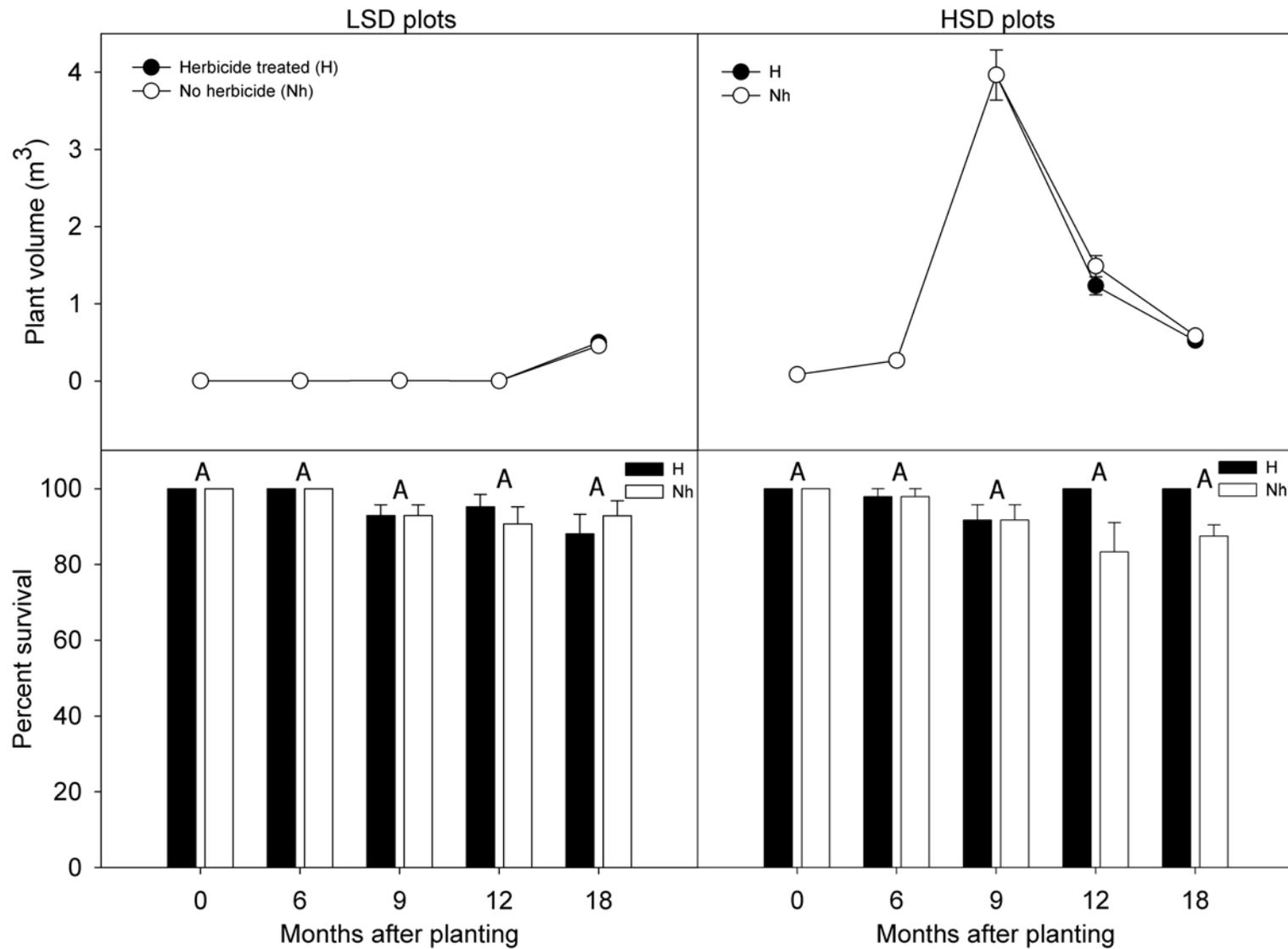


Figure 3-9. *Muhlenbergia capillaris* – the effects of planting regime and herbicide application on plant volume and survival. Mean of 6 replications followed by standard error.

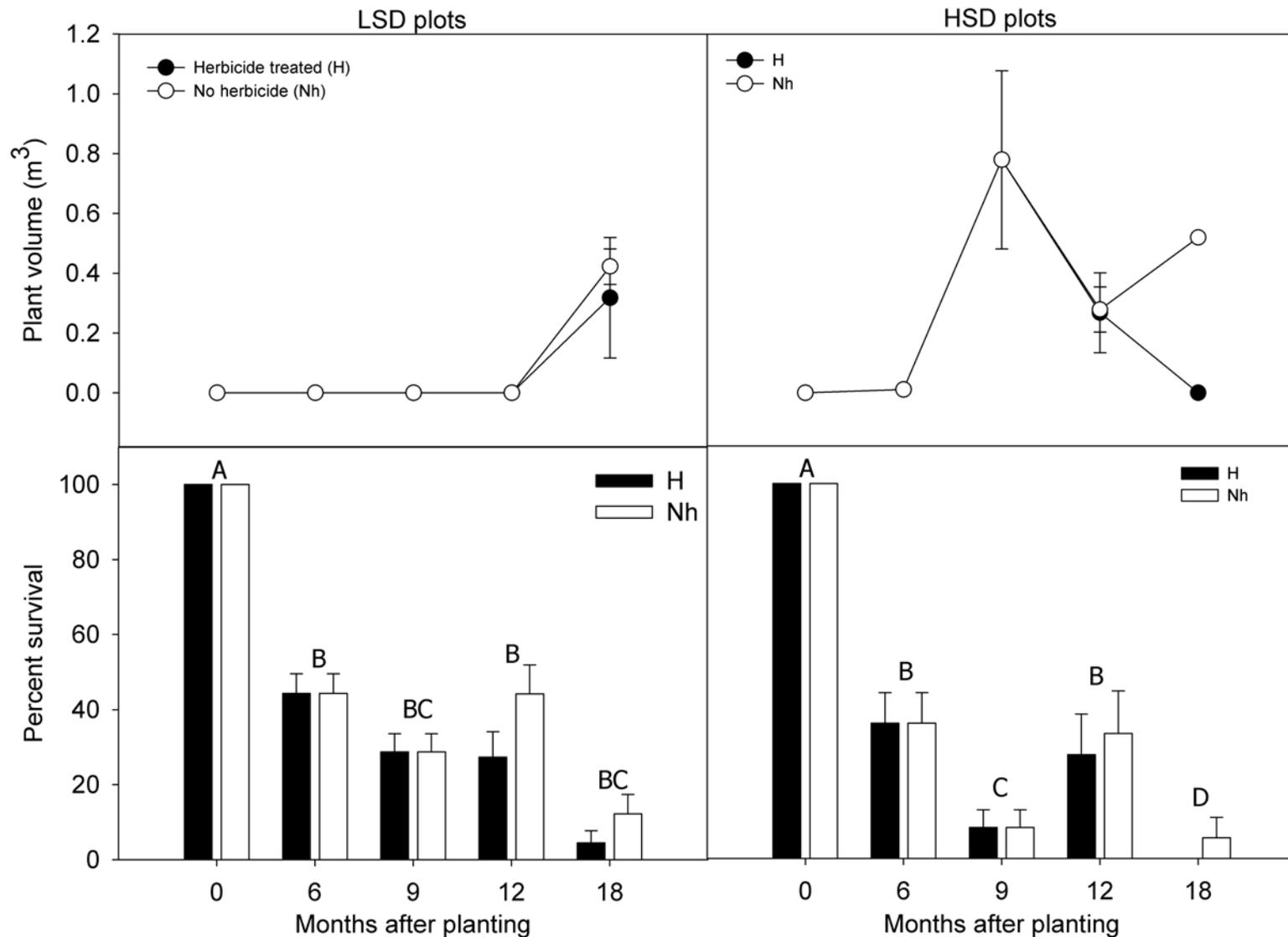


Figure 3-10. *Panicum anceps* – the effects of planting regime and herbicide application on plant volume and survival. Mean of 6 replications followed by standard error.

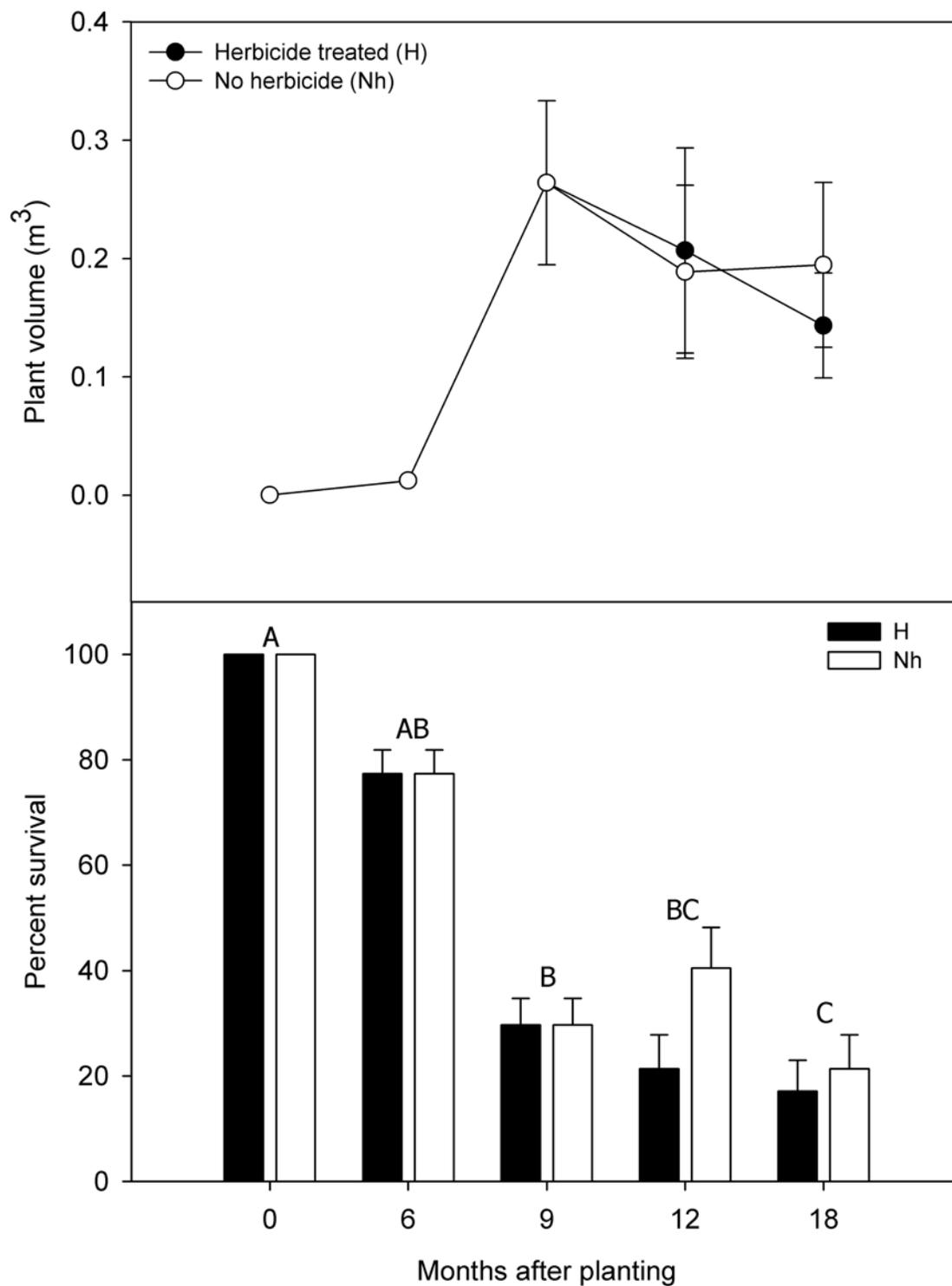


Figure 3-11. *Pityopsis graminifolia* – the effects of herbicide application on plant volume and survival. Mean of 6 replications followed by standard error.

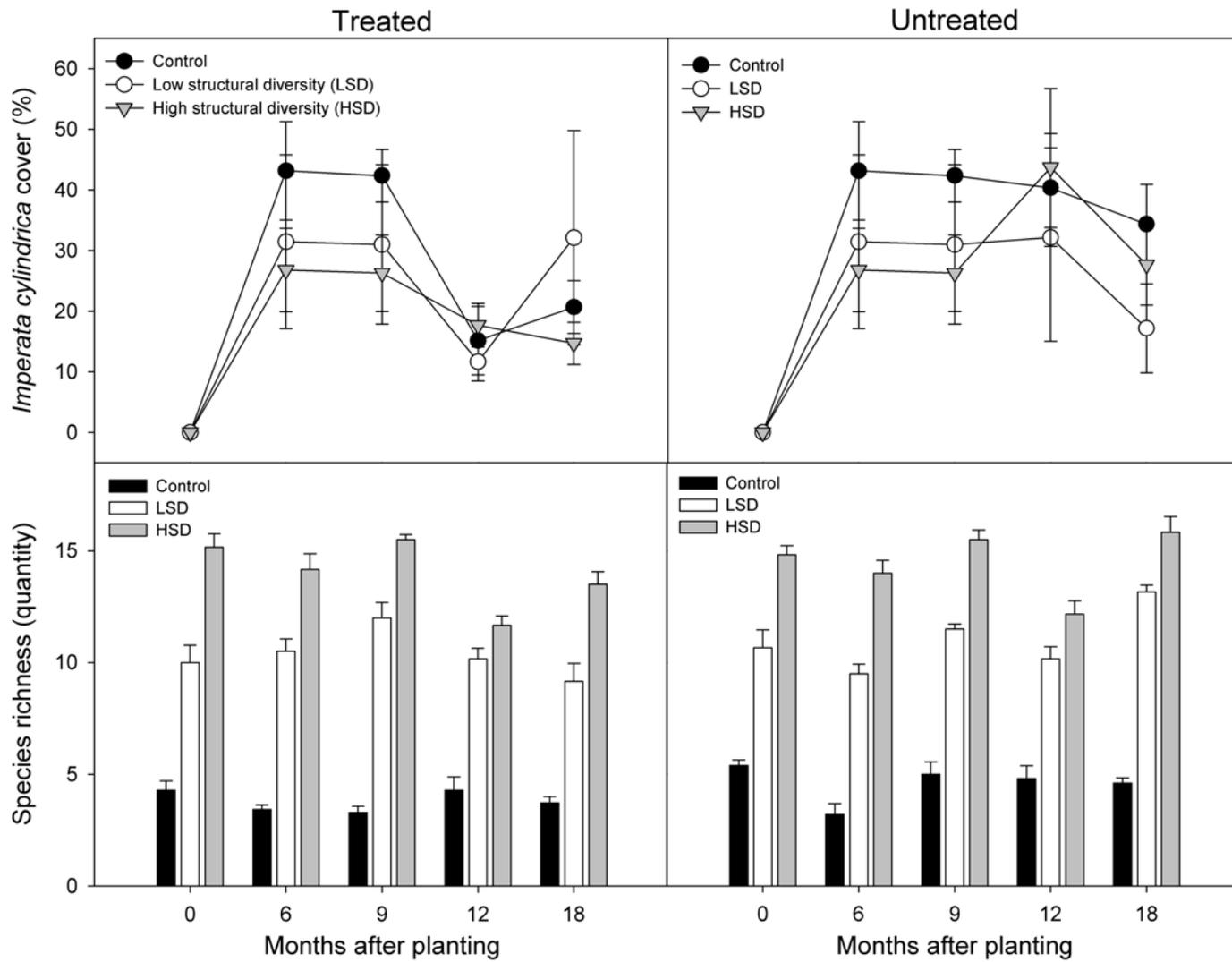


Figure 3-12. Effects of planting regime and herbicide application on species richness and percent cover of *I. cylindrica*. Mean of 6 replications followed by standard error.

## CHAPTER 4 CONCLUSIONS

Florida's landscape consists of unique and highly valued natural conservation areas including: forests, flatwoods, prairies, swamps, and marshes. Rapid population growth makes Florida susceptible to land disturbances. Opportunistic plant species, such as *Imperata cylindrica* (cogongrass), *Salix caroliniana* (coastalplain willow) and *Typha* spp. (cattail), have become problematic by displacing Florida's native plant communities. Removal of species that preclude native plant communities is important to conserving Florida's unique ecosystems. Though rarely attempted in practice, revegetation after invasive species removal assists in restoration of native plant communities.

Little is known about revegetation attempts after invasive species removal. Planting density, planting elevation, propagule size, and herbicide application may play an important role in successfully revegetating a disturbed ecosystem. The research previously stated shows that planting at sparse densities increases the likelihood of survival for *Juncus polycephalos*, *Pluchea rosea*, *Cyperus haspan*, *Eleocharis atropurpurea*, and *Fuirena breviseta*, most likely because competition is reduced. Planting elevation was another parameter investigated by this research. We note that several species preferences emerged, even for the small difference in hydrologic condition that resulted from the elevation differential tested in this study. Larger propagule size plants establish more easily, and may buffer harsh environmental conditions for newly establishing species.

It is well known that for heavily invaded sites, broadscale herbicide treatment for initial removal of invasive species increases the likelihood of native species

establishment. Active revegetation by planting native species following invasive species removal is likely critical and, as we found in this study, also deters *I. cylindrica* from further spreading. High structural diversity (HSD) plantings are recommended as greater volumes and survival rates were observed for species planted in this regime. Follow-up herbicide application reduced *I. cylindrica* cover by 30%, but had no effect on native species performance at the end of the study period in HSD planting regimes, possibly due to non-target effects of the herbicide application on native species in the HSD plantings. To reduce non-target effects of the herbicide, extreme care is required to avoid planted species during application. Therefore, it is recommended to carefully decide whether follow-up herbicide treatments should be applied. This research also suggests a two-stage planting effort may be a more effective approach than a single planting event. For instance, 1) plant native grasses, 2) selectively control undesirable forbs (planted grasses will be unaffected), and 3) plant again with native forbs. Because undesirable species may persist past an initial selective control herbicide application, plan for potentially multiple selective control efforts (these may take a year or more) prior to planting subsequent native species.

Initial removal of invasive species is one of the first steps in successfully restoring a native plant community. After removal, actively revegetating native plant communities, taking into account species preferences for environmental influences, should be a priority. Follow-up herbicide treatments should be applied appropriately. On-going monitoring to track plant community development will allow identification of further interventions necessary to restore these plant communities.

APPENDIX A

Table A-1. Number of plants per species present in seedbank at TFMA 0, 3, 6, and 9 months after planting in the *I. cylindrica* patch, edge of planting area, and inside the planting area.

Species	In planting area	Edge of planting area	In <i>I. cylindrica</i> patch
0 months after planting			
<i>Gnaphalium purpureum</i>	79	24	29
<i>Oenothera laciniata</i>	2	0	1
<i>Oxalis</i> spp.	2	4	4
<i>Cyperus</i> spp.	15	7	14
3 months after planting			
<i>Gnaphalium purpureum</i>	0	0	0
<i>Oenothera laciniata</i>	0	0	1
<i>Oxalis</i> spp.	5	0	0
<i>Cyperus</i> spp.	2	25	4
<i>Indigofera hirsuta</i>	50	22	27
<i>Eupatorium capillifolium</i>	1	1	1
<i>Aeschynomene</i> spp.	0	1	1
6 months after planting			
<i>Gnaphalium purpureum</i>	181	138	157
<i>Oenothera laciniata</i>	0	0	0
<i>Oxalis</i> spp.	0	0	0
<i>Cyperus</i> spp.	0	0	1
<i>Indigofera hirsuta</i>	6	0	0
<i>Eupatorium capillifolium</i>	0	0	1
<i>Aeschynomene</i> spp.	2	0	0
<i>Sida rhombifolia</i>	2	6	1
<i>Wahlenbergia marginata</i>	74	97	62
9 months after planting			
<i>Gnaphalium purpureum</i>	135	134	360
<i>Oenothera laciniata</i>	0	0	0
<i>Oxalis</i> spp.	3	4	3
<i>Cyperus</i> spp.	2	5	3
<i>Indigofera hirsuta</i>	0	0	0
<i>Eupatorium capillifolium</i>	0	0	0
<i>Aeschynomene</i> spp.	0	0	0
<i>Sida rhombifolia</i>	0	0	0
<i>Wahlenbergia marginata</i>	133	239	93

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## BIOGRAPHICAL SKETCH

Kathryn was born and raised in Homestead, Florida and attended the University of Florida (UF). Her research career began at UF where she worked in a plant tissue culture and micropropagation lab studying the effects of plant growth regulators and basal salts on inducing root and shoot organogenesis in the aquatic plant *Aponogeton madagascariensis* under the supervision of Dr. Michael Kane. Although she loved laboratory research, she decided field research was more fitting since her greatest passions exist in the outdoor environment.

Kathryn graduated from UF in 2007 with a major in Interdisciplinary Studies with a concentration in Environmental Horticulture Operations. She joined the Plant Restoration, Conservation, and Propagation Biotechnology Program in the Environmental Horticulture Department at UF in February 2006. She was fortunate enough to venture in wetland and upland restoration projects. In her spare time Kathryn enjoys hunting, fishing, photography, crocheting, playing various instruments, and writing biographies in the third person.