A STUDY ON PROPAGATION METHODS FOR TWO NATIVE WILDFLOWERS: *POLYGONELLA POLYGAMA* AND *POLYGONELLA ROBUSTA*

BY

ALISON HEATHER

A THESIS PRESENTED TO THE GRADUATE SCHOOL
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To everyone who has helped me with this project, even if it was just a word of encouragement

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PROPAGATION METHODS OF TWO NATIVE WILDFOWERS: *POLYGONELLA POLYGAMA* AND *POLYGONELLA ROBUSTA*

By

Alison Heather

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Chair: Hector E. Perez
Cochair: Sandra B. Wilson
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The perennial nature, prolific white to pink flower racemes, and attractive foliage and form of October flower (*Polygonella polygama* (Vent.) Engelm. & A. Gray [Polygonaceae]) and sandhill wireweed (*Polygonella robusta* (Small) G.L. Nesom & V.M. Bates [Polygonaceae]) suggest that these wildflowers could have significant ornamental and landscape potential if an effective propagation method can be developed. Prior to germination experiments, both species were tested for viability using Triphenyltetrazolium chloride (TZ). Tests indicate the seed viability was variable (*P. polygama*= 77%, *P. robusta*= 54%). Initial germination tests showed that both species germinated best in cooler temperatures (22^{o}C day and 11^{o}C night temperatures). Both species exhibited physiological dormancy. Germination was greatest when seeds were treated with 100 or 1000ppm GA_3 (*P. polygama* = 35%, *P. robusta* = 58%) and kept in an incubator at 22/11 ^{o}C. Germination of both species at simulated seasonal temperatures, carried out in a move-along experiment, indicated that seeds require a period of warm stratification before germination commences at cooler temperatures. Additionally, *P. polygama* seeds stratified in 5^{o}C for 2 weeks prior to planting out in the

greenhouse showed an improvement in germination. However, poor collection timing and improper post-harvest storage may decrease germination.

Propagation by stem cuttings may decrease production time, improve uniformity, and widen collection times. Experiments were conducted to determine the effects of Indole-3-butyric acid (IBA) and 1-Naphthaleneacetic acid (NAA) on rooting softwood cuttings of *P. polygama* and *P. robusta* collected from natural populations in central and south Florida. Softwood cuttings of each species were quick dipped with nine different concentrations of K-IBA:K-NAA (0:0, 0:250, 0:500, 500:0, 500:250, 500:500, 1000:0, 1000:250, 1000:500 ppm). Root initiation and quality were assessed after 6 weeks (*P. polygama*) or 8 weeks (*P. robusta*) under intermittent mist. When *P. polygama* (south) cuttings were treated with 1000:250 IBA:NAA ppm, rooting reached 63%. The highest rooting of 80% was achieved for *P. robusta* (south) when treated with 500:250 IBA:NAA ppm. Significant site × IBA × NAA interactions occurred for root index and percent rooting of *P. robusta*. However, most measured responses were not significantly different among auxin treatments.

CHAPTER 1
INTRODUCTION AND LITERATURE REVIEW

History of Florida and Native Plants

Florida has been inhabited by Europeans and their descendents since 1565, with their impact being fully visible with the cession of Florida to England in 1763 (Myers and Ewel, 1990). Though Native Americans had lived in Florida for centuries prior to European settlement, their impact on the state was less severe compared to the changes seen today. Though Native Americans used fire to clear understories for agriculture and improve passage, thus mimicking the effects of natural wildfire, they also first began the practice of fire suppression (Delcourt, 1987). Landscapes where fire has been suppressed may have higher intensity wildfires due to fuel accumulation, preventing the beneficial post-burn effects that most ecosystems are adapted to. The arrival of European settlers marked the beginning of the drastic transformation of the Florida landscape, including a lack of understanding of fire. Since the settlers arrival there have been three major events which have further altered ecosystems of Florida: ubiquitous clearing of land for agriculture and silviculture to support the expanding population, conversion of 80% of Lake Wales ridge for the cultivation of citrus (Christman and Judd, 1990), and attempted drainage of Lake Okeechobee and the Everglades (Myers and Ewel, 1990). Since civilization has entered Florida, urban development has taken over half of Florida's pine flatwoods (Taylor, 1998). The scrub ecosystem, which is home to many wildflowers, has suffered great losses.

There are many ways to define a native plant; the U.S. Fish and Wildlife Service (2001) considers a species native to a particular ecosystem if "other than a result of introduction, (it has) historically occurred or currently occurs in that ecosystem". The

Florida Native Plant Society (FNPS) specifies that a plant is native to Florida if it has occurred within the state's boundary before European contact (FNPS, 2003). In 1992 it was estimated that there were approximately 2,870 plants native to Florida (Marinelli, 1994). Wunderlin and Hansen (2003) list 4,200 species of native or naturalized ferns and seed plants in their Atlas of Florida Vascular Plants, making Florida the third most floristically diverse state in the United States. Florida's distinctive native flora has multiple origins. Plants occurring in South Florida are often also found in the Bahamas, West Indies, and South America. Those from North Florida can often be found in Texas and throughout the Midwest and Appalachian Mountains. Florida also possesses many endemic native plants. An endemic plant is unique to a particular region and can be a measure of species richness in a given area (Taylor, 1998). For example, the Central Florida Ridge is a unique area of relict Pleistocene epoch dunes composed primarily of scrub ecosystem. Forty species of endemic plants occur in this region, thus providing one of the highest rates of endemism in North America (Christman and Judd, 1990).

Economics of Florida Native Plants

In 2005, sales in the Florida environmental horticulture sector reached over $15 billion. Of these sales, 10.5% were native species (Hodges and Haydu, 2006), an increase of 3.5% since 1997 (Hodges and Haydu, 1999). The popularity of native plants appears to be increasing as evidenced by growing sales, establishment of more native plant society chapters (FNPS, 2003) and wider plant availability listed by the Association of Florida Native Nurseries (AFNN, 2009). In addition, there are several books that have been written in recent years that concentrate only on Florida native landscaping (Walton and Schiller, 2007; Osorio, 2001; Haehle and Brookwell, 1999; Huegel, 1995). Moreover, groups that specifically focus on wildflowers have been

formed such as the Florida Wildflower Foundation and the Wildflower Seed and Plant Growers Association, Inc. The Florida Wildflower Foundation's mission is to "protect and replenish native wildflowers while increasing public knowledge of them as vital members of the state's delicately balanced ecosystems" (Florida Wildflower Foundation, 2009). Currently the Wildflower Seed and Plant Growers Association, Inc. works with the Florida Wildflower Foundation to popularize the production of wildflowers with over a dozen active seed producers across the state (Wildflower Seed and Plant Growers Association, Inc., 2003; J. Norcini, personal communication).

Ecological Benefits of Florida Native Plants

Native plants attract a wide variety of birds and insects. Observational studies indicate that alien plants support fewer insect species than natives (Tallamy, 2004). Ornamentals commonly promoted by the horticulture industry are selected because they are "pest free" (Mack and Erneberg, 2002). A decline in the number of insect species will invariably cause the decline of insectivores, which could continue through the food web (Tallamy, 2004). As Huegel (1995) simply put "native plants and diversity of wildlife are intertwined". Wildlife use plants for both food and cover (Huegel, 1995). An example of this dependency is the gopher apple (*Licania michauxii* [Chrysobalanaceae]), which received its common name because of the nourishment it provides to the gopher tortoise. Furthermore, creating a landscape enriched with native plants can promote genetic diversity and reduce habitat fragmentation (Marinelli, 1994).

Although Scheiber et al. (2008) found no difference in the amount of water required for postestablishment growth between native and exotic shrubs, anecdotal evidence suggests that Florida native plants that are properly selected require less water, fertilizer, and pesticides than non-natives. This conclusion is supported by the

knowledge that native plants adapt to the nutrient poor soils, unique rainfall patterns and intense sunlight (Walton and Schiller, 2007; Haehle and Brookwell, 1999).

Native Wildflowers

The National Wildlife Research Center describes a wildflower as a flowering plant that is native to a specific geographical area or habitat, capable of growing without the assistance of humans (Paulson, 1989). Therefore, once an area that fits the plant's desired condition is seeded with wildflowers, it should be maintained with little effort (Paulson, 1989; Phillips, 1985). Florida native wildflowers often thrive in the hot and humid summers found in the state, unlike most industry termed "perennials" which often don't survive more than two years (Walton and Schiller, 2007; Harper-Lore and Wilson, 2000).

The wildflower industry boom began in 1982 with Lady Bird Johnson's opening of the National Wildflower Research Center in Austin, Texas (Milstein, 2005). Since then, interest in wildflowers has spread to highway departments, homeowners, and restoration specialists. Wildflowers are chosen for landscape use because they are easy to cultivate, often showy perennials (Miles, 1976). Wildflowers have primarily been used for roadside beautification and erosion control (Milstein, 2005). Considering this niche, the most widely utilized wildflower species have been relatively inexpensive, easy to grow, and adaptable to a wide range of habitats and conditions (Milstein, 2005).

In cultivation, wildflowers often flourish due to lack of competition from neighboring plant species (Phillips, 1985). Hammond et al. (2007) reported that firewheel (*Gaillardia pulchella* [Asteraceae]) grown individually in landscape plots were up to 2.5 times larger than those found in their native habitat. Further, Frances (2008) found reduced above and below ground biomass in tickseed (*Coreopsis* spp. [Asteraceae]) when planted in

pots with other tickseed plants or bahiagrass (*Paspalum* spp. [Poaceae]). In this study, intraspecific competition decreased biomass more than interspecific competition (Frances, 2008). Weed competition is one primary reason wildflowers do not establish within a year or two of planting (Norcini and Aldrich, 2004). Native plants are also commonly less aggressive, which makes them more susceptible to weed intrusions (Pfaff et al., 2002).

Native Wildflowers of Florida

Wildflowers exist in most Florida ecosystems. They dominate the understory of pine flatwoods and can be found on open sandhills and sand pine scrub (Taylor, 1998). Harsh environments such as flatwoods and dry prairies can support a diverse understory with wildflowers such as tarflower (*Befaria racemosa* [Ericaceae]), yellow bachelor's button (*Polygala rugelii* [Polygalaceae]), fall-flowering ixia (*Nemastylis floridana* [Iridaceae]), and scare-weed (*Baptisia simplicifolia* [Fabaceae]) (Myers and Ewel, 1990). Scrubs and sandhills, that usually consist of soils of the order entisol derived from quartz sand, generally have a more sparse ground cover that includes species such as gopher apple (*Licania michauxii* [Chyrsobalanceae]), milk peas (*Galactia* spp. [Fabaceae]), and garberia (*Garberia heterophylla* [Asteraceae]) (Myers and Ewel, 1990). Fire is a vital component in maintaining the stability of many Florida ecosystems. In the absence of fire due to intentional fire suppression or habitat fragmentation, the ecosystem is disrupted and pest species not adapted to fire repopulate the area. Native wildflowers can be replaced by low growing shrubs due to fire suppression (Taylor, 1998).

Wildflower seed mixes used by restoration specialists and homeowners often contain a variety of species specific to one habitat. This allows users to broadcast

wildflower seed easily and effectively. Many wildflower handbooks stress the importance of selecting wildflower species of known provenance or ecotype, then planting the species within climates similar to ones they are adapted to (Miles, 1976). Taking advantage of provenance and ecotype often results in improved health and vigor of the plant. Genotypes from a local population are more likely to be tolerant of those stresses (Booth and Jones, 2001). One study found that Firewheel (*Gaillardia pulchella* [Asteraceae]) plants derived from a seed source from West Florida were intolerant of South Florida conditions (Hammond et al., 2007). A similar response was seen when the species red clover (*Trifolium pratense* [Fabaceae]),orchardgrass (*Dactylis glomerata* [Poaceae]), and narrowleaf plantain (*Plantago lanceolata* [Plantaginaceae]) planted in test sites across Europe were found to have a home site advantage and a decrease in transplant performance with an increase in distance from the collection site (Joshi et al., 2001). Norcini et al. (2001) found that local seeds from lyreleaf sage (*Salvia lyrata* [Lamiaceae]) had a higher survival percentage as compared to non local seed sources. Also, local seeds from tickseed (*Coreopsis lanceolata* [Asteraceae]) and lyreleaf sage began flowering and reached full flower earlier as compared to non local ecotypes (Norcini et al., 2001). Another study comparing six wildflower species collected from outside vs. inside Florida found that those from outside the state adapted poorly to North Florida conditions, based on features such as length of flowering period, flower number, and disease incidence (Norcini et al., 1998). Harper-Lore and Wilson (2000) clarify that climate and geological variation may be more important to plant establishment than geographical distance. Elevation is another important consideration when planting. Lippit et al. (1994) recommend that seeds be planted in the same zone

within 150 m of elevation. Even when a wildflower is native to an area, it should, like any other plant, be studied for the ideal soil type, moisture, and pH requirements to determine if it is appropriate for a planting site (Paulson, 1989).

There are some issues associated with collecting plants from a local source. A limited collection area due to urban development makes collection more difficult and time consuming. Collecting seed in the wild can be complicated because uneven ripening can exist at a single location (Pfaff et al., 2002).

Landscape Potential of Two Florida Native Wildflowers

There are currently 300-500 species of wildflowers commercially available, representing only 3-5% of the country's native population (Milstein, 2005). October flower (*Polygonella polygama* (Vent.) Engelm. & A. Gray [Polygonaceae]) and sandhill wireweed (*Polygonella robusta* (Small) G.L. Nesom & V.M. Bates [Polygonaceae]) are two Florida native wildflowers that have limited commercial availability. Many Florida wildflower handbooks neglect this genus entirely, with a few offering only limited descriptions. However, Haehle and Brookwell (1999) note the aesthetic appeal of the genus, stating that *Polygonella* spp. would make "a lovely addition to a wildflower garden". Both species have distinct characteristics that differentiate them from other commercially available species, making them excellent options for homeowners and restoration specialists looking for unique native wildflowers (Table 1).

P. polygama var. *polygama* is a perennial, widespread wildflower that is found in sandhills, flatwoods, and scrubs, in full to partial sun. The spatulate to linear light green leaves are approximately 0.5 to 2 cm (0.2 to 0.8 in) long and 0.25 cm (0.1 in) wide. The stem has some jointing visible every 2 to 3 cm (0.8 to 1.2 in). Its prolific, miniature, cream to yellow flowers usually appear in late fall on a terminal raceme 2 to 5 cm (0.8 to

2 in) long, thus garnering its common name October flower. Distribution includes the coastal plain regions of the southeastern United States (PLANTS Database, 2008). It is frequently found in open spaces with surrounding flora such as scrub oaks (*Quercus* spp. [Fagaceae]), gopher apple (*Licania michauxii* [Chrysobalanaceae]), saw palmetto (*Serenoa repens* [Arecaceae]), false rosemary (*Ceratiola ericoides* [Empetraceae]), and pines (*Pinus* spp. [Pinaceae]) (Wunderlin and Hansen, 2003; Osorio, 2001; Taylor, 1998). Wunderlin and Hansen (2003) also describes a second, though less common variety, *Polygonella polygama* var. *brachystachya* (Meisn.) that is found mostly in flatwoods and has leaves 0.5-1.0 mm wide.

A second *Polygonella* species, *P. robusta,* is more commonly found in open sand, full sun environments along the coasts of Florida (PLANTS Database, 2008). This mounding perennial has linear shaped leaves 3 to 5 cm (1.2 to 2 in) long and 0.25 cm (0.1 in) wide. The stems appear to be jointed due to a sheathing petiole, with fibrous hairs at each node. Like *P. polygama,* it is also floriferous, but with terminal pink to cream miniature flowers that appear sporadically throughout the year on racemes 3 to 10 cm (1.2 to 4 in) long. Though the species has only been found in Florida, it is well distributed throughout the state. Accompanying flora include: saw palmetto (*Serenoa repens* [Arecaceae]), turkey oak (*Quercus laevis* [Fagaceae]), gopher apple (*Licania michauxii* [Chyrsobalanaceae]), prairie clover (*Dalea* spp. [Fabaceae]), yucca (*Yucca* spp. [Agavaceae]), and some xerophytic grasses (PLANTS Database, 2008; Wunderlin and Hansen, 2003; Osorio, 2001; Taylor, 1998). Though neither species is widespread in cultivation, some native nurseries in Florida have attempted to grow *P. polygama* and

P. robusta from seed. However, germination is often under 25% (N. Bissett, personal communication).

The ornamental potential and limited propagation knowledge of *P. polygama* and *P. robusta* warrants further investigation. The overall objective of this thesis project is to determine effective sexual and asexual propagation methods of *P. polygama* and *P. robusta* to increase their availability and use in urban landscapes and restoration projects.

Propagation by Seed

Genetic diversity during sexual reproduction results from independent assortment of chromosomes and crossing over during meiosis and fusion of gametes during fertilization. Plants grown from seed will therefore be genetically diverse compared to plants from cuttings. Genetic diversity within populations for outplanting is a goal in many restoration projects. Using direct seeding methods for projects also eliminates the production process in the nursery. This may reduce labor costs and other expenses as maintenance of mature plants would be unnecessary.

Germination is loosely defined as emergence of the radicle through the seed coat (Bradbeer, 1988). From a physiological perspective, germination begins with water uptake by the seed and ends with the elongation of the embryonic axis (Bewley and Black, 1994). Unless dormant, seeds will germinate if appropriate moisture, temperatures, and aerobic conditions are sensed (Bradbeer, 1988; Bewley and Black, 1994). To germinate and establish readily, seeds should have high viability and vigor. However, viability and vigor may be reduced when seeds are not collected and stored correctly. Seeds should be collected when the fruit is mature, usually indicated by a change in color. After collection, seeds should be dried quickly and stored in a cool

place at a moisture level low enough to prevent premature germination, the formation of disease, and seed aging (Justice and Bass, 1978; Lippit et al., 1994). However, levels of desiccation and cold that are too low may damage seeds of some species (Justice and Bass, 1978).

Dormancy is a seed trait that prevents germination when conditions are favorable for germination but the probability of seedling survival is low (Baskin and Baskin, 2001; Fenner and Thompson, 2005). Baskin and Baskin (2001) recommend testing for dormancy by subjecting seeds to a range of simulated seasonal temperatures for a period of four weeks. Seeds are considered dormant if no or little germination occurs at the end of the four week period (Finch-Savage and Leubner-Metzger, 2006; Baskin and Baskin, 2001; and Bradbeer, 1988). When this test is extended for longer than four weeks it is possible that the seed would have broken dormancy, thus rendering the test results inconclusive (Baskin and Baskin, 2004a).

Five types of dormancy are currently recognized. Physical dormancy (PY) results from water impermeable seed or fruit coats. Physiological dormancy (PD) can be characterized by the inability of embryos to rupture covering structures such as testa, endosperm, perisperm, and/or pericarp (Bradbeer, 1988; Baskin and Baskin, 2004a). Physiological dormancy may be caused by an embryo with a low growth potential, covering structures have low oxygen permeability, or covering structures that are resistant to pressure exerted by elongating embryos change over time (Baskin and Baskin, 2001). Embryos that are undifferentiated or underdeveloped at shedding are considered morphologically dormant (MD). Morpho-physiological dormancy (MPD) is a combination of PD and MD and is commonly found in wildflowers of temperate

deciduous forests (Baskin and Baskin, 2004a). Finally, seeds with combinational dormancy (PY + PD) have impermeable coats and some physiological inhibition of the embryo (Baskin and Baskin, 2004a). Dormancy in native wildflowers may last anywhere from several weeks to many months, with some species possessing more than one type of dormancy mechanism (Bradbeer, 1988; Baskin and Baskin 2004a). Dormancy has been found to occur more commonly in seeds of native plants than the seeds of cultivated plants (Paulson, 1989).

There are several treatments that can be applied to alleviate dormancy and promote germination. Scarification to the testa or fruit tissues can improve imbibition and overcome PY. For the Florida native wildflower summer farewell (*Dalea pinnata* [Fabaceae]), mechanical scarification was more successful in overcoming dormancy than acid scarification (Perez et al., 2009). Sulfuric acid scarification was successful however in increasing germination in some woody plant species of Texas (Vora, 1989). Using warm and/or cold stratification, soaking the seeds in gibberellic acid (GA), or allowing the seeds to after-ripen by storing in a cool and dry environment can alleviate PD. In two rare species of buckthorn (*Rhamnus* spp. [Rosaceae]), germination was increased when the seeds collected in the summer received cold stratification for at least one month (Sharma and Graves, 2005). For Leavenworth's tickseed (*Coreopsis leavenworthii* [Asteraceae]) physiological dormancy was broken by exposing the seed to specific temperature ranges, either cooler temperatures or cooler temperatures followed by warmer temperatures (Kabat et al., 2007). Elevated temperatures that alleviate dormancy in some species can be damaging in others, as seen in some species of Australian native forbs (Willis and Groves, 1991). In this same study, other species

showed an increase in germination when treated with GA, though it did not overcome any dormancy mechanisms (Willis and Groves, 1991). GA was helpful in overcoming the inhibitory effect of light on native Australian understory species (Bell et al., 1995). Using GA or stratification can also allow the embryos to further develop and overcome MD. It is suggested that MPD and PY + PD can be overcome by one or more of the previously recommended treatments (Baskin and Baskin, 2004a).

Propagation by Cuttings

Asexual, or vegetative, propagation may be used when growing plants from seed is impractical or not possible due to non viable or dormant seed (Ingram and Yeager, 1990). In some cases, plants are propagated asexually to determine the sex of the finished plant. In hollies, female plants are desired for their red berries which are aesthetically pleasing and attract wildlife. Producing plants using asexual propagation will result in a genetic clone of the parent plant. This method is commonly used in the horticulture industry to maintain the characteristics desired in a popular ornamental. Asexual propagation may also be used to widen production times that are usually restricted by seed availability (Guo et al., 2009). Finally, plants grown from cuttings often require less maintenance and time to produce a finished product (Phillips, 1985, Graham et al., 2006).

Plant development is regulated by six types of hormones: auxins, gibberellins, cytokinins, ethylene, abscisic acid, and brassinosteroids (Graham et al., 2006). Auxins are plant hormones that play a role in many plant activities, including root initiation and development. Auxins accumulate in the epidermal cells of root initials during the formation of lateral roots (Taiz and Zeiger, 2006), although the effects of auxin on root formation are not universal. The four major advantages of applying auxin to vegetatively

propagated tissues are an increased rooting percent, reduced time to root initiation, increased quality of roots, and greater uniformity of the cutting (Macdonald, 1986). Once the roots are formed, the auxin concentrations are highest in the root tip, promoting the production of cells at the meristem (Taiz and Zeiger, 2006). Auxins typically interact with other plant hormones; in the case of cytokinins, the ratio determines the initiation of root or shoot. Examples of naturally occurring auxins are indole-3-acetic acid (IAA) and 4-chloroindole-3-acetic acid (4-Cl-IAA) (Taiz and Zeiger, 2006). Indole-3-butyric acid (IBA), previously believed to be synthetic, was also found to occur naturally in plants as a conversion product of IAA. The synthetic auxin 1-Naphthaleneacetic acid (NAA), in conjunction with IBA, has been shown to be more effective in promoting rooting than IAA. Root initiation of the Florida native beach sunflower (*Helianthus debilis* ssp. *debilis* 'Flora Sun' [Asteraceae]) improved when cuttings were treated with 2000 ppm of IBA (Norcini and Aldrich, 2000). However, regardless of the concentration the use of auxin did not improve the survival or growth of the plant during container production (Norcini and Aldrich, 2000). Auxin application may also be deleterious in high concentrations, often causing damage to the tissue resulting in discoloration or death (Macdonald, 1986). Growth abnormalities such as leaf epinasty, stem curvature, growth inhibition, and intensified leaf pigmentation may also occur with increasing auxin concentrations (Grossmann, 2000).

It is also important to consider cutting material when choosing an auxin concentration. Stem cuttings can be taken at different stages of vegetative growth. Those taken from the current season's growth can be either softwood or semi-hardwood (Ingram and Yeager, 1990). Softwood cuttings are taken from succulent plant tissue that

is from the new spring and summer growth (Ingram and Yeager, 1990). Softwood cuttings are commonly used in vegetative propagation because the tissue has yet to become lignified and the shoots are in an active state of growth (Macdonald, 1986). In contrast, semi-hardwood cuttings are of matured growth from the current season's growth (Ingram and Yeager, 1990). Hardwood growth is matured growth that is from previous season's growth (Ingram and Yeager, 1990). In a study on the Florida native rusty blackhaw (*Viburnum rufidulum* [Adoxaceae]), cuttings from hardwood treated with 9000 ppm of IBA resulted in 100% rooting while cutting from softwood treated with 6000 ppm of IBA reached 87% rooting (Griffin, 2008), suggesting that hardwood cuttings often require higher concentrations of auxin as compared to softwood. Griffin (2008) also found that softwood cuttings that received 0 ppm IBA still rooted minimally, while hardwood cuttings exhibited no rooting.

It is recommended that cuttings of perennial plants should be taken in late spring as new growth is beginning to harden (Miles, 1976). They should also be collected before flowering. During floral initiation and anthesis a plant will reallocate resources towards production, development, and function of flowers instead of roots. O'Rourke (1940) observed that hardwood cuttings of blueberry (*Vaccinium atrococcum* [Ericaceae]) with flower buds rooted poorly when compared to cuttings containing only vegetative buds. Cutting success may also be dictated by collection time after flowering. Guo et al. (2009) found that cuttings taken from Peony (*Paeonia* 'Yang Fei Chu Yu' [Paeoniaceae]) at 70 days after flower had the highest rooting percentage when compared to 40 and 10 days after flower. Regardless of cutting type, age, or location, many studies stress the importance of collecting cuttings from healthy stock plants, as

this has been directly correlated to rooting success (Guo et al., 2009; Druege et al., 2004; Thetford et al., 2001)

After collection, cuttings are placed in moist media to facilitate growth of adventitious roots. Root initiation and survival of the cuttings can be augmented by the media. The media serves the dual function of keeping the cuttings moist while also maintaining good drainage. False rosemary (*Ceratiola ericoides* [Empetraceae]), a species native to the Florida scrub and sandhills, showed minimal effect of root initiation when treated with IBA and NAA (Thetford et al., 2001). However, when comparing different media, it was found that the cuttings were most successfully rooted in a media with good drainage (Thetford et al., 2001), a result not surprising for a species that naturally occurs in open sand. Thus media must be tailored to the moisture requirements of the species.

In order for the plant to properly photosynthesize it requires sunlight, oxygen, and water. Cuttings lack the root system required for the uptake of water from the soil, thus dessication of stem and leaf tissues must be avoided. Misting the cuttings helps manage the temperature of the cuttings and limit water loss by transpiration. However, cuttings that are kept too wet may be susceptible to fungal or bacterial pathogens (Miles, 1976). Ensuring proper water levels in the cuttings enables the plant to successfully undergo photosynthesis. Carbohydrates can then be utilized in the development of adventitious roots. Another factor considered important by some is the proportion of endogenous nitrogen. Druege et al. (2004), found a positive correlation between the level of internal nitrogen and the amount of total sugars produced via photosynthesis for root production in geraniums (*Geranium* spp. [Geraniaceae]). They

found that cuttings with low levels of nitrogen and carbon resulted in greater leaf senescence (Druege et al., 2004).

Experiment Preface

This thesis will investigate methods of propagation of two native wildflowers that would make attractive additions to the horticulture industry. Chapter 2 will focus on the propagation of *Polygonella polygama* and *Polygonella robusta* by seed. Propagating native plants by seed is often the most desirable method to ensure genetic diversity. However, dormancy mechanisms often prevent optimal germination. Based on preliminary research on related species and discussions with native plant growers, I hypothesize that seeds of both species will be dormant at shedding and after storage, thus justifying investigation of dormancy breaking treatments.

Vegetative propagation offers an alternative when seeds are not viable, or the seeds are resistant to germination treatments. In Chapter 3, propagation of *P. polygama* and *P. robusta* by stem cuttings will be discussed. I hypothesize that cuttings will show improved rooting when treated with a 2:1 combination of IBA and NAA. This is the recommended ratio for the optimal rooting of semi-hardwood cuttings.

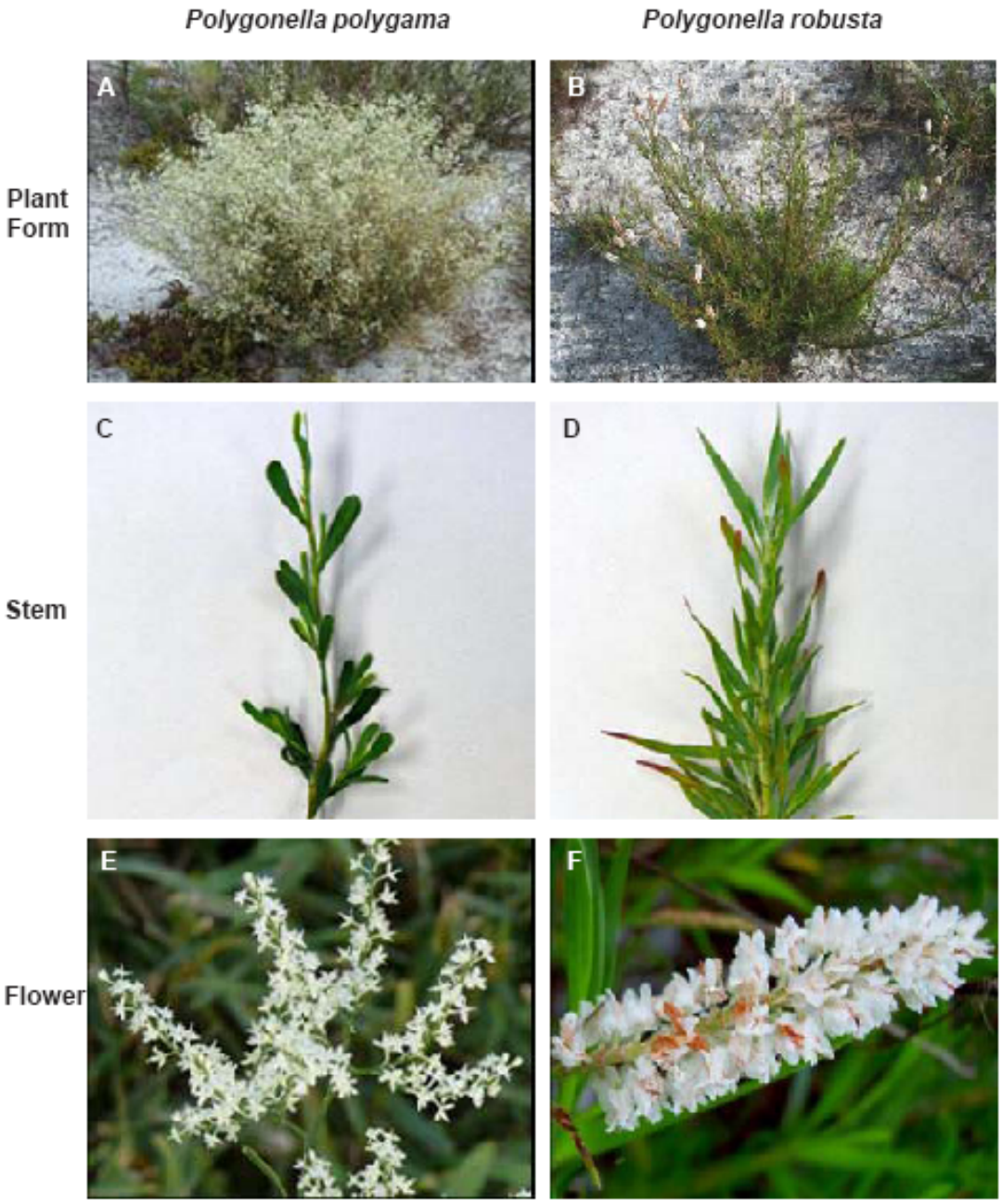

Figure 1-1. Differences in the morphological characteristics and distribution of *Polygonella polygama* (left column) and *Polygonella robusta* (right column). Plant form and habit of *P. polygama* is more upright (A) versus the low growing *P. robusta* (B). Leaves of *P. polygama* are spatulate in shape (C), but linear for *P. robusta* (D). The flowers of *P. polygama* are yellow to creamy white (E) and pink to white on *P. robusta* (F). Plant distribution, noted by green color, is statewide for *P. polygama* and prevalent along the coast (G), where *P. robusta* has been found in fewer counties, but also frequently along the coast (H). Photo credits: A. Heather, K. Muller, K. Ruder, S. Wilson, and S. Woodmansee. Maps credits: www.florida.plantatlas.usf.edu.

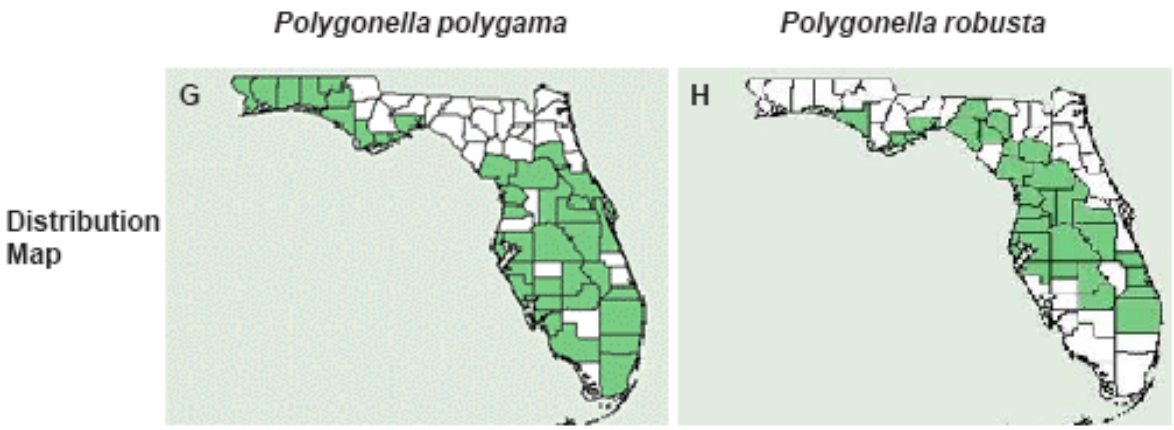

Figure 1-1. Continued.

CHAPTER 2
GERMINATION AND DORMANCY STUDIES OF *POLYGONELLA POLYGAMA* AND *POLYGONELLA ROBUSTA*

Introduction

Seed dormancy, a phenomenon commonly associated with native plants, delays germination of seeds when conditions are not favorable for seedling establishment (Baskin and Bakin, 2004a; Paulson, 1989; Baskin and Baskin, 2001; Vleeshouwsers et al., 1995; Bewley and Black, 1994). While this benefits the seed and species by preventing wasted resources, it presents challenges to propagation efforts. Seed dormancy is classified into five categories (Baskin and Baskin, 2004b). Physically dormant (PY) seeds have water impermeable seed or fruit coats. Seeds with embryos that are undifferentiated or underdeveloped at shedding are considered morphologically dormant (MD). Seeds with physiological dormancy (PD) have covering structures that prevent the embryo from emerging. PD can also be attributed to low growth potential of the embryo, reduced oxygen permeability of the covering structures, changes in resistance to penetration of covering structures, or endogenous levels of plant hormones such as abscisic acid (ABA) and gibberellins (GA) (Bradbeer, 1988; Baskin and Baskin, 2004a; Baskin and Baskin, 2001). Morpho-physiological dormancy (MPD) is a combination of PD and MD, where the embryo is both underdeveloped and the covering structures provide resistance to further embryo development. MPD is commonly found in wildflowers of temperate deciduous forests (Baskin and Baskin, 2004b). Combinational dormancy (PY + PD) refers to seeds that have impermeable coats and some physiological inhibition of the embryo (Baskin and Baskin, 2004b).

With the exception of species within *Coreopsis*, *Rudbeckia*, *Gaillardia*, and *Dalea* (Perez et al., 2009; Rukuni, 2008; Kabat et al., 2007; Norcini and Aldrich, 2007;

Danielson, 2005) dormancy and germination characteristics are largely unknown for Florida native wildflowers. Dormancy in wildflowers can last from a few weeks to many months; and some species possess more than one type of dormancy (Bradbeer, 1988; Baskin and Baskin, 2004a). The presence of dormancy can be investigated by incubating freshly shed, mature seeds at various simulated seasonal temperatures for 4 weeks, calculating the rate of water uptake, looking at seed and/or fruit coat anatomy and measuring embryo growth (Baskin and Baskin, 2004a).

In addition to dormancy, improper seed storage can hinder native wildflower production. When seeds are kept in dry storage for long periods of time, viability may be reduced and the ability to germinate lost (Baskin and Baskin, 2001). Factors that are most important to successful seed storage are temperature, relative humidity, and storage time. Seeds can be dried naturally, using sunlight and wind, without significant loss of quality in the commercial production of some species (Black et al., 2006). However, prolonged hot and humid weather can accelerate seed deterioration (Priestly, 1986). These types of conditions are prevalent in Florida and present a major challenge for seed storage within the state. If proper storage conditions are unknown, but wildflower germplasm must be stored, then the recommendation is that the sum of the storage temperature (^{o}F) and relative humidity should be less than 100 (Norcini and Aldrich, 2007; Harrington, 1972). It should be noted that the duration of viability under these conditions is unknown for many wildflower species and after-ripening may occur under certain storage conditions.

After-ripening, which can occur in dry seeds of some species, is defined as the progressive loss of dormancy in dry mature seed (Black et al., 2006). This effect may

terminate dormancy when oxygen concentration and temperature increase and moisture levels decrease (Bewley and Black, 1994). This method is often used as a treatment to overcome physiological dormancy; however in some species with physical dormancy, it can also be beneficial. Baskin and Baskin (2001) explain that as seeds after-ripen, the seed coat can dry and crack, thus becoming more permeable with time, and allowing water to enter and seeds to germinate more easily (Baskin and Baskin, 2001). Alternatively, seeds with higher water contents may lose viability or enter a secondary dormancy (Bewley and Black, 1994). In a study on several Florida native tickseed (*Coreopsis* spp. [Asteraceae]), after-ripening effects were seen on all species, even with freshly harvested, less dormant seed populations (Norcini and Aldrich, 2007). Lanceleaf tickseed (*Coreopsis lanceolata* [Asteraceae]) dormancy was alleviated most effectively when the seeds were kept at 23% relative humidity and 17-19 ^{o}C (63-66 ^{o}F) (Norcini and Aldrich, 2007). In contrast, maximum after-ripening of wild oat (*Avena fatua* [Poaceae]) seeds was observed at around 40 ^{o}C (104 ^{o}F), and 10-12% relative humidity. Tang et al. (2009) found that dwarf rocket (*Olimarabidopsis pumila* [Brassicaceae]) seeds dried to 5.6% moisture content had the highest germination when incubated for 8 weeks at 30 ^{o}C (80 ^{o}F), and an additional 8 weeks at 4 ^{o}C.

October flower (*Polygonella polygama* (Vent.) Engelm. & A. Gray [Polygonaceae]) is a perennial wildflower with widespread distribution across Florida. It is commonly found in sandy scrub where it receives full to partial sun. The inflorescence consists of prolific, miniature, cream to yellow flowers. These flowers usually appear in mid to late fall, with seeds maturing and dropping in winter. In contrast, sandhill wireweed (*Polygonella robusta* (Small) G.L. Nesom & V.M. Bates [Polygonaceae]) is more

commonly found in open sand, full sun environments on the coasts of Florida. This mounding perennial is also very floriferous, but with terminal pink to cream colored miniature flowers covering each raceme. Flowers appear sporadically throughout the year with fruit appearing soon after (PLANTS Database, 2008; Wunderlin and Hansen, 2003; Osorio, 2001; Taylor, 1998). These features make both native *Polygonella* species attractive candidates for the horticulture industry. Grower interest may be reduced however, if germination is delayed due to seed dormancy. Developing an efficient, cost effective propagation plan for the seed production of *Polygonella* species may improve availability and increase use in the landscape. The objective of this study was to determine the extent of germination in *P. polygama* and *P. robusta* seeds. The following questions are addressed: 1) Are seeds dormant after harvest and subsequent storage; 2) What type(s) of dormancy may be present; and 3) How can dormancy be alleviated and germination promoted?

Materials and Methods

Seed Collection and Storage

Achenes, henceforth referred to as seeds, of *Polygonella polygama* were collected from a native stand in Brooksville, Florida (28° 51' 25"N, 82° 25' 49"W) on February 15, 2008. This source is referred to as *P. polygama* (C), indicating the central collection site. Seeds were spread out in a single layer and exposed to ambient laboratory conditions (~ 24 °C (75 ^{o}F), RH unknown) for 45 days before being cleaned of floral remains. They were then stored in a zip top plastic bag and placed again on the laboratory bench top. Initial germination and viability experiments commenced approximately 2 months later. An additional source of *P. polygama* seeds were collected from Pensacola, Florida (30° 18' 48"N, 81° 24' 39"W) on November 12, 2008. This source is referred to as *P.*

polygama (N), indicating the northern collection site. Seeds were spread out in a single layer and exposed to laboratory conditions as stated previously. Germination and viability experiments began approximately 5 months later. Seeds of *Polygonella robusta* were collected on April 10, 2008 in Hobe Sound, Florida (27^{o} 01' 11"N 80^{o} 06' 38"W). After collection, the seeds were cleaned, dried, and stored in plastic zip top bag in ambient laboratory conditions described above, for almost 4 months before undergoing testing.

Pre-Germination Viability Assay

Seed viability was examined using a triphenyltetrazolium chloride (TZ) staining test before germination experiments commenced. Procedures were adapted from the Tetrazolium Testing Handbook (Peters, 2000). Four replications of twenty-five seeds (n=100) each were nicked with a scalpel on the proximal end of the seed. Each replicate of nicked seeds was then placed into its own beaker containing 5 mL of 1% TZ solution (pH = 7). After 48 h of dark incubation at 35 ^{o}C (95 ^{o}F), the seeds were removed and triple rinsed with 15 mL of deionized water. When data could not be immediately collected, the seeds were placed in 5 mL of deionized water and kept in the refrigerator at 5 ^{o}C for up to two weeks. Staining patterns were viewed by bisecting the seed longitudinally and examining the endosperm and embryo under a dissecting microscope at 15× magnification. If embryos were lacking, unstained, or black, they were considered non-viable. If the entire embryo was stained a light pink, dark pink, or red, they were considered viable.

Initial Germination Tests

Four replications per treatment of 25-seeds (n = 800) of *P. polygama* (C) and *P. robusta* were surface sterilized in a 20% bleach solution for 10 min, followed by a triple

rinse in deionized water. Seeds were placed in 32 Petri dishes (10.0 cm) on top of two sheets of blue blotter paper (8.9 cm diameter Steel Blue Seed Germination Blotter, Anchor Paper Company, Minnesota, U.S.A.) moistened with approximately 15 mL of deionized distilled water. Dishes were sealed with laboratory film. The seeds were then exposed to one of four simulated seasonal temperatures. The 12 h alternating temperatures used were 22/11 °C (72/52 °F), 27/15 °C (81/59 °F), 29/19 °C (84/66 °F), and 33/24 ^{o}C (93/75 °F). These temperatures represent the seasons of Florida during winter, early spring or late fall, early fall or late spring, and summer, respectively. For each treatment, four replicates were exposed to a 12 h daily photoperiod (80 μmol m^{-2} s^{-1}, cool white fluorescent light), with the warmer temperature occurring during the lighted period. The remaining four replicates were incubated in the dark by covering Petri dishes with two layers of aluminum foil. Treatments for initial germination were arranged in a 2 (illumination) × 4 (temperature) factorial. A randomized complete block design was used for this study. The experiment was carried out for 4 wk with germination counts taken once per week for the light treatment and at 4 wk for dark incubated seeds. After 4 wk, any remaining non-germinated seeds were assayed for viability as described previously with seeds that molded during the germination experiment counting as non viable seeds.

Water Uptake by Intact and Scarified Seeds

The extent to which imbibition occurred was determined by measuring the increase in fresh mass on scarified and non-scarified seeds. Seeds of *P. polygama* (C) and *P. robusta* were scarified by nicking the pericarp with a scalpel on the proximal end. The dry mass of four replications of 25-seed (n=100) was measured gravimetrically. After initial mass determinations (T_0), seeds were placed into dishes containing two

sheets of moistened blotter paper. After 0.25 h seeds were removed from the dishes, lightly blotted with a paper towel, and weighed to determine the fresh mass. Seeds were returned to their respective dishes, with the process repeating at 0.5, 0.75, 1-12, 24, and 48 h. Fresh mass increase was calculated using the formula $[(W_i – W_n)/ W_n] \times 100$, where W_i and W_n are the masses of imbibed and non-imbibed tissues (at T_0), respectively. Seeds with obvious signs of fungal contamination or radicle emergence were noted and removed and mass determination was adjusted accordingly. This experiment utilized a completely randomized design.

Seed Anatomy and Morphology

Seeds of *P. polygama* (C) and *P. robusta* were excised from their exocarps under a dissecting microscope using a scalpel, then nicked on the proximal and distal ends. Seeds that buckled when pressure was applied, thus identified as unfilled, and those with obvious signs of insect damage were excluded. Separated fruit coats (n = 10) and seeds (n = 10) were incubated for 24 h at 20 ^{o}C in plastic vials containing 1 mL of Trump's fixative. Following fixation, tissues were transferred to a buffer solution (0.1M cacodylate), microwaved at 180 watts for 45 sec with a vacuum at a pressure of 80 kPa, then allowed to sit for 15 min. After the buffer was removed, tissues were post-fixed in 20% osmium tetroxide for 24 h. Tissues were rinsed twice in buffer, then three times in deionized water. Samples were microwaved with a vacuum as before, then allowed to cool for 10-15 min between each rinse. The seeds underwent dehydration using a graded EtOH series where the samples were held in each grade for 10-15 min, followed by two final steps in 100% acetone. Tissues were infiltrated with a graded Spurr's resin acetone series. Samples were heated in a microwave set to 250 watts with a vacuum at 80 kPA for 3 min, with at least one hour between each step. After the final infiltration

step, seeds were removed and placed in molds with fresh 100% Spurr's resin. The resin was allowed to polymerize in an oven set to 40 °C for 48 h (Bozzola and Russell, 1999). Thick sections (ca. 680 nm) were made using a rotary microtome. Sections were placed on microscope slides and stained with Toluidine Blue. The slides were then examined under a light microscope at 40×. Images were captured using a digital camera (Olympus BH2-RFCA with a QImaging camera model Retiga 2000R FAST). Images were cropped and adjusted for color using QCapture Pro software (QImaging Corporation, Surrey, British Columbia, Canada).

Images of seed longitudinal sections, obtained from light microscopy studies, were used to calculate embryo : seed ratios. Images were uploaded into Adobe Photoshop (Adobe Systems Inc., San Jose, California, USA) and the lengths of (n = 10) embryos and associated seeds were taken using the measurement feature. Images of the fruit coat were examined to determine thickness based on the number of cells.

Requirements for Warm or Cold Stratification

The 'move-along' experiment (Baskin and Baskin, 2003) was used to determine the extent to which seeds required warm or cold stratification. Four replications of 25-seeds of *P. polygama* (C) and *P. robusta* were used in six temperature treatments (n=600). Seeds were surface sterilized and placed on blue blotter paper in Petri dishes. Treatments were adapted from Baskin and Baskin (2003) to mimic seasonal changes in Florida. The treatments consisted of four control chambers set to 22/11, 27/15, 29/19, or 33/24 °C; and two move-along treatments that began with the summer temperature (33/24 °C) or the winter temperature (22/11 °C) (Table 2-1). Germination data was collected once per week for one year.

Effect of Cold Stratification on Seeds Propagated in the Greenhouse

Six replications of 1 seed (n=48) of *P. polygama* (N) were placed in plastic zip-top bags containing moistened vermiculite. The bags were then placed in a dark refrigerator at 5 ^{o}C (35 ^{o}F) for 0, 2, 4 or 8 wk. Seeds were then removed from the bags and half were surface sterilized using a 20% bleach solution for 10 min. Sterilized and non sterilized seeds were sown in trays containing Fafard 2P. The trays were then placed in a misthouse where they received mist for 8 sec every 10 min for the first week, and then 5 sec every 20 min for subsequent weeks. Treatments for cold stratification were arranged in a 2 (sterilization) × 4 (stratification duration) factorial. A randomized complete block design was used for this study. Emergence data was collected once a week for six weeks.

Effects of Gibberellic Acid on Seeds Propagated in the Laboratory and Greenhouse

Four 25-seed replicates (n=500) of *P. polygama* (C) and *P. robusta* were placed on blue blotter papers moistened with gibberellic acid (GA_3) solutions of 0, 1, 10, 100, or 1000 ppm for 24 h. After imbibition, seeds were surface sterilized with 20% bleach solution for 10 min. All seeds were then incubated at 22/11 ^{o}C (72/52 °F) with a 12 h photoperiod. Germination data was collected once a week for four weeks.

The effect of GA_3 on germination was tested again in the greenhouse on seeds of *P. polygama* (N). Six replications of 1 seed (n=48) were placed on blotters moistened with 0, 10, 100, or 1000ppm of GA_3. After 24 h, half of the seeds were sterilized with 20% bleach solution for 10 min while the remaining half were not sterilized additionally, then all sown in trays containing Fafard 2P. The trays were then placed in a misthouse where they received mist for 8 sec every 10 min for the first week, and then 5 sec every

20 min for subsequent weeks. Treatments were arranged in a 2 (sterilization) × 4 (GA_3 concentration) factorial. A randomized complete block design was used for this study. Emergence data was collected once a week for six weeks.

Initial Seedling Emergence and the Effect of Different Propagation Media

Additional germination tests were carried out in the greenhouse to test seedling emergence under simulated production conditions. To test for emergence, seeds of *P. polygama* (N) were separated into sterilized and non sterilized treatments. Each treatment consisted of six replications of 6-seeds (n=72). Those that were sterilized were placed in a 20% bleach solution for 10 min. After sterilization, both treatments were sown into 72- cell trays filled with a peat based germination media (Fafard 2P, Conrad Fafard Inc., Massachusetts, U.S.A.). The trays were then placed in a misthouse where they received mist for 8 sec every 10 min for the first week, and then 5 sec every 20 min for subsequent weeks. A randomized complete block design was used for this study. Emergence data was collected weekly for six weeks.

Seeds (n=72) of *P. polygama* (N) were subjected to an additional experiment to assess the effects of media on germination. Thirty-six seeds were surface sterilized with 20% bleach solution for 10 min while the remaining half received no bleach (six replications of 6-seeds). Seeds were then sown on top of moistened media and lightly covered with a 0.5 cm (0.2 in) layer of the same media. The medias used consisted of a peat based soilless mix (Fafard 2P, peat:perlite 3:2), fine quartz sand approximately 1mm in diameter (QUIKRETE Play Sand, Atlanta, Georgia, U.S.A), and a “native mix”. The native mix consisted of approximately 40% fine pine bark, 25% Fafard 2P, 25% sand, and 10% vermiculite. Fafard 2P was chosen because the composition is common to production media. Pure sand was used because both species are known to occur in

areas with soil comprised largely of sand. Finally, the native mix was used because it simulates the composition of media used by Florida native nurseries. The trays were then placed in a misthouse where they received mist for 8 sec every 10 min for the first week, and then 5 sec every 20 min for subsequent weeks. Treatments were arranged in a 2 (sterilization) × 3 (media type) factorial. A randomized complete block design was used for this study. Emergence data was collected weekly for six weeks. Data was analyzed using class comparisons for media treatments.

Data Analysis

For experiments carried out in the lab incubators, seeds were recorded as germinated when radicle emergence was at least 2 mm in length. For these experiments the final percent germination was adjusted by removing contaminated seeds from the calculation. However, seeds with fungal contamination were considered non viable in the post germination viability test. To determine the emergence percent for the seeds in the greenhouse, data was collected when the hypocotyl was visible above the soil line, usually more than 2 mm. Germination rate was estimated for all experiments by determining the mean germination time (MGT). Data were transformed using the arcsine of the square root when the range in percent germination was greater than 40 (Little and Hills, 1978). Non transformed data are presented. Analysis of variance was performed using the PROC GLM procedure in SAS v. 9.1 (SAS Institute, Cary, North Carolina).

Results

Pre-Germination Viability Assay and Initial Germination Tests

TZ staining indicated the initial viability of all sources to be greater than 50% (Table 2-2). All viable seeds of *P. polygama* (C) and *P. robusta* germinated to 100%

when incubated at 22/11°C. Moreover, germination ranged between 73 and 88% for viable seeds of either species incubated at 29/19°C (Table 2-3). However, the lowest germination (> 6%) occurred at 27/15 ^{o}C or 33/24 ^{o}C for viable *P. polygama* seeds. Germination was somewhat higher (~ 30-63%) for *P. robusta* seeds incubated at these temperatures. No germination was observed at any temperature combination for seeds of either species incubated in the dark (data not presented). However, according to the post-germination TZ test for seeds in complete darkness, less than 5% of *P. polygama* and 12% of *P. robusta* remained viable (Table 2-4).

Germination rate is presented in Table 2-3 as mean germination time (MGT). Mean germination time for seeds of *P. polygama* at 27/15 and 29/19 ^{o}C was almost twice as rapid compared to MGT at 22/11 ^{o}C. However, the MGTs were not significantly different ($F_{2,6} = 1.77$; $p = 0.25$). In seeds of *P. robusta*, MGT was not significantly different between treatments ($F_{2,6} = 1.11$; $p = 0.39$).

Water Uptake by Intact and Scarified Seeds

Fresh mass increased for both species and scarification treatments, indicating uptake of water (Figure 2-2). Initially, *P. polygama* (C) seeds that were scarified showed the greatest increase in fresh mass; however, by the end of the 48 h experiment the scarified seeds showed a 61% increase from their dry mass as compared to 74% increase in the seeds that were non-scarified. This difference between scarification treatments was significantly different ($F_{1,3} = 12.9$; $p = 0.04$). *P. robusta* showed a similar pattern, where scarified seeds initially had a greater increase. In contrast to *P. polygama*, at the end of the 48 h experimental period, the difference between scarified and non-scarified seeds of *P. robusta* was not significant ($F_{1,3} = 2.59$; $p = 0.21$).

Seed Anatomy and Morphology

The average length of *P. polygama* (C) embryos was 1.6 ± 0.1 mm, with seeds averaging 1.7 ± 0.1 mm. Embryos of *P. robusta* averaged 1.5 ± 0.2 mm, with seeds averaging 1.6 ± 0.2 mm (Figure 2-3A). Therefore, embryo : seed ratios were 0.95 ± 0.01 and 0.94 ± 0.01 for *P. polygama* (C) and *P. robusta*, respectively. Moreover, fully developed embryos (i.e. cotyledons, embryonic axis, and radicle) were clearly distinguishable in longitudinal sections (Figure 2-3A).

Neither species' seed nor fruit coats contained cells with lignified walls, nor palisade layers of macrosclerids or osteosclerids (Figure 2-3B and 2-3C). Instead, the cell layers in the seed coats were observed to be one to two cells thick. Additional images of only the fruit coat show layers two to four cells thick (data not shown).

Requirements for Warm or Cold Stratification

After one year, seeds of *P. polygama* (C) incubated in control chambers showed reduced germination as compared to the move-along treatments (Figure 2-4). Seeds in the move-along chambers had a final germination of about 79% and 67% when started in summer and winter temperatures, respectively. For both move-along treatments, a dramatic increase in the slope of the germination was visible when the incubator was changed from warmer to cooler temperatures (illustrated in Figure 2-4 by black arrows). The lowest germination percentages occurred for seeds incubated at 29/19 and 33/24 ^{o}C, where germination reached 15% and 0%, respectively. *Post-hoc* mean separation showed that final germination percent at different simulated seasonal temperatures were significantly different ($F_{5,18} = 35.02$; $p < 0.01$). Mean germination time was shortest for seeds in the 22/11 ^{o}C treatment (115 days) and longest for 27/15 ^{o}C (236 days). MGT for the move-along treatments fell in the middle at 180 days for summer and 213

days for winter. *Post-hoc* mean separation showed that all treatments were significantly different ($F_{5,18} = 7.02$; $p < 0.01$).

Seeds of *P. robusta* in the move-along chambers had a final germination of 67% when begun in summer temperatures and 73% when begun in winter temperatures (Figure 2-4). Again both move-along treatments had an increase in germination when seeds were moved from warmer to cooler temperatures. However, the slope of the line for *P. robusta* was not as steep as was seen for *P. polygama.* In the control chambers, seeds performed the poorest when kept in 33/24 ^{o}C, reaching a final germination of less than 20%. A *post-hoc* mean separation showed that final germination percentage at simulated seasonal temperatures were significantly different ($F_{5,18} = 4.90$; $p = 0.005$). Germination rates, as determined by MGT, were much different from *P. polygama.* All seeds that germinated in 22/11 and 33/24 ^{o}C did so in less than 20 days, whereas it took nearly 90 days for both move-along treatments. A *post-hoc* mean separation showed that mean germination times were significantly different from each other ($F_{5,18} = 8.34$; $p < 0.001$).

Effect of Cold Stratification on Seeds Propagated in the Greenhouse

Figure 2-5 displays the range of percent emergence for seeds of *P. polygama* (N) treated with 0, 2, 4, or 8 wk of cold (5°C) stratification. For non sterilized and sterilized treatments, emergence was greatest when seeds were stratified for 2 wk (39 and 31%, non-sterilized and sterilized, respectively). No emergence was observed for seeds treated in 8 wk of cold stratification, regardless of sterilization. Class comparisons of the main effects of weeks in cold stratification were significant ($F_{3,40} = 20.1$; $p < 0.01$), but sterilization was not ($F_{1,40} = 0.0$; $p = 0.89$). The difference between 2 wk vs. 0 wk was significant ($F_{7,40} = 7.08$; $p = 0.01$), whereas 4 wk vs. 0 wk was not significant ($F_{7,40} =$

0.23; $p = 0.63$). The interaction of stratification × sterilization was not significant ($F_{3,40} = 2.5$; $p = 0.07$). Trends observed for emergence percent found a linear decreasing trend when seeds were not sterilized ($t_{2,19} = -3.6$, $p < 0.01$) and a convex quadratic form when sterilized ($t_{2,19} = -2.58$, $p = 0.02$). Emergence rate, determined by MGT, was shortest for the control treatment (less than 10 days for both sterilization treatments) (Figure 2-5). A convex quadratic trend was observed in MGT for the sterilized treatment ($t_{1,23} = -2.58$, $p = 0.02$) while a negative linear trend was seen in the unsterilized treatment ($t_{1,23} = -3.60$, $p < 0.01$).

Effects of Gibberellic Acid on Seeds Propagated in the Laboratory and Greenhouse

P. polygama (C) seeds treated with 1000 ppm of GA_3 reached a final germination of 33%, which was nearly double that of the control (16%) (Figure 2-6). When regressed, the trend in germination was a linear increase ($t_{1,19} = 4.79$, $p < 0.01$). There was no trend observed in MGT.

GA_3 concentrations of 100 and 1000 ppm had a positive effect on the germination percent of seeds of *P. robusta* (Figure 2-7). The trend seen in this data was a linear increase ($t_{1,19} = 2.48$, $p = 0.02$). There was no trend when observing MGT.

For seeds of *P. polygama* (N) sown in the greenhouse (Figure 2-7) no trends were observed in GA_3 concentration for sterilized. The highest germination percent (58%) occurred in seeds that were not sterilized and were treated with 10 ppm. The lowest percent germination (33%) occurred when seeds were not sterilized and treated with 100 ppm. The main effects of sterilization ($F_{1,40} = 0.5$; $p = 0.48$) and GA_3 treatments were not significantly different ($F_{2,40} = 0.2$; $p = 0.92$). The interaction of sterilization × treatment was also not significant ($F_{3,40} = 1.69$; $p = 0.18$). Mean germination time also

had no observed trend due to GA_3 concentration or sterilization treatment. Germination took 25 days when treated with 10 ppm of GA_3 and no sterilization. The shortest period occurred when seeds were treated with 1000 ppm of GA_3 and sterilized (14 days). Again, the main effects of sterilization and GA_3 treatments were not significantly different ($F_{1,40} = 0.0$; $p = 0.85$ and $F_{2,40} = 0.2$; $p = 0.89$, respectively). The interaction of sterilization x treatment was also not significant ($F_{3,40} = 1.78$; $p = 0.17$).

Initial Seedling Emergence and the Effect of Different Propagation Media

In the greenhouse, unsterilized seeds of *P. polygama* (N) emerged less (17%) then sterilized seeds (28%). However, a post-hoc mean separation showed that the sterilization treatments were not significantly different ($F_{1,11} = 1.74$; $p = 0.22$). When comparing MGT, again the treatments were not significantly different ($F_{1,10} = 0.54$; $p = 0.48$).

Sterilized seeds of *P. polygama* (N) responded with the greatest percent emergence (~81%) when sown on sand (Figure 2-1). As seen in Table 2-5, the sterilization treatments did not significantly affect emergence percent, whereas media did ($F_{2,30} = 33.8$; $p < 0.01$). Class comparisons of the main effects of treatment show that the difference between native mix vs. Fafard 2P was not significant ($F_{1,29} = 0.04$; $p = 1.00$), whereas sand vs. Fafard 2P was significant ($F_{1,29} = 29.68$; $p < 0.01$) (Table 2-5). The mean germination time was fastest for seeds sown on sand (16 days when not sterilized). Again, the MGTs of native mix vs. Fafard 2P was not significant ($F_{1,29} = 0.00$; $p = 0.96$) whereas significant differences in MGT occurred for sand vs. Fafard 2P ($F_{1,29} = 6.29$; $p = 0.02$).

Discussion

Physical dormancy does not occur in seeds of *P. polygama* nor *P. robusta* because both intact and scarified seeds imbibed water at similar rates and to similar final fresh mass percetages. Seeds with PY do not regularly imbibe water (Perez et al., 2009; Jayasuriya et al., 2007; Baskin et al., 2006b, Turner et al., 2005, Baskin and Baskin, 2001). Although the final increase in fresh mass was statistically different for scarified vs. non scarified seeds of *P. polygama*, the difference is not large enough to be considered biologically significant as both seeds regularly imbibed water. On the contrary, scarification of six physically dormant genera of Rhamanceae showed that seeds treated first in hot water had a steady increase or an initial dramatic increase in fresh mass whereas non treated increased only minimally (Turner et al., 2005). Regular increases in fresh mass were also observed for seeds of *P. robusta*. These patterns of fresh mass increase for both scarification treatments and species are similar to what was observed in the non-physically dormant seeds of woollyjoint prickly pear (*Opuntia tomentosa* [Cactaceae]) when placed on a wet substrate (Orozco-Segovia et al., 2007). Additionally, both *Polygonella* species lacked the anatomy typically observed in species reported to possess PY. Light microscopy images of the physically dormant whitestar (*Ipomoea lacunosa* [Convolvulaceae]) show the presence of long sclereid cells perpendicular to the seed coat (Jayasuriya et al., 2007). Moreover, two species of *Stylobasium* spp. (Surianaceae) displayed thickened outer layer of palisade cells (Baskin et al., 2006b).

Likewise, morphological dormancy is not present for either *Polygonella* species. For example, the embryo : seed ratio was close to 1 indicating fully mature embryos that did not require additional time to develop. The embryo : seed ratio for the

morphologically dormant bishop's weed (*Aegopodium podagraria* [Apiaceae]) was 0.14 (Vandelook et al., 2009). The images of the *Polygonella* species were similar to morphologically dormant seeds of Haskap (*Lonicera caeulea* var. *emphyllocalyx* [Caprifoliaceae]) that were allowed to mature after 3 weeks in incubation to promote embryo growh (Phartyal, 2009).

Despite the lack of PY and MD, dormancy has long been known to occur in some members of the Polygonaceae (Ransom, 1935). Viability and initial germination studies indicate some form of dormancy is present in seeds of both *P. polygama* and *P. robusta* after harvest and subsequent storage. For example, over 20% of *P. polygama* collected in north Florida remained unstained during initial viability testing (Table 2-2), but these embryos appeared intact, suggesting that a portion of the seed population was dormant (Baskin et al., 2006a; Norcini et al., 2006; Peters, 2000). Furthermore, all temperature treatments except for 22/11 ^{o}C, had seeds that remained ungerminated that stained pink or red in the post-germination TZ test. These stained seeds were considered dormant and reached 33% when *P. polygama* was kept in 33/24 ^{o}C (Table 2-3). The occurrence of dormancy is also supported by the initially low germination occurring at each initial incubation temperature (Table 2-3). An initial germination test on both species found that emergence percent reached nearly 86% for *P. polygama* and 68% for *P. robusta* when held in a 20/10 ^{o}C incubator for 16 wk (S. Wilson, personal communication). Though seeds did not germinate in when subjected to the dark treatment, it is not believed that dormancy prevented the germination. Interestingly, both species, regardless of temperature, had low viability when subjected to a post TZ test (Table 2-4). This low viability may indicate that seeds will not survive in their natural

seed bank. Since scrub and sandhill ecosystems are known to have a low germinable seed bank, future germination studies using buried seed should be carried out on these species.

Seeds of both species were exposed to various simulated seasonal temperatures in a move-along study. Sudden increases in germination were observed when temperatures were changed from warmer to cooler (33/24 to 29/19 to 27/15 ^{o}C). Final germination of the seeds kept in the cooler incubators (27/15 and 22/11 ^{o}C) was not as high as the move-along treatments. The increase in germination as temperature decreases, indicates that seeds require a period of warm temperatures followed by cool for maximum germination. Since final germination percent was not as high for the winter control as it was for both move-along treatments, this indicates that the succession of fall to winter conditions maximizes germination. This trend was seen in Hawaiian seeds of Hamakua clermontia (*Clermontia pyrularia* [Campanulaceae]) where 12 wk at 25/15 ^{o}C followed by 20/10 ^{o}C resulted in 90% germination (Baskin et al., 2005). Dormant seeds of Virginia blue bells (*Mertensia virginiana* [Boraginaceae]) also responded with greater germination in cooler temperatures, reaching 91% when incubated at 5 ^{o}C as compared to 0% in 25/15 ^{o}C (Baskin and Baskin, 2003).

Seeds receiving 2 weeks of cold stratification and sown in the greenhouse reached a final germination greater than the control. The improvement of germination using cold stratification was also seen on initially dormant wild buckwheat (*Eriogonum* [Polygonaceae]) seeds (Meyer and Paulsen, 2000). The same pattern was observed in seeds of wild buckwheat (*Polygonum convolvulus* [Polygonaceae]), where stratification at low temperatures of 2 or 10 ^{o}C was believed to increase embryo growth potential

thus overcoming dormancy and promoting germination (Metzger, 1992). The common name of *P. polygama* is October flower, referencing its flower time. Seeds of *P. polygama* are usually shed in fall when temperatures are still relatively warm in Florida, meaning that seeds are initially exposed to relatively warm temperatures then low temperatures as the seasons progress supporting the germination patterns observed during the move-along experiment. Though *P. robusta* flowers more sporadically throughout the year, a common time for the seeds to reach the shedding stage is late summer/early fall (A. Heather, personal observation).

Gibberellic acid is used to improve germination in seeds with physiological dormancy. GA_3 is thought to increase the activity of cell wall degrading enzymes allowing the radicle to emerge, induce the synthesis of other enzymes that transport nutrients necessary for embryonic growth, and act as an antagonist to ABA, which has been shown to play a significant role in the maintenance of physiological dormancy (Baskin and Baskin, 2001; Nicolas et al., 1996). Germination of *Polygonella* seeds also improved following the application of GA_3, suggesting that physiological dormancy was alleviated and germination enhanced with this treatment. For example, final germination was almost two times greater in seeds of both species when treated with 1000 ppm of GA_3 compared to controls (Figure 2-6 and 2-7). This finding was similar to what was seen in beech (*Fagus sylvatica* [Fagaceae]) seeds where dormancy was overcome by a long period in cold temperatures or the application of 100 *μM* GA_3 (Nicolas et al., 1996). Once their physical dormancy was broken, germination in some physiologically dormant seeds of sumac species (*Rhus* spp. [Anacardiaceae]) treated with 500 or 1000 mg/L GA_3 was more than 5 times greater than the control (Li et al., 1999). Unlike what was

observed in the controlled setting of the incubators, percent germination was not positively correlated with GA_3 concentration when applied on seeds grown in the greenhouse. This inconsistency may be explained by the very warm temperatures that occurred in the greenhouse (daytime highs were often > 35 ^{o}C) versus the cooler temperatures used in the incubators (set to reach a maximum of 22 ^{o}C).

Using the classification system suggested by Baskin and Baskin (2004b) and the results discussed above, it is suggested that seeds of *P. polygama* and *P. robusta* exhibit non-deep physiological dormancy after harvest and subsequent storage. Characteristics of non-deep PD include promotion of germination when GA is applied, dormancy break occurring with the use of warm or cool stratification, and after-ripening occurring during storage (discussed below). Seeds exhibiting non-deep PD may require as little as 5 days at cold stratification to promote germination (Baskin and Baskin, 2001). Seeds of *P. polygama* and *P. robusta* responded to 2 weeks of 5 ^{o}C when sown in the greenhouse. Sumac species (*Rhus* spp. [Anacardiaceae]) exhibiting non-deep PD showed improved germination when cold stratified 7, 15, or 25 days (Li et al., 1999). Baskin and Baskin (2004b) go on to delineate between five types of non-deep PD. It is concluded that *Polygonella* species in this study fall under type 2 non-deep physiological dormancy. As seeds move from dormancy to non-dormancy, species that display type 2 will germinate as temperatures move from high to low.

The obvious issue that persists for this study is the factor of storage. Seeds were collected and then kept in the lab (approximately 25 ^{o}C or 77 ^{o}F) for 2-5 months prior to experimentation. Though other members of Polygonaceae have had substantial success in maintaining viability in storage (Japanese knotweed [*Fallopia japonica*])

(Forman and Kesseli, 2003), it is unknown if *P. polygama* or *P. robusta* responded as well. If germination experiments had begun immediately after collection, results may have been dramatically different as seeds are typically the most deeply dormant at the shedding stage (Baskin and Baskin, 2001; Bewley and Black, 1994). However, it is possible that seeds of the *Polygonella* species studied here experienced the loss of dormancy over time due to the process of after-ripening (Foley, 2001). As after-ripening proceeds, seeds become more germinable over a wider range of conditions (Bewley and Black, 1994). This includes the ability to germinate more readily for a greater number of temperatures, and a greater sensitivity to chemical treatments such as GA's (Foley, 2001). Sometimes storage (i.e. after-ripening) is used as a treatment to increase germination when dormancy mechanisms are unknown. Seeds of marigold (*Calendula* spp. [Asteraceae]) germinated at higher rates after being stored for multiple years in a cool, dry environment (Widrlechner, 2007).

Aside from the possible types of dormancy present in *Polygonella* seeds and how to alleviate these and promote germination, the experiments reported here investigated the role of propagation media in germination. Though the experiment utilized only 72 seeds and was carried out once, it was concluded that sand was the most effective germination media (Figure 2-1). This study can be supported by a germination study carried out in a greenhouse in Fort Pierce on Fafard germination media. There, seeds (n = 100) emerged to only 4% for *P. polygama* in May and 25% for *P. robusta* in December (S. Wilson, personal communication). This is also ecologically supportable as both species are found to naturally occur on areas previously disturbed and consisting of open sand (S. Woodmansee, personal communication). Both species are

also found in areas commonly associated with fire, indicating that organic matter found in the topsoil would be consumed in the burn. However, using only sand as a germination media in the nursery setting is impractical due to its high bulk density and low water holding capacity. Instead, it would be recommended to use a media with high drainage and low organic matter.

Several general conclusions can be drawn from these studies. First, the results support that both species possess non-deep physiological dormancy after harvest and subsequent storage. Non-deep physiological dormancy in these species may be alleviated most effectively by cold stratification at 5 ^{o}C for 2 weeks or the application of warmer, summer like temperatures followed by cooler, winter like temperatures. Second, dormancy may also be alleviated by periods of indoor storage. However, it must be stressed that the effects of storage temperature, storage relative humidity, and storage duration on possible after-ripening or seed viability of *Polygonella* species remains unknown at this time. Finally, in the nursery setting, seeds will have the highest rate and percent germination when sown on sand based media.

Table 2-1. Change in temperatures detailed in the move-along experiment with number of weeks seeds spent in incubation at each temperature.

Weeks at temperature	Move-Along treatments		Control treatments			
	22/11 °C Winter	33/24 °C Summer	22/11 °C	27/15 °C	29/19 °C	33/24 °C
12	↓	↓	↓	↓	↓	↓
	27/15 °C Early Spring	29/19 °C Early Fall	22/11 °C	27/15 °C	29/19 °C	33/24 °C
4	↓	↓	↓	↓	↓	↓
	29/19 °C Late Spring	27/15 °C Late Fall	22/11 °C	27/15 °C	29/19 °C	33/24 °C
4	↓	↓	↓	↓	↓	↓
	33/24 °C Summer	22/11 °C Winter	22/11 °C	27/15 °C	29/19 °C	33/24 °C
12	↓	↓	↓	↓	↓	↓
	29/19 °C Early Fall	27/15 °C Early Spring	22/11 °C	27/15 °C	29/19 °C	33/24 °C
4	↓	↓	↓	↓	↓	↓
	27/15 °C Late Fall	29/19 °C Late Spring	22/11 °C	27/15 °C	29/19 °C	33/24 °C
4	↓	↓	↓	↓	↓	↓
	22/11 °C Winter	33/24 °C Summer	22/11 °C	27/15 °C	29/19 °C	33/24 °C
12						

Table 2-2. Pre-germination viability of all seed sources tested using 1% TZ solution. Total viability determined by adding total number of seeds stained red and pink. Data is presented as mean percent ± standard error.

	Tetrazolium test (%)					
Species	Red	Pink	White	Black	Empty	Total viable (%)
P. polygama (C)	65.5 ± 4.7	11.9 ± 2.6	1.0 ± 1.0	21.6 ± 6.6	0.0 ± 0.0	77.4 ± 6.3
P. polygama (N)	66.7 ± 3.6	11.5 ± 5.9	21.8 ± 5.1	0.0 ± 0.0	0.0 ± 0.0	78.1 ± 5.1
P. robusta	47.3 ± 6.7	6.2 ± 1.1	3.0 ± 1.0	8.2 ± 3.9	35.3 ± 5.9	53.5 ± 6.8

Table 2-3. Germination and TZ viability testing of *Polygonella polygama* (C) and *Polygonella robusta* seeds. Dormant (%) determined by total viable-final germination. Total viable via pregermination TZ found by dividing Final germination by Total viable. Mean percentages and standard errors are shown.

Species and temperature (^{o}C)	Final germination (%)	Dormant (%)	Total viable (%)	Total viable via pregermination TZ (%)	Mean germination time (d)
P. polygama					
22/11	30.1 ± 5.3	0.0 ± 0.0	30.1 ± 5.3	100.0 ± 0.0	14.4 ± 1.8
27/15	1.4 ± 1.4	20.0 ± 6.4	21.4 ± 6.3	6.3 ± 6.3	7.0 ± 7.0
29/19	17.2 ± 3.0	5.8 ± 5.8	23.0 ± 8.1	87.5 ± 12.5	12.3 ± 1.8
33/24	0.0 ± 0.0	33.5 ± 12.2	33.5 ± 12.2	0.0 ± 0.0	-
LSD(0.05)	9.6	23.0	24.1	16.1	4.3
P. robusta					
22/11	59.4 ± 17.6	0.0 ± 0.0	59.4 ± 17.6	100.0± 0.0	9.3 ± 1.3
27/15	55.9 ± 9.2	32.1 ± 5.7	88.0 ± 7.4	62.6 ± 7.9	9.6 ± 0.4
29/19	26.1 ± 11.5	17.1 ± 10.2	43.2 ± 20.1	73.3 ± 16.3	8.2 ± 1.2
33/24	8.3 ± 8.3	22.2 ± 15.7	30.6 ± 17.8	30.0 ± 30.0	7.0 ± 7.0
LSD (0.05)	27.9	24.4	32.0	3.4	2.4

Table 2-4. Post-germination viability from the dark treatment at all temperatures tested using a 1% TZ solution. Total viability determined by adding total number of seeds stained red and pink. Data is presented as mean percent ± standard error.

Species and temperature (°C)	Red	Pink	White	Black	Empty	Total viable (%)
P. polygama						
22/11	1.0 ± 1.0	0.0 ± 0.0	31.0 ± 4.4	3.0 ± 1.0	17.0 ± 1.0	1.0 ± 1.0
27/15	3.0 ± 1.9	1.0 ± 1.0	36.0 ± 15.1	1.0 ± 1.0	8.0 ± 4.3	4.0 ± 2.3
29/19	1.0 ± 1.0	2.0 ± 2.0	15.0 ± 3.4	4.0 ± 1.6	12.0 ± 4.0	3.0 ± 3.0
33/24	0.0 ± 0.0	0.0 ± 0.0	42.0 ± 11.8	4.0 ± 4.0	14.0 ± 7.4	0.0 ± 0.0
P. robusta						
22/11	0.0 ± 0.0	0.0 ± 0.0	7.0 ± 3.4	2.0 ± 2.0	7.0 ± 7.0	0.0 ± 0.0
27/15	6.0 ± 3.8	0.0 ± 0.0	8.0 ± 4.3	4.0 ± 2.8	7.0 ± 4.7	6.0 ± 3.8
29/19	10.0 ± 4.2	1.0 ± 1.0	4.0 ± 2.8	3.0 ± 1.0	13.0 ± 4.4	11.0 ± 5.0
33/24	4.0 ± 1.6	1.0 ± 1.0	3.0 ± 1.9	4.0 ± 1.6	12.0 ± 1.6	5.0 ± 2.5

Table 2-5. Analysis of variance table of emergence percent and rate for class comparison between sterilization and media treatments of *P. polygama* (N).

Source	df	Emergence MS	Emergence *p*-value	MGT MS	MGT *p-value*
Model	5	4406.4	<0.001	417.6	0.14
Sterilization	1	31.0	0.75	1.1	0.88
Media	2	10040.0	<0.001	199.6	0.02
Native mix v. Fafard2P	1	0.0	1.00	0.1	0.96
Sand v. Fafard2P	1	15060.1	<0.001	6.3	0.02
Sterilization x Media	2	960.6	0.05	12.0	0.77

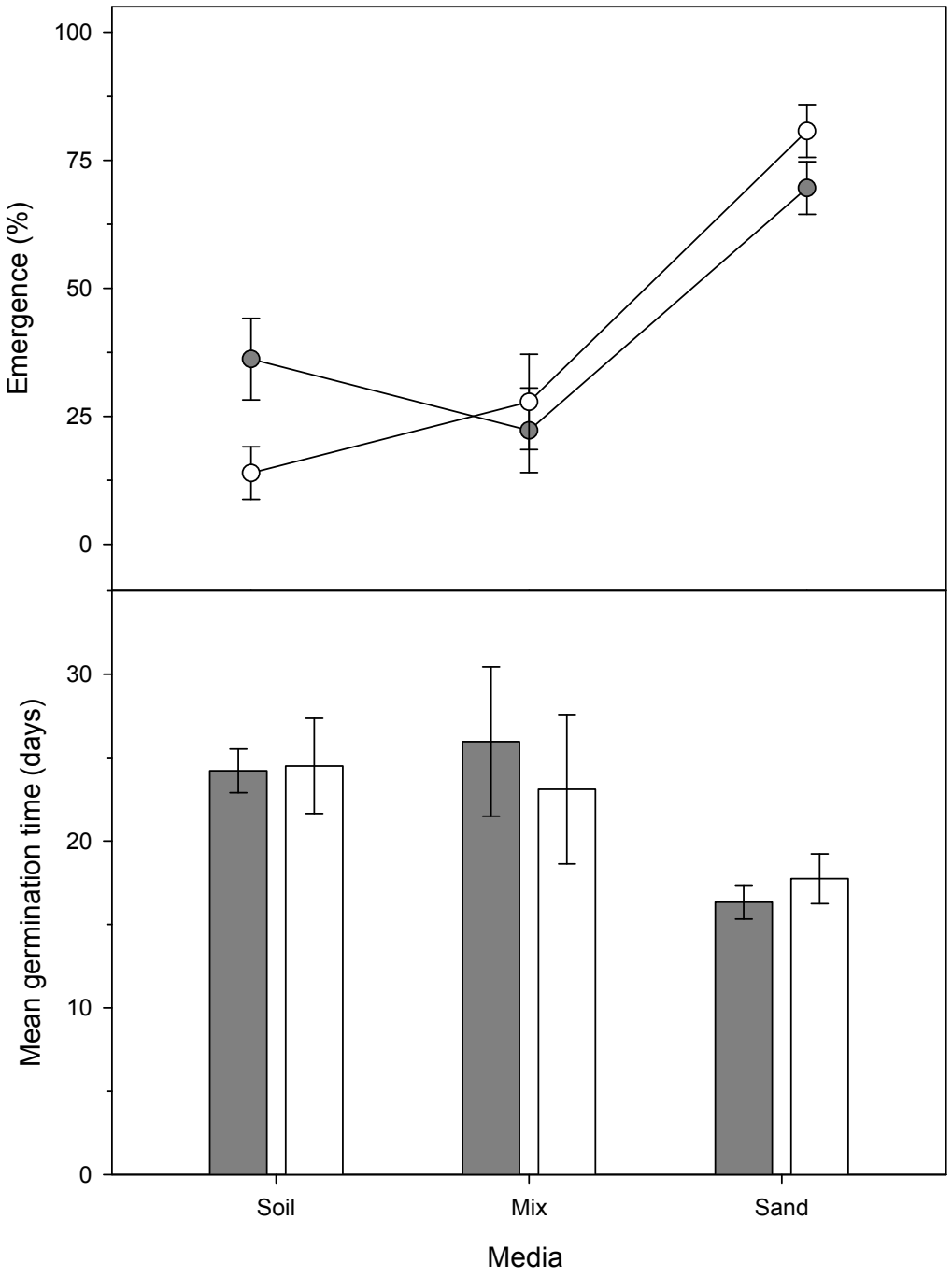

Figure 2-1. Emergence percent as an effect of media types and surface sterilization on seeds of *P. polygama* (N). The native mix consisted of 40% fine pine bark, 25% Fafard 2P, 25% sand, and 10% vermiculite. Circles and columns in dark gray were non-sterilized treatments, while open bars denote sterilized treatments. Error bars represent the standard error of the mean.

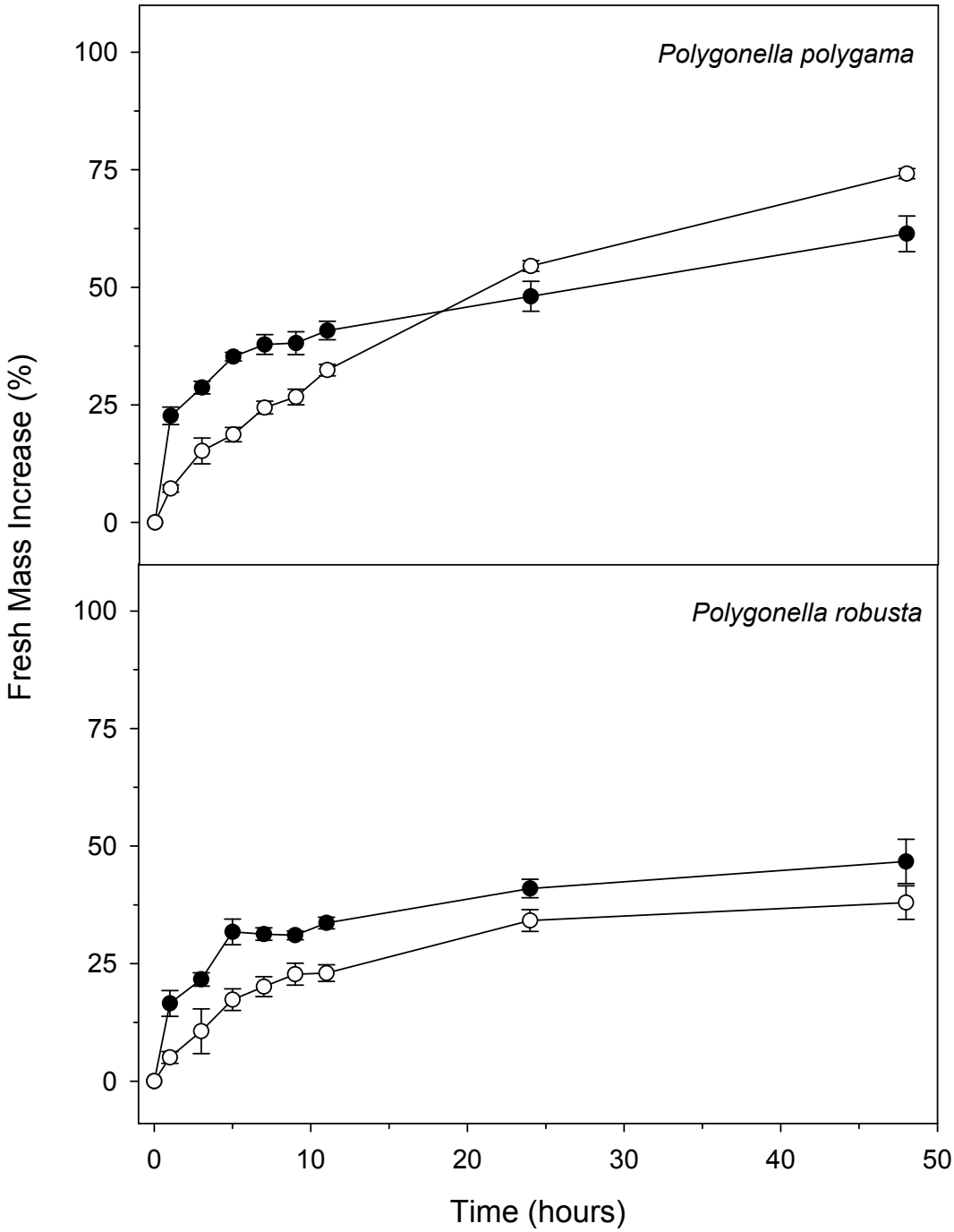

Figure 2-2. Increase in fresh mass of scarified (solid circles) or non-scarified (open circles) seeds of *P. polygama* (top), or *P. robusta* (bottom). Error bars represent standard error of the mean.

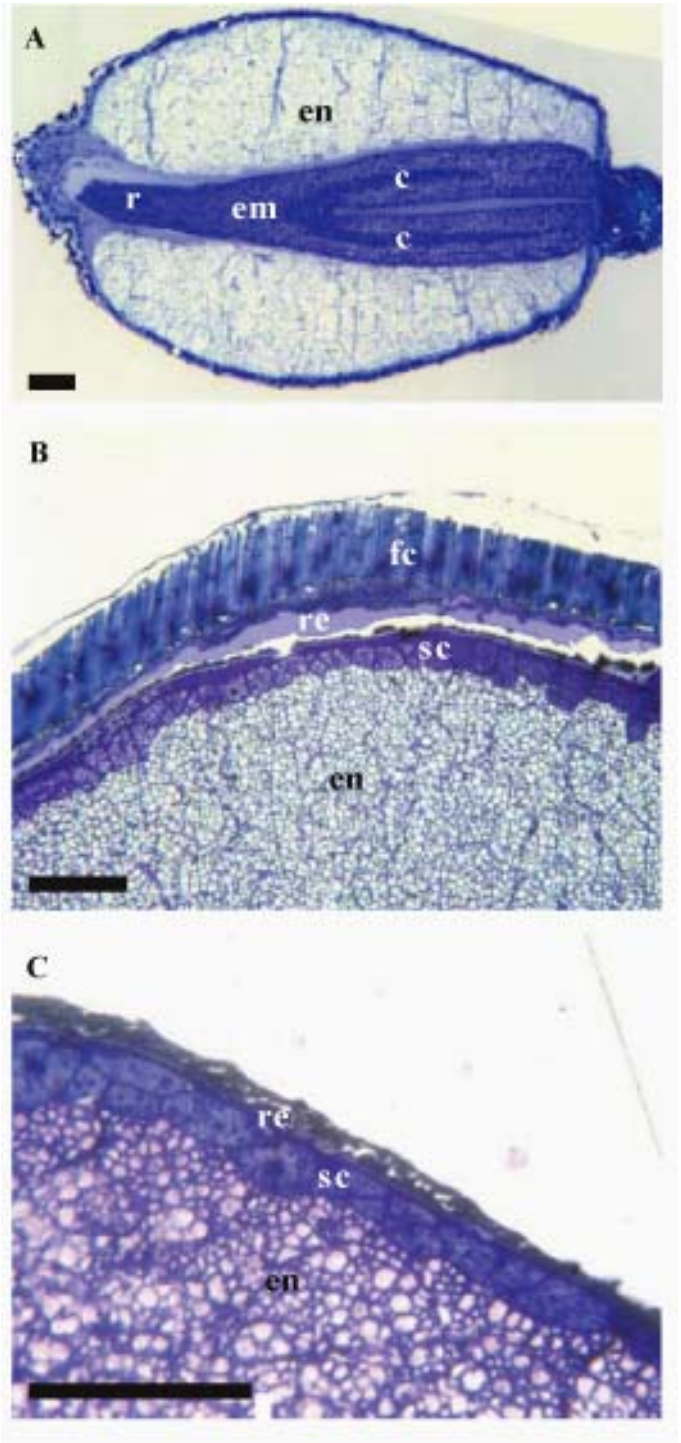

Figure 2-3. Thick sections of *Polygonella* seeds. (A) Longitudinal section of *P. polygama* seed showing fully developed embryo (em) with cotyledons (c) and radicle (r) surrounded by the endosperm (en). Magnification = 20×. (B) The fruit coat (fc), remaining resin layer (re), seed coat (sc) and endosperm (en) of *P. polygama.* Magnification = 40×. (C) Close up of the endosperm (en), seed coat (sc) and resin layer (re) of *P. polygama*. Magnification = t 100×. (C). Black bars represent 0.1 mm.

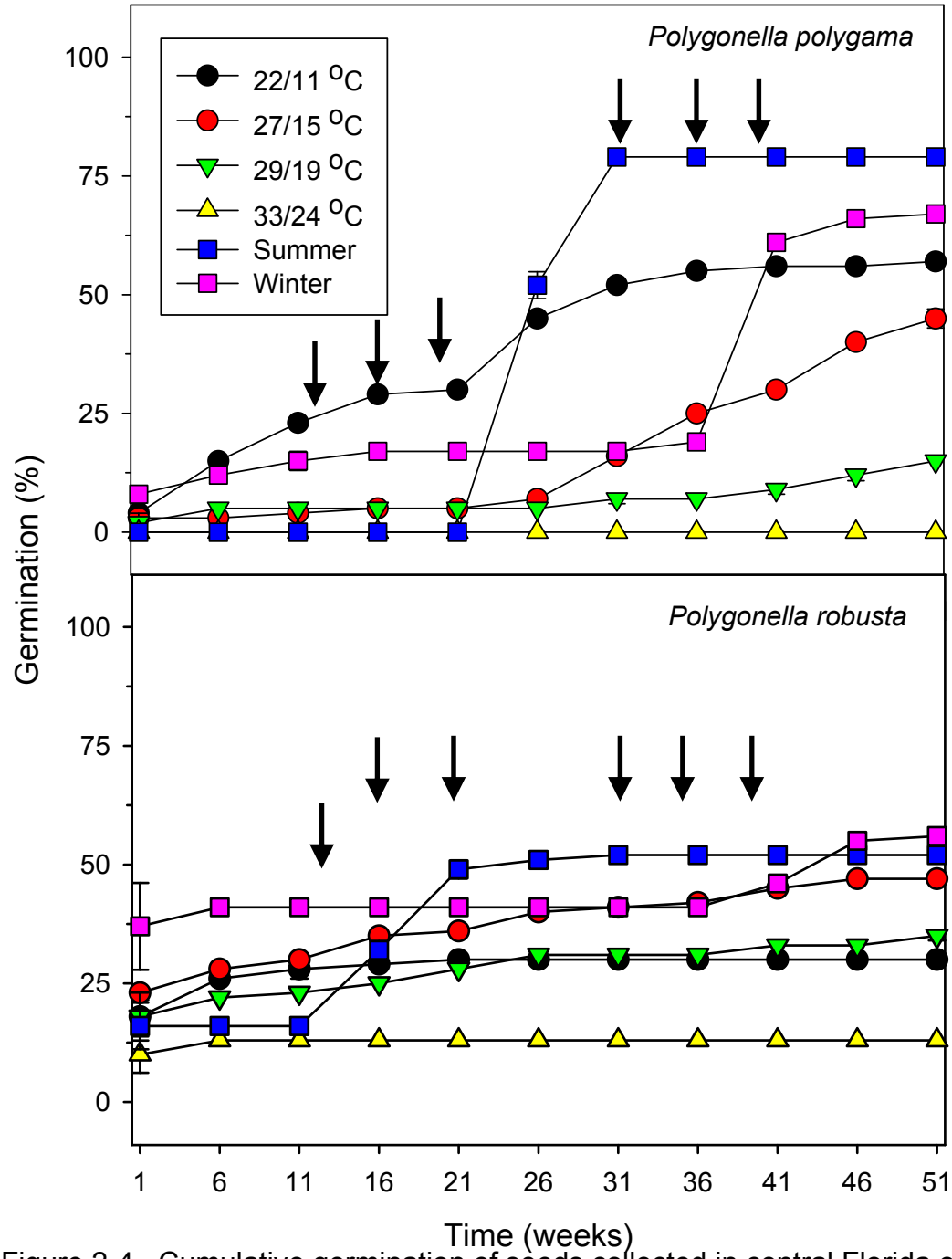

Figure 2-4. Cumulative germination of seeds collected in central Florida of *P. polygama* (top) and *P. robusta* (bottom) incubated at simulated seasonal temperatures. The key shows the four control temperatures and the two move-along treatments beginning at 33/24 °C for summer and 22/11 °C for winter. Arrows indicate when move-along treatments changed temperatures.

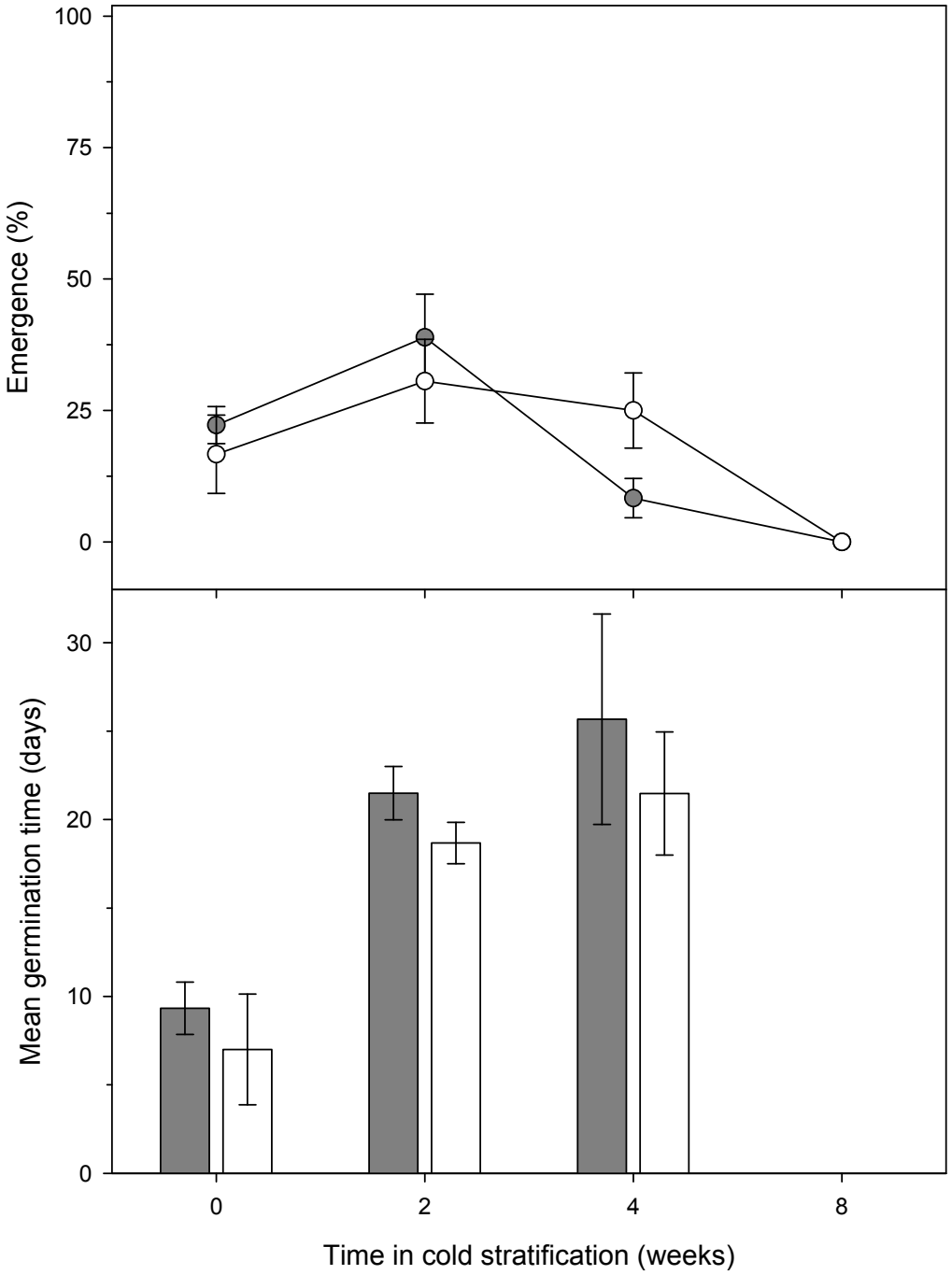

Figure 2-5. Final emergence percent (top line graph) and mean germination time (bottom bar graph) of *P. polygama* (N) seeds after moist stratification in the dark at 5 ^{o}C for 0, 2, 4, or 8 wk. Circles and columns in dark gray were treatments not sterilized, while white was sterilized. Error bars represent standard error of the mean.

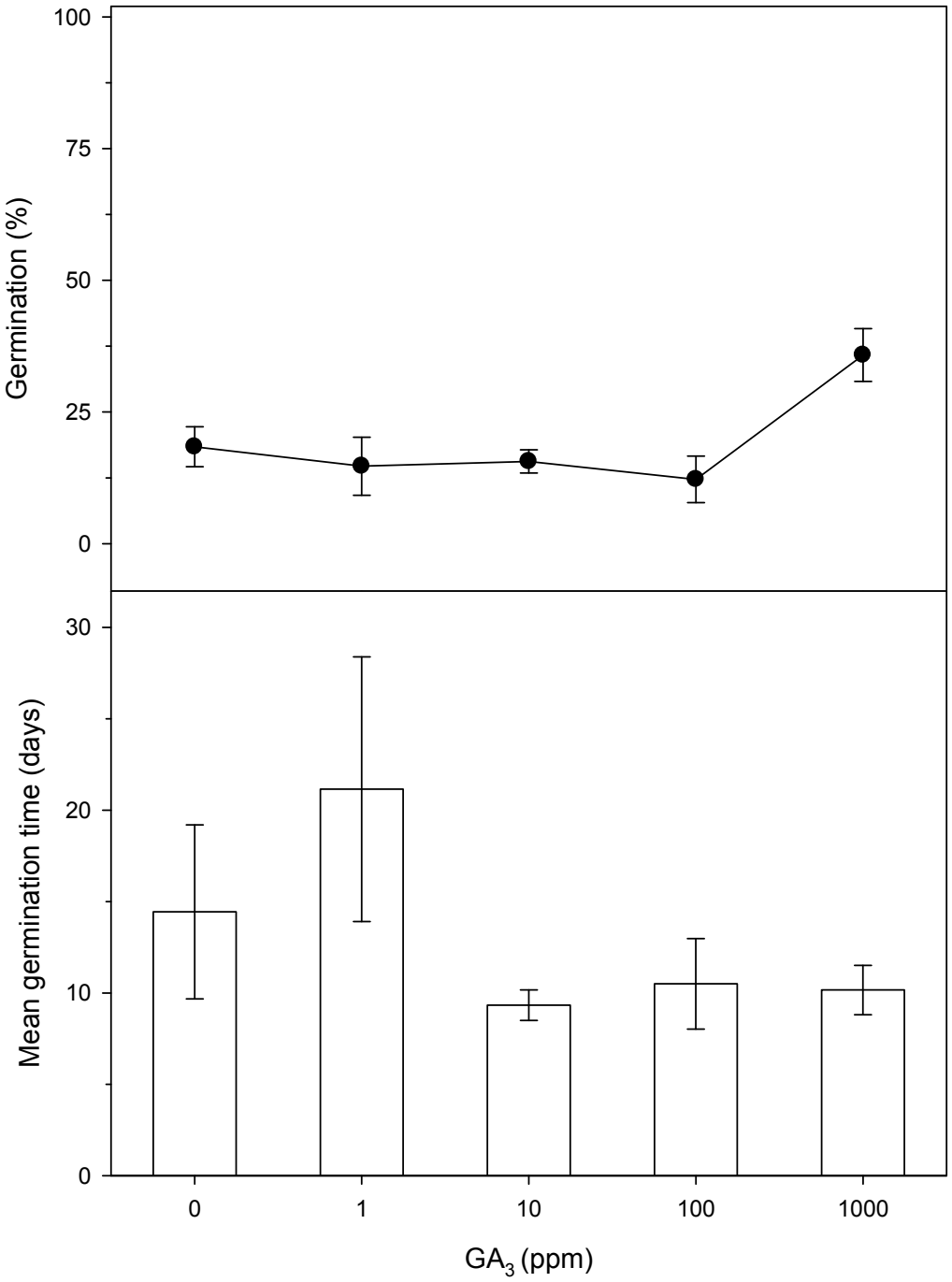

Figure 2-6. Final germination percent (top line graph) and mean germination time (bottom bar graph) of *P. polygama* (C) seeds after soaking for 24 h in varying concentrations of GA_3 and subsequent incubation at the constant temperature of 22/11 ^{o}C (72/52 ^{o}F) for 28 days.

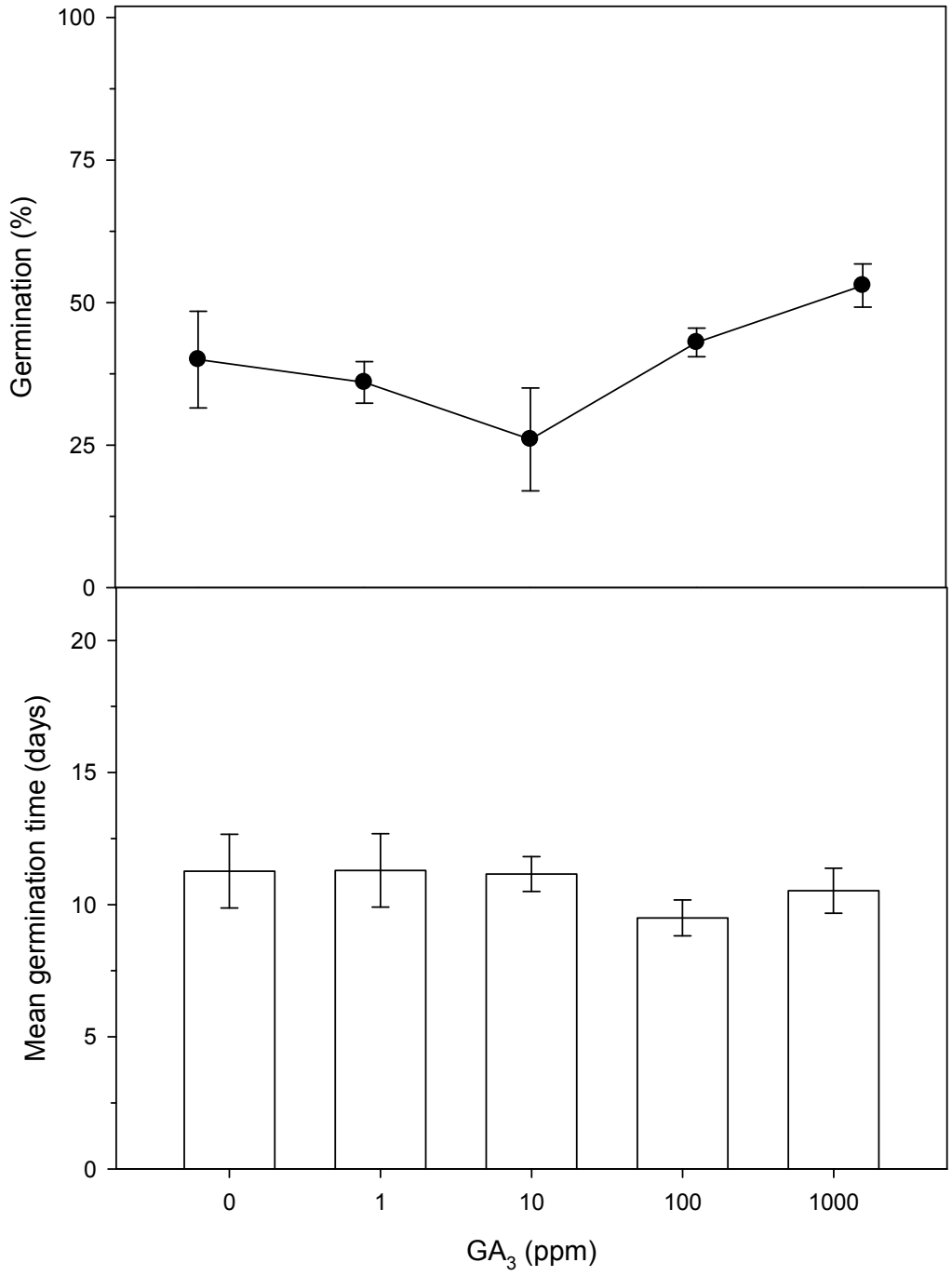

Figure 2-7. Final germination percent (top line graph) and mean germination time (bottom bar graph) of *P. robusta* seeds after soaking for 24 h in varying concentrations of GA_3 and subsequent incubation at the constant temperature of 22/11 ^{o}C (72/52 ^{o}F) for 28 days.

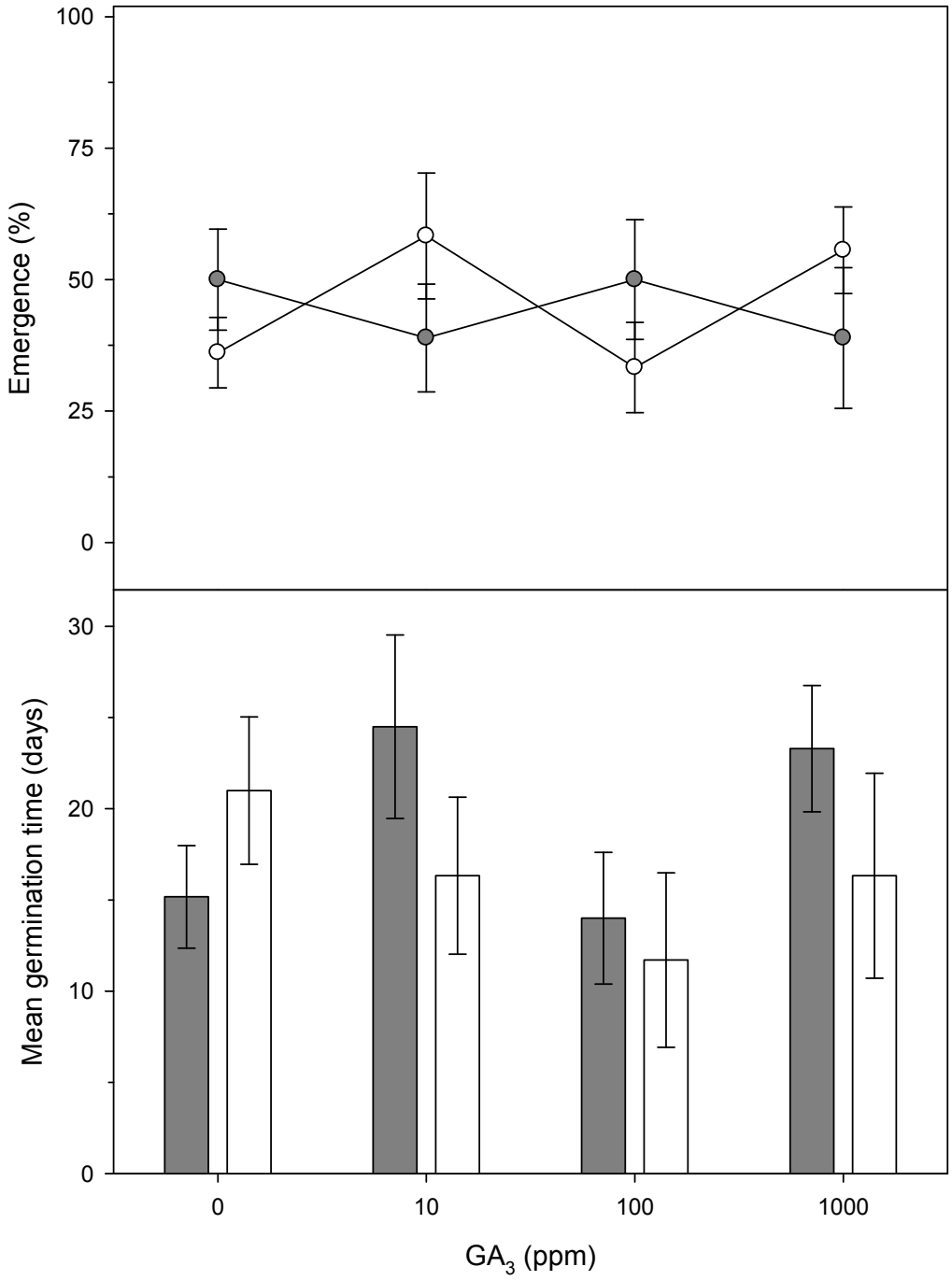

Figure 2-8. Final emergence percent (top line graph) and mean germination time (bottom bar graph) of *P. polygama* (N) seeds after soaking for 24 h in varying concentrations of GA_3 and subsequent growth in the misthouse for 42 days. Circles and columns in dark gray were treatments not sterilized, while white was sterilized. Error bars represent standard error of the mean.

CHAPTER 3
EFFECT OF AUXIN APPLICATION ON ROOTING OF SOFTWOOD CUTTINGS OF *POLYGONELLA POLYGAMA* AND *POLYGONELLA ROBUSTA*

Introduction

Even when seeds are collected at the appropriate time, properly stored, and subjected to favorable conditions, dormancy mechanisms often exist that delay germination (Baskin and Baskin, 2004a; Finch-Savage and Leubner-Metzger, 2006; Bradbeer, 1988). Though both October flower (*Polygonella polygama* (Vent.) Engelm. & A. Gray [Polygonaceae]) and sandhill wireweed (*Polygonella robusta* (Small) G.L. Nesom & V.M. Bates [Polygonaceae]) flower prolifically, propagation by seed can be difficult due to physiological dormancy that is characterized by delayed, erratic, or reduced germination (Heather et al., 2009). In addition, seed collection of *Polygonella* spp. from natural populations can be restricted by varying seasonal conditions, management practices, or narrow harvest windows (Ingram and Yeager, 1990). As an alternative to producing natives by seed, vegetative propagation can often improve efficiency, enhance product uniformity, generate finished plants quicker and with greater reliability. Vegetative propagation, or asexual propagation, is the production of a new plant from a cutting of a parent plant. For this research project, stem cuttings were taken from plants in wild populations and then rooted in media trays kept in a misthouse.

Auxins are plant growth regulating substances that are frequently applied to the basal end of fresh cuttings to induce the formation of adventitious roots. For instance, rooting of softwood cuttings of Indian rosewood (*Dalbergia sissoo* [Fabaceae]) was enhanced when 100 mg/L of either indole-3-butyric acid (IBA) or 1-naphthaleneacetic acid (NAA) was applied (Puri and Verma, 1996). Auxins such as IBA and NAA are

commonly used in the propagation industry. For example, the commercially popular liquid rooting product known as Dip ‘N Grow (Dip‘N Grow Inc., Clackamas, OR) is a combination of auxins at a ratio of two parts IBA to one part NAA. Though this combination is often used in the industry, most researchers utilize the chemicals independently to optimize species specific rooting responses. Both IBA and NAA elicited similar results in percent rooting and mortality when applied to Fraser fir (*Abies fraseri* [Pinaceae]), though NAA produced a greater number of and longer roots (Rosier et al., 2004). In one experiment on sugar maple (*Acer saccharum* [Sapindaceae], combinations of IBA and NAA were used in a 1:1 ratio, though this ratio did not produce longer or a greater percentage of roots as compared to IBA or NAA alone (Alsup and Cole, 2004).

Vegetative propagation has served as a reliable alternative for several other native coastal or scrub species including false rosemary (*Ceratiola ericoides* [Empetraceae]) (Thetford et al., 2001), dune sunflower (*Helianthus debilis* [Asteraceae]) (Norcini and Aldrich, 2000), and for the micropropagation of sea oats (*Uniola paniculata* [Poaceae]) (Valero-Aracama et al., 2007). Thetford et al. (2001) recommend IBA and NAA concentrations below 5000 ppm for rooting false rosemary. Norcini and Aldrich (2000) found 2000 ppm IBA was optimal to induce adventitious roots on dune sunflower. To our knowledge, vegetative propagation protocols have not been developed for *Polygonella* species. In unpublished preliminary data of *P. polygama* using only IBA, M. Thetford (personal communication) found that the control cuttings rooted at 83% and increased to 86-98% with 1000 to 5000 ppm of IBA. Additionally, *P. robusta* cuttings exposed to 1000 to 5000 ppm K-IBA produced more roots than those treated with 0

ppm K-IBA. In these preliminary studies, rooting substrate (Fafard 3P mix or perlite:vermiculite) did not affect rooting (M. Thetford, personal communication).

The overall objective of this study was to determine if effective methods could be developed to root *P. polygama* and *P. robusta* cuttings collected from different sites in Florida. Specific objectives were to (1) evaluate the effectiveness of various auxin concentrations and combinations on rooting, and (2) determine if collection site influences rooting success.

Materials and Methods

Cutting source and auxin treatments

Natural populations of *P. polygama* and *P. robusta* were identified and the habitats they were growing in were characterized by assessing neighboring species, burn history, population number, population health, disturbance affinity, and distribution (Table 3-1). *P. polygama* is often found along the edge of scrub and mesic habitats (Table 3-2). *P. robusta* is found growing in full sun on sandhills. Both species are commonly associated with highly disturbed areas (S. Woodmansee, personal communication). Cuttings of *P. polygama* and *P. robusta* were collected from locations in central or south Florida during the summer of 2008 and 2009 (Table 3-1, Table 3-2). Terminal (i.e. softwood) stem cuttings, approximately 10 cm (3.9 in) in length, were collected in the morning from approximately 35 plants of each species. Pruning shears were surface sterilized with 95% ethanol in between species. All cuttings were immediately placed between moist paper towels in zip top plastic bags. Plastic bags were then wrapped with newspapers and stored between icepacks in a foam cooler for transportation to the processing site in Gainesville. The cuttings were stored inside the cooler for up to 24 h prior to sticking.

Prior to treatment, cuttings were trimmed to 11.5 cm (4.5 in) and the foliage removed from the basal 3 cm (1.2 in) of each cutting. Nine auxin treatments were formulated from 10,000 ppm stock solutions of indole-3-butyric acid (K-IBA) and 1-naphthaleneacetic acid (K-NAA) that were diluted to appropriate concentrations with distilled water. The basal 1 cm (0.4 in) of each cutting was quick dipped for 8 sec in one of nine IBA:NAA solutions (0:0, 0:250, 0:500, 500:0, 500:250, 500:500, 1000:0, 1000:250, 1000:500 ppm) and allowed to air dry prior to sticking (Table 3-3). Cuttings were inserted 2 cm (0.8 in) deep into 72-plug cell trays filled with pre-moistened soilless Fafard 2P media (Fafard Inc., Apopka, FL). The trays were placed in a mist house where intermittent mist operated 8 sec every 10 min during the daytime. After 2 weeks, mist was reduced to 5 sec every 20 min. Greenhouse temperature was set at 27 ^{o}C (80 ^{o}F) with a natural photoperiod of 14 h and an approximate photon flux of 300 $\mu mol \cdot m^{-2} \cdot s^{-1}$ at the propagation bench.

Data Collection

Cuttings were removed from mist after 6 weeks (*P. robusta*) or 8 weeks (*P. polygama*). Media was carefully removed from roots by rinsing with tap water. For each experiment, rooting was assessed using four parameters: a visual root quality rating (rooting index), rooting percentage, root length, and root number. A cutting was considered rooted if it had one adventitious root ≥ 0.5 cm in length. Rooting index was based on a scale from 1 to 5, where 1= dead, 2= alive without roots, 3= light rooting that does not hold media, 4= medium rooting that holds media that is mostly removed with a light shake, and 5= heavy rooting that holds onto media that must be removed with washing. Rooting percentage was calculated using the number of cuttings that received

a rating of 3, 4, or 5. Root length was recorded as the longest primary root from each cutting.

Experimental Design and Data Analysis

For each species, a split plot experimental design was used with the collection site as the main plot and the auxin treatments as the subplot. The subplots were arranged in a randomized complete block design with 6 cuttings per treatment (n=270). Each auxin treatment was replicated 5 times. Root percent data was transformed by taking the arcsine of the square root. However, untransformed data are presented. Data were subjected to ANOVA and trend analysis using SAS v.9.1 (SAS Institute, Cary, North Carolina). Significance of main effects and interactions was determined using SAS PROC MIXED. Trend analysis was carried out using SAS PROC GLM.

Results

Polygonella polygama

At 6 weeks, the *P. polygama* cuttings did not produce sufficient roots as compared to what had been observed in *P. robusta* at 6 weeks, therefore the experiment was extended for an additional 2 weeks. At 8 weeks, regardless of collection site, no single auxin treatment produced roots that were consistently best among all measured response variables (Table 3-4). Average root index is shown in Figure 3-2 and Figure 3-3. The root index across both sites and all auxin combinations was somewhat low and did not exceed a rating of 2.8. Root index reached 2.6 ± 0.2 and 2.5 ± 0.1 when cuttings from the central Florida source were treated with 500:0 and 0:500 (IBA:NAA), respectively. For the southern source of *P. polygama,* the greatest root index of 2.8 ± 0.4 and 2.8 ± 0.2 was achieved when cuttings were treated with 500:0 and 1000:500 IBA:NAA, respectively. Rooting percentage ranged from 27-63% for both collection sites

of *P. polygama*. The greatest rooting for the central collection source of 60 ± 11.3 % was reached when cuttings were treated with 500:0 IBA:NAA. For cuttings collected from the southern source, rooting ranged from 40-63% with the greatest rooting of 63.3 ± 9.7 % receiving the treatment of 1000:250 IBA:NAA.

Neither source produced more than 5 roots per cutting. The greatest number of roots (4.2) was reached when cuttings from south Florida were treated with 500:0 IBA:NAA. The least number of roots per cutting (2.3) was obtained when the central collection was treated with 500:250 IBA:NAA. Number of roots for cuttings collected in south Florida ranged from 3.8 to 5.1. The greatest number of roots of 5.1 ± 0.7 was reached when treated with 1000:500 IBA:NAA. Finally, root length varied from about 2 to 5 cm. The longest root length, 5.2 ± 0.3 cm, was reached when cuttings from central Florida were treated with 1000:0 IBA:NAA. The shortest root length was also found in the central source for cuttings in the control group.

Both rooting percent and root index for the central source of *P. polygama* had the greatest response when treated with IBA at 500:0. Root index, percent and number all responded the least to IBA:NAA at 500:250. For root length, the 1000:0 and 1000:500 IBA:NAA treatments produced roots that were more than twice the length of the control plants. Cuttings taken from the south Florida source had similar rooting success compared to cuttings from the central Florida source (Table 3-4). For example, root index reached 2.8 at 500:0 and 1000:500 IBA:NAA. Root index, percent and number were lowest when treated with 0:250.

As seen in Table 3-4, only one interaction was significant for *P. polygama*, IBA × NAA for root index. When IBA was held constant at 0 ppm, a concave quadratic trend

was observed. In comparison, a convex quadratic curve and an increasing linear trend were seen when NAA was held constant at 0 and 250 ppm, respectively (Table 3-6).

Polygonella robusta

Regardless of collection site, no single auxin treatment produced roots that were consistently best among all measured traits (Table 3-5). Averages of each root index treatment are depicted in Figure 3-4 and Figure 3-5. The root index ranged from 1.6 to 3.6. The cuttings collected from the central source had the greatest root index when treated with 1000:0 IBA:NAA (3.3 ± 0.3). Those collected in south Florida reached a maximum root index of 3.6 ± 0.3 when treated with 500:250 IBA:NAA. Percent rooting was highly variable, ranging from 10-80%. Less variation among treatments occurred from the central source, with greatest percent rooting (76.7 ± 11.3%) at 1000:0 IBA:NAA. When cuttings collected in south Florida were treated with 500:250 IBA:NAA, rooting percent reached 80.0 ± 6.2%.

The number of roots per cutting for *P. robusta* ranged from 3 to nearly 11. Both central and south Florida cuttings produced the greatest number of roots when treated with 1000:250 IBA:NAA (9.6 ± 1.7 and 10.9 ± 2.0, respectively).The range for root length varied greatly from about 2 to 10 cm. The greatest root length occurred with the 1000:250 IBA:NAA treatment for central Florida cuttings (8.3 ± 1.0 cm) or 0:250 IBA:NAA for south Florida cuttings (10.1 ± 0.3 cm).

Considering all rooting factors, cuttings from the central source performed best when treated with 1000:0 IBA:NAA. The central source had the lowest rooting for all factors when treated with 0:500 IBA:NAA. Cuttings from the south source performed best when treated with 500:250 IBA:NAA. Lowest percent rooting and root index were found when south Florida cuttings were treated with 0:500 IBA:NAA.

As seen in Table 3-5, some interactions are significant for *P. robusta*. For the central source, there is a significant linear decrease in rooting index when IBA concentration was zero and NAA concentration increased. Alternatively, a significant linear increase in rooting index occurred when IBA concentration increased but NAA remained at 500ppm. However, the rooting index for cuttings from south Florida can be explained using a quadratic function when IBA concentrations were held constant but NAA concentration increased. In these instances the shape of the quadratic curves were concave when IBA was held constant at 0 ppm and convex for 500 and 1000 ppm. A quadratic trend, convex in shape, was observed when NAA was held constant at 250 ppm and IBA increased (Table 3-7). For the interaction of site × IBA × NAA, similar trends were seen for percent rooting as was seen for root index (Table 3-8). An increasing linear trend was visible for cuttings collected in central Florida when NAA was held constant at 250 ppm and IBA increased. When IBA was held constant and NAA increased, percent rooting for the southern source could all be described by a quadratic curve. When IBA was held at 0 ppm the shape can be described as concave, whereas when IBA is held at 500 and 1000 ppm the shape is convex. When NAA is held constant at 0 ppm, the percent rooting is shaped as a quadratic concave curve. Alternately, NAA held at 250 ppm yields a quadratic convex curve.

When looking at root number, the interaction of IBA × NAA was significant. Only one trend was visible over increasing IBA concentrations, and one trend for increasing NAA concentrations (Table 3-9). When IBA was held constant at 1000 ppm a quadratic curve, convex in shape, was evident with increasing NAA concentrations. In contrast, holding NAA constant at 250 ppm, created an increasing linear trend with increasing

IBA concentrations. Finally, trends in root length were evident by Site × IBA and Site × NAA interactions (Table 3-10). When Site × IBA was pooled over NAA the central site had an increasing linear trend when IBA concentrations were increasing. In contrast, for Site × NAA pooled over IBA, a convex quadratic curve was observed with increasing NAA concentrations.

Discussion

Results for both species revealed no single auxin treatment to be consistently most effective in promoting the formation of adventitious roots. However, for both species collected from both locations, improved rooting was often seen when IBA was greater than 0 ppm, regardless of NAA concentration.

Overall, cuttings taken from *P. robusta* had a greater response to auxin application, with larger root index results and percent rooting than *P. polygama.* Rooting hormones have been shown to have variable effects based on species. In a study on fifteen taxa of snowbells (*Styrax* spp. [Styracaceae]), it was found that rooting of 9 species was influenced by the application of IBA, five of which were positive (Griffin and Lasseigne, 2005). The improved rooting of *P. robusta* may be explained in part by the condition of the plants at the collection site. The southern site was characterized by a recently cleared scrub habitat with surrounding pines, oaks, and saw palmettos. The plants collected there appeared healthier with greater rates of new, green growth and thicker stems.

Another potential influence in rooting success may be explained by collection time. The initial collection was done in late summer of 2008 due to environmental setbacks such as drought and fire. This meant that the cuttings were collected just a few months prior to flowering. Many of the south *P. polygama* cuttings flowered during the 8 weeks

in the mist house, while cuttings from south *P. robusta* flowered soon after data collection in the greenhouse (less than 10 weeks after collecting). This suggests that the cuttings may have allocated carbohydrates to flowering instead of rooting. This translocation of energy has been known as early as 1940 (O'Rourke) when hardwood cuttings of blueberry (*Vaccinium atrococcum* [Ericaceae]) with flower buds did not root as well as those with vegetative buds. Though levels of rain and fire make it difficult to collect only softwood cuttings, higher concentrations of rooting hormones may be required to induce adventitious rooting based on time of collection. Sharma and Aier (1989) found that when semi-hardwood plum cuttings were collected in the summer they needed only 2000 ppm of IBA to reach optimal rooting. This concentration had to be increased to 3000 ppm when cuttings were taken in the fall or when dormant.

Finally, hormone concentration has been known to improve rooting success. Often when rooting trends were described linearly, this indicates that using even higher levels of auxins might produce greater rooting. Only when the results were explained quadratically can it be assumed that the optimal auxin concentration was used. For cuttings of *P. robusta* collected in south Florida, both root index and percent rooting were described quadratically for IBA concentrations (Table 3-7 and Table 3-8). However, the lack of significance for *P. polygama* may indicate that a greater number of auxin treatments with a greater number of replications at higher concentrations, should have been used (Table 3-5). It is also possible that other factors, such as misthouse conditions and propagation media may have contributed to variable results. The misthouse used had very low light and overhead misters that frequently got clogged, resulting in occasional inconsistent watering. The media used here was only a peat

based soilless media. In previous propagation work done on *P. polygama* also using a perlite/vermiculite media with K-IBA concentrations at 1000 and 2000 ppm and higher light levels, a significant interaction was observed between propagation media and root length (M. Thetford, personal communication). Increase in rooting at higher light levels would be expected as both species grow well in full sun.

Future experiments on these species should be carried out to test other factors that are important to the success of root formation. Cutting maturity, substrate, moisture, auxin concentration, year, and stock plant management can significantly affect root potential (Blythe et al., 2007; Thetford et al., 2001). These experiments only utilized tip cuttings collected in the summer. Thetford (2008) found that rooting percentage, rate, and number of roots did not differ with Fafard or perlite/vermiculite substrates. Since both *Polygonella* species are native to sandy sites, better drainage may improve rooting. The auxin concentrations used in this experiment were also fairly low. In order to make appropriate recommendations, additional experiments should be conducted with higher concentrations of both NAA and IBA individually and combined.

Table 3-1. Collection date, site location, and site information for cuttings of *Polygonella polygama* (October flower) and *Polygonella robusta* (sandhill wireweed).

Species	Collection date	Site name	City	Location	Ecosystem type	GPS coordinates
Polygonella polygama	8/28/2008	Haney Creek Preserve	Stuart	South	scrub	27° 13' 35"N, 80° 15' 16"W
Polygonella polygama	6/9/2009	Withlacoochee State Forest	Brooksville	Central	scrub/sandhill	28° 51' 25"N, 82° 25' 49"W
Polygonella robusta	7/21/2008	Jonathan Dickinson State Park	Hobe Sound	South	scrub	27° 01' 11"N 80° 06' 38"W
Polygonella robusta	6/9/2009	Pine Ridge proposed landfill	Orlando	Central	sandhill	28 ° 29' 32''N 81 ° 3' 27''W

Table 3-2. Collection site characteristics for cuttings of *Polygonella polygama* (October flower) and *Polygonella robusta* (sandhill wireweed). Observations were noted by the co-collector, S. Woodmansee (personal communication).

Species	Location	Dominating species	Fire influence	General observations
Polygonella polygama	South	*Pinus clausa,* scrub oaks (*Quercus* spp.), *Serenoa repens, Ceratiola ericoides*	Fire suppressed, though recent wildfire	~100 individuals, little to no exotic plant influence, foot trail nearby
Polygonella polygama	Central	*Andropogon* spp., *Quercus geminata, Pinus clausa, Pityopsis, Aristida* spp.	Fire suppressed in scrub, sandhill burned recently	~300 individuals, plants declining slightly, though expected to improve with increased rain and fire
Polygonella robusta	South	*PInus clausa,* scrub oaks (*Quercus* spp.) *Serenoa repens, Ceratiola ericoides*	Regular fire regime	~200 individuals occur in recently cleared area, healthy
Polygonella robusta	Central	*Pinus palustris, Serenoa repens, Quercus geminata, Selaginella* spp., *Liatris tenuifolia*	Fire suppressed	~400 individuals, disturbed area to become landfill in a few years, plants slightly smaller

Table 3-3. Cuttings of October flower (*Polygonella polygama*) and sandhill wireweed (*Polygonella robusta*) were quick dipped in one of the following indole-3-butyric acid (IBA) 1-naphthaleneacetic acid (NAA) used.

IBA Concentration (ppm)	NAA Concentration (ppm)		
	0	250	500
0	0 , 0	0 , 250	0 , 500
500	500 , 0	500 , 250	500 , 500
1000	1000 , 0	1000 , 250	1000 , 500

Table 3-4. Effects of K-IBA and K-NAA treatments on rooting softwood cuttings of October flower (*Polygonella polygama*) collected from central (top) or south (bottom) Florida populations. Data presented as means ± standard error.

Site	Treatment	Root index (scale 1-5)	Rooting (%)	Root number	Root length (cm)
Central	0 IBA, 0 NAA	2.0 ± 0.1	30.0 ± 6.2	3.3 ± 1.1	2.2 ± 0.3
Central	500 IBA, 0 NAA	2.6 ± 0.2	60.0 ± 11.3	3.6 ± 0.2	4.0 ± 1.0
Central	1000 IBA, 0 NAA	2.2 ± 0.1	43.3 ± 8.5	4.2 ± 0.3	5.2 ± 0.3
Central	0 IBA, 250 NAA	1.9 ± 0.2	36.7 ± 9.7	2.6 ± 0.5	4.6 ± 0.8
Central	500 IBA, 250 NAA	1.8 ± 0.2	26.7 ± 11.3	2.3 ± 0.1	4.2 ± 1.6
Central	1000 IBA, 250	2.4 ± 0.3	46.7 ± 16.2	3.6 ± 0.5	4.4 ± 0.3
Central	0 IBA, 500 NAA	2.5 ± 0.1	56.7 ± 4.1	3.2 ± 0.4	3.8 ± 0.6
Central	500 IBA, 500 NAA	2.3 ± 0.3	50.0 ± 15.8	3.3 ± 1.8	3.0 ± 0.5
Central	1000 IBA, 500	2.1 ± 0.4	43.3 ± 17.2	2.8 ± 0.4	4.9 ± 0.8
South	0 IBA, 0 NAA	2.7 ± 0.3	53.3 ± 9.7	3.8 ± 0.7	2.6 ± 0.6
South	500 IBA, 0 NAA	2.8 ± 0.4	60.0 ± 13.5	4.0 ± 0.7	3.7 ± 0.5
South	1000 IBA, 0 NAA	2.2 ± 0.1	40.0 ± 6.7	4.1 ± 1.0	2.7 ± 0.5
South	0 IBA, 250 NAA	1.8 ± 0.2	26.7 ± 11.3	4.0 ± 0.8	4.8 ± 0.7
South	500 IBA, 250 NAA	2.6 ± 0.3	50.0 ± 13.9	4.1 ± 0.8	2.7 ± 0.4
South	1000 IBA, 250	2.5 ± 0.2	63.3 ± 9.7	4.2 ± 0.2	4.5 ± 0.5
South	0 IBA, 500 NAA	2.6 ± 0.3	50.0 ± 9.1	4.1 ± 0.7	2.8 ± 0.6
South	500 IBA, 500 NAA	2.4 ± 0.2	56.7 ± 8.5	4.6 ± 0.8	3.4 ± 0.7
South	1000 IBA, 500 NAA	2.8 ± 0.2	60.0 ± 12.5	5.1 ± 0.7	2.9 ± 0.5
Site		*	NS	*	NS
NAA		NS	NS	NS	NS
Site × NAA		NS	NS	NS	NS
IBA		NS	NS	NS	NS
Site × IBA		NS	NS	NS	NS
IBA × NAA		*	NS	NS	NS
Site × IBA × NAA		NS	NS	NS	NS

Non-significant (NS) at $\propto$=0.05 (*), 0.01 (**), or 0.001 (***).

Table 3-5. Effects of K-IBA and K-NAA treatments on rooting softwood cuttings of Sandhill wireweed (*Polygonella robusta*) collected from central (top) or south (bottom) Florida populations. Data presented as means ± standard error.

Site	Treatment	Root index (scale 1-5)	Rooting (%)	Root number	Root length (cm)
Central	0 IBA, 0 NAA	3.0 ± 0.5	56.7 ± 19.4	8.9 ± 1.7	6.3 ± 1.7
Central	500 IBA, 0 NAA	3.0 ± 0.3	60.0 ± 12.5	7.7 ± 0.9	7.3 ± 1.7
Central	1000 IBA, 0 NAA	3.3 ± 0.3	76.7 ± 11.3	7.6 ± 1.0	7.9 ± 1.4
Central	0 IBA, 250 NAA	2.9 ± 0.2	66.7 ± 5.3	6.2 ± 0.7	6.1 ± 2.2
Central	500 IBA, 250	2.8 ± 0.4	60.0 ± 15.5	7.5 ± 1.6	8.2 ± 1.9
Central	1000 IBA, 250	2.8 ± 0.6	53.3 ± 17.8	9.6 ± 1.7	8.3 ± 1.0
Central	0 IBA, 500 NAA	1.6 ± 0.4	20.0 ± 12.3	5.8 ± 2.4	2.9 ± 0.7
Central	500 IBA, 500	2.7 ± 0.5	53.3 ± 13.3	7.7 ± 0.8	6.1 ± 0.8
Central	1000 IBA, 500	2.9 ± 0.2	63.3 ± 9.7	7.3 ± 0.4	8.1 ± 1.0
South	0 IBA, 0 NAA	2.8 ± 0.3	50.0 ± 11.8	7.4 ± 1.7	2.3 ±1.1
South	500 IBA, 0 NAA	2.1 ± 0.2	13.3 ± 8.2	3.0 ± 0.0	1.7 ± 0.8
South	1000 IBA, 0 NAA	2.1 ± 0.1	16.7 ± 5.3	4.1 ± 0.5	4.4 ± 0.6
South	0 IBA, 250 NAA	2.1 ± 0.1	10.0 ± 6.7	5.0 ± 0.0	10.1 ± 0.3
South	500 IBA, 250	3.6 ± 0.3	80.0 ± 6.2	8.5 ± 1.4	6.9 ± 0.8
South	1000 IBA, 250	2.9 ± 0.4	50.0 ± 14.9	10.9 ± 2.0	4.8 ± 0.5
South	0 IBA, 500 NAA	2.9 ± 0.2	53.3 ± 6.2	6.4 ± 1.2	6.8 ± 1.9
South	500 IBA, 500	2.4 ± 0.4	23.3 ± 16.3	9.4 ± 4.3	7.6 ± 0.2
South	1000 IBA, 500 NAA	2.3 ± 0.2	23.3 ± 11.3	9.2 ± 1.6	4.2 ± 1.3
Site		NS	***	*	NS
NAA		NS	NS	NS	*
Site × NAA		*	NS	*	*
IBA		NS	NS	NS	NS
Site × IBA		NS	NS	NS	*
IBA × NAA		NS	*	*	NS
Site × IBA × NAA		**	***	NS	NS

Non-significant (NS) at $\propto$=0.05 (*), 0.01 (**), or 0.001 (***).

Table 3-6. Trend analysis of *P. polygama* root index. Main effects equaled the source locations. Sub-plots equaled factorial combinations of IBA and NAA. ANOVA indicated that the interaction of IBA × NAA was significant.

		NAA				
IBA		0[z]	250	500	*L*	*Q*
0		2.33	1.85	2.58	*NS*	**
500		2.70	2.17	2.37	*NS*	*NS*
1000		2.20	2.47	2.43	*NS*	*NS*
	L	*NS*	*	*NS*		
	Q	*	*NS*	*NS*		

[z]Non-significant (NS) or significant at ∝=0.05 (*), 0.01 (**), or 0.001 (***).

Table 3-7. Trend analysis of *P. robusta* root index. Main effects equaled the source locations. Sub-plots equaled factorial combinations of IBA and NAA. ANOVA indicated that the site × IBA × NAA interactions were significant.

			NAA[z]				
	IBA[z]		0	250	500	*L*	Q
Central	0		3.00	2.93	1.60	*	*NS*
	500		2.97	2.83	2.67	*NS*	*NS*
	1000		3.30	2.77	2.93	*NS*	*NS*
		L	*NS*	*NS*	*		
		Q	*NS*	*NS*	*NS*		
South	0		2.83	2.07	2.90	*NS*	**
	500		2.07	3.57	2.43	*NS*	**
	1000		2.13	2.90	2.27	*NS*	*
		L	*NS*	*NS*	*NS*		
		Q	*NS*	**	*NS*		

[z]Non-significant (NS) or significant at ∝=0.05 (*), 0.01 (**), or 0.001 (***).

Table 3-8. Trend analysis of *P. robusta* percent rooting. Main effects equaled the source locations. Sub-plots equaled factorial combinations of IBA and NAA. ANOVA indicated that the site × IBA × NAA interactions were significant.

			NAA[z]				
	IBA[z]		0[z]	250	500	*L*	*Q*
Central	0		52.06	55.02	20.59	*NS*	*NS*
	500		51.13	54.00	47.23	*NS*	*NS*
	1000		67.18	47.23	53.36	*NS*	*NS*
		L	*NS*	*NS*	*		
		Q	*NS*	*NS*	*NS*		
South	0		45.00	11.87	46.95	*NS*	**
	500		14.11	66.26	20.23	*NS*	***
	1000		21.51	47.87	22.82	*NS*	*
		L	*NS*	*NS*	*NS*		
		Q	*	***	*NS*		

[z]Non-significant (NS) or significant at ∝=0.05 (*), 0.01 (**), or 0.001 (***).

Table 3-9. Trend analysis of *P. robusta* root number. Main effects equaled the source locations. Sub-plots equaled factorial combinations of IBA and NAA. ANOVA indicated that the interaction of IBA × NAA was significant.

		NAA				
IBA		0[z]	250	500	*L*	*Q*
0		8.18	5.59	6.08	*NS*	*NS*
500		5.34	8.00	8.53	*NS*	*NS*
1000		5.83	10.27	8.27	*NS*	*
	L	*NS*	**	*NS*		
	Q	*NS*	*NS*	*NS*		

[z]Non-significant (NS) or significant at ∝=0.05 (*), 0.01 (**), or 0.001 (***).

Table 3-10. Trend analysis of *P. robusta* root length. Main effects equaled the source locations. Sub-plots equaled factorial combinations of IBA and NAA. ANOVA indicated that the Site × IBA pooled over NAA (top) and Site × NAA pooled over IBA (bottom) interactions were significant.

	Site	
IBA	Central	South
0	5.11	6.38
500	7.19	5.37
1000	8.08	4.45
L	*	*NS*
Q	*NS*	*NS*
NAA	Central[z]	South
0	7.16	2.80
250	7.53	7.23
500	5.69	6.17
L	*NS*	*NS*
Q	*NS*	*

[z]Non-significant (NS) or significant at ∝=0.05 (*), 0.01 (**), or 0.001 (***).

Figure 3-1. Representation of 5 root index categories, from left to right, where 5= heavy rooting, 4= medium rooting, 3= light rooting, 2= alive with no roots and 1=dead. Cuttings are from *P. polygama* collected in south Florida.

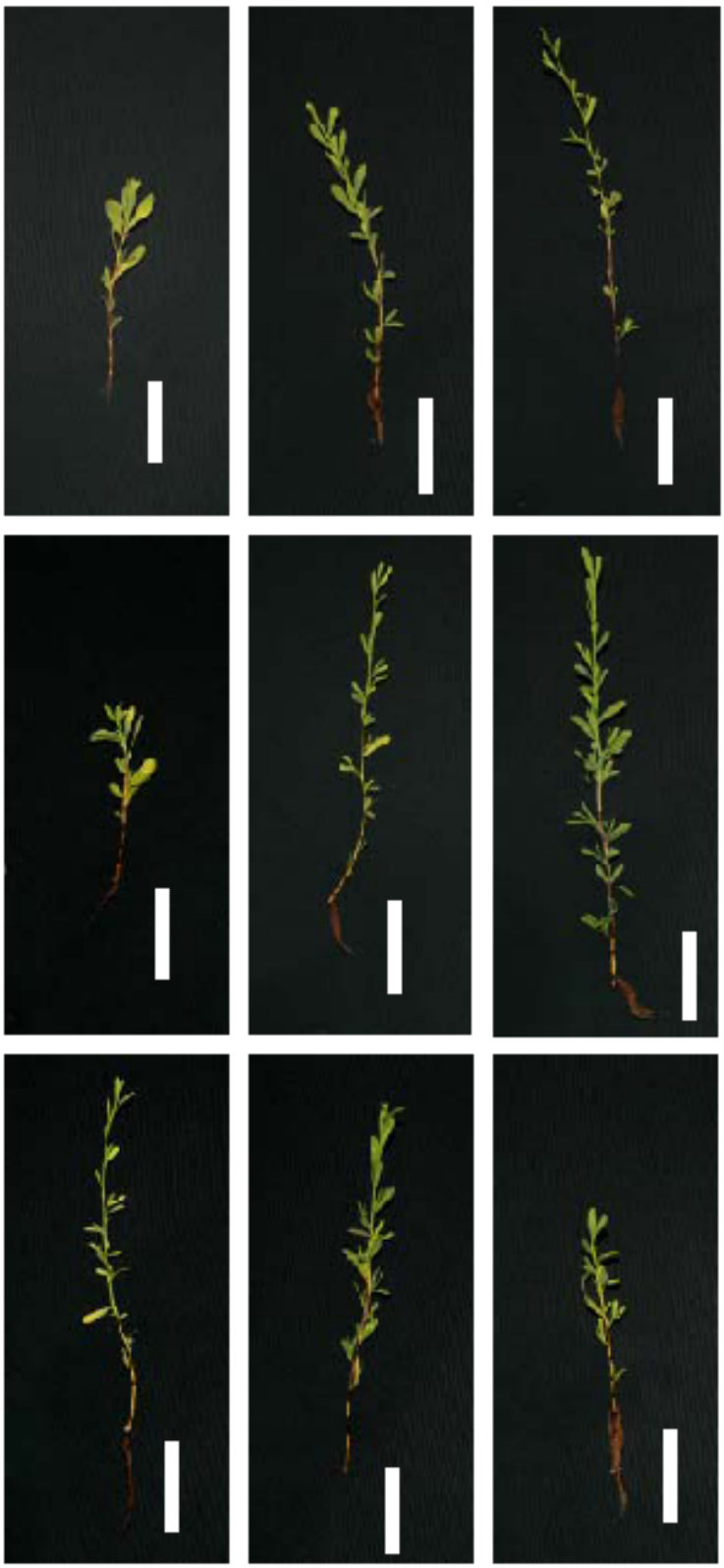

Figure 3-2. Representative central Florida *P. polygama* cuttings 8 wk after being quick dipped in various rooting hormone concentrations. Starting at the top row, from left to right, NAA:IBA ppm, 0:0, 0:500, 0:1000, 250:0, 250:500, 250:1000, 500:0, 500:500, and 500:1000. Scale bars represent 4 cm (1.6 in).

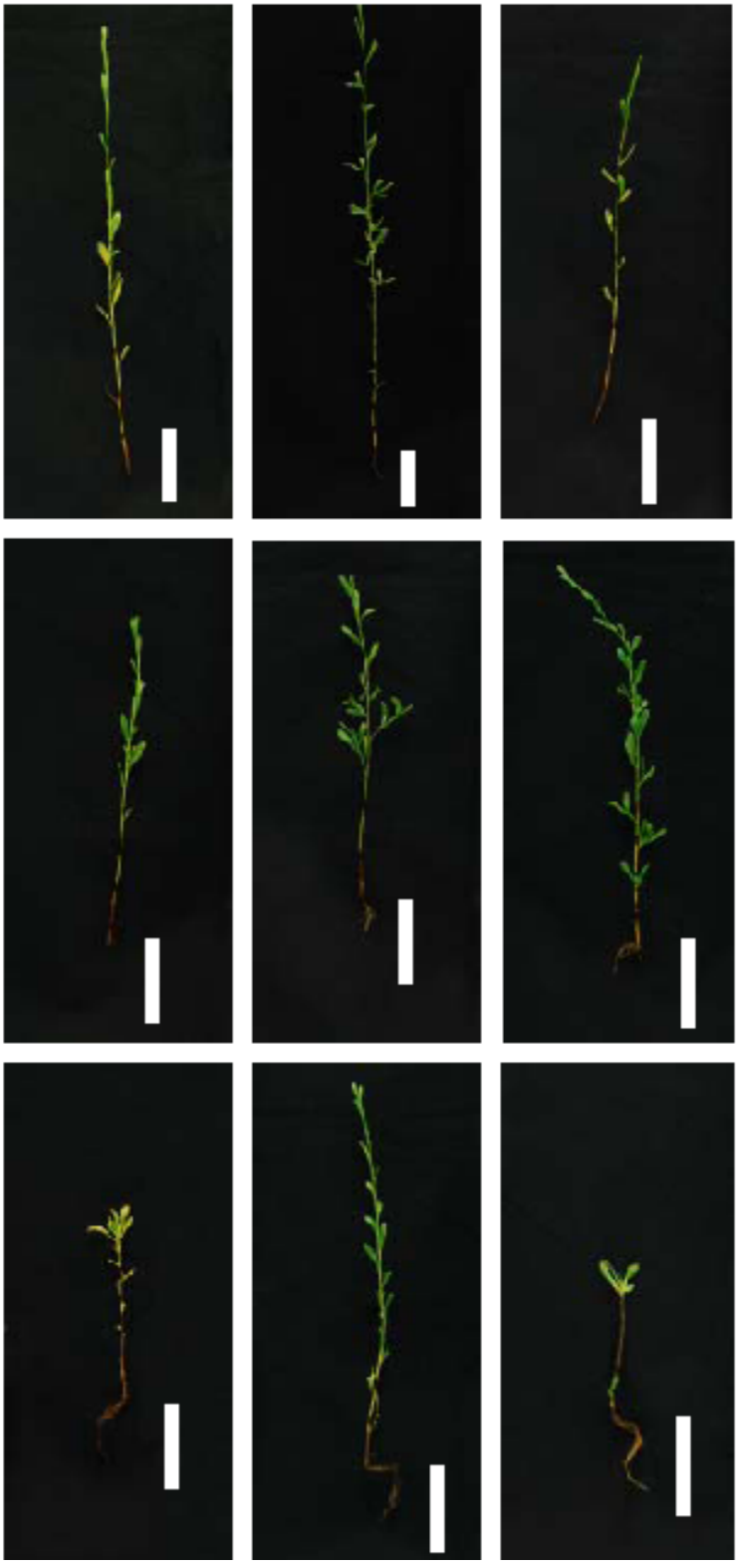

Figure 3-3. Representative south Florida *P. polygama* cuttings 8 wk after being quick dipped in various rooting hormone concentrations. Starting at the top row, from left to right, NAA:IBA ppm, 0:0, 0:500, 0:1000, 250:0, 250:500, 250:1000, 500:0, 500:500, and 500:1000. Scale bars represent 4 cm (1.6 in).

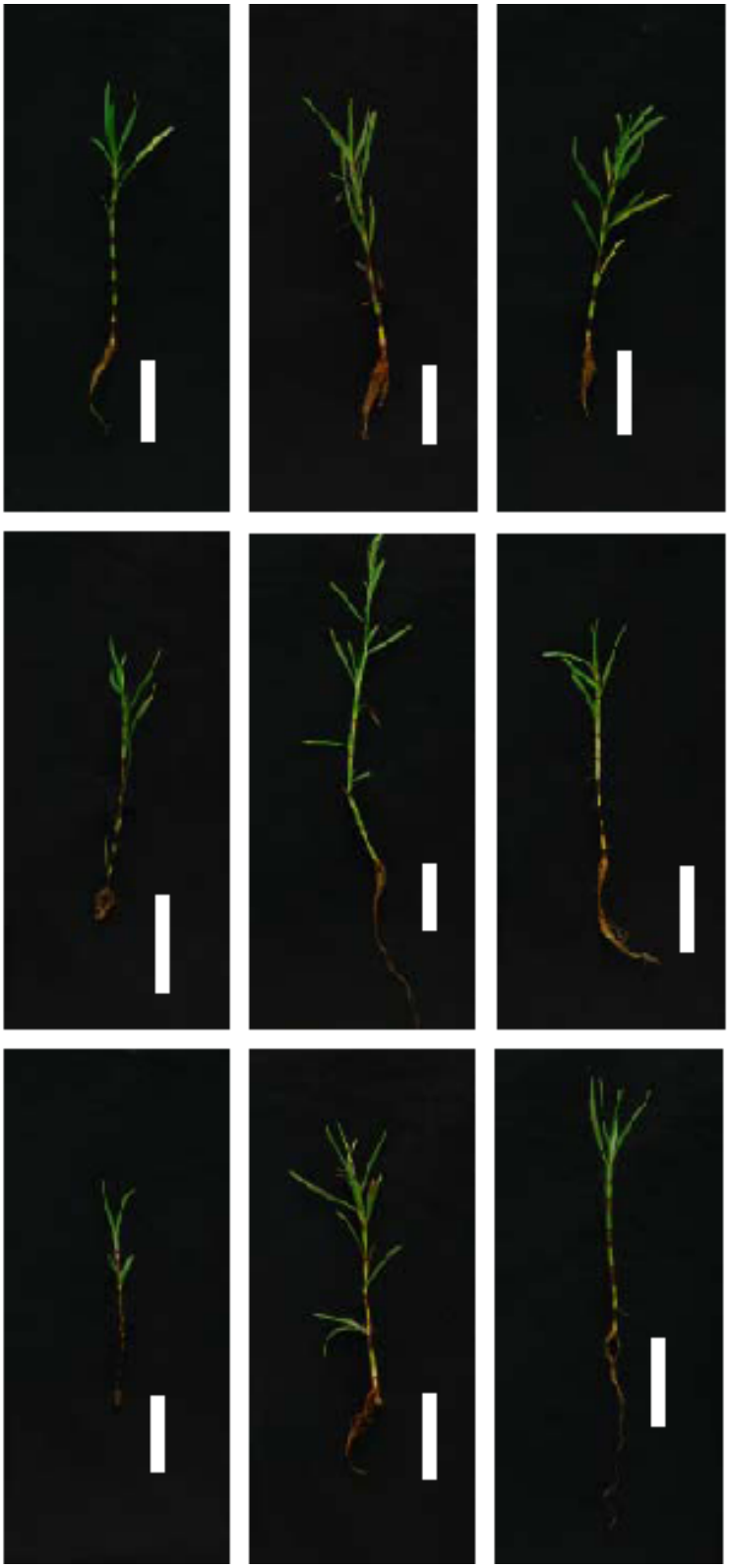

Figure 3-4. Representative central Florida *P. robusta* cuttings 6 wk after being quick dipped in various rooting hormone concentrations. Starting at the top row, from left to right, NAA:IBA ppm, 0:0, 0:500, 0:1000, 250:0, 250:500, 250:1000, 500:0, 500:500, and 500:1000. Scale bars represent 4 cm (1.6 in).

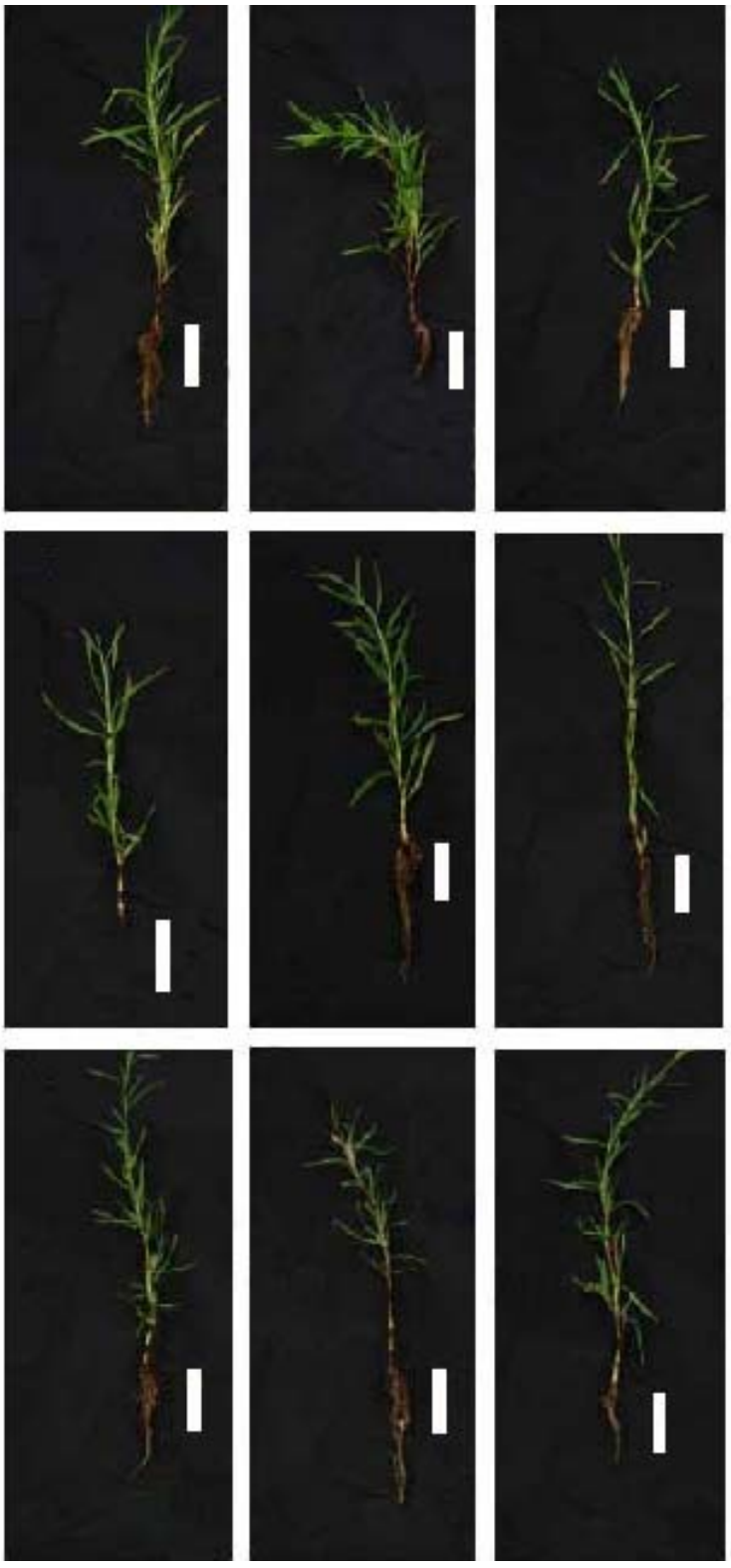

Figure 3-5. Representative south Florida *P. robusta* cuttings 6 wk after being quick dipped in various rooting hormone concentrations. Starting at the top row, from left to right, NAA:IBA ppm, 0:0, 0:500, 0:1000, 250:0, 250:500, 250:1000, 500:0, 500:500, and 500:1000. Scale bars represent 4 cm (1.6 in).

CHAPTER 4
CONCLUSIONS

The intent of this thesis was to find effective methods of propagating October flower (*Polygonella polygama* (Vent.) Engelm. & A. Gray [Polygonaceae]) and sandhill wireweed (*Polygonella robusta* (Small) G.L. Nesom & V.M. Bates [Polygonaceae]) to enable commercial production for use in landscape and restoration. Both species have tremendous value aesthetically and would provide ecological benefits to the natural landscape.

The first method investigated was sexual, or seed propagation (Chapter 2). Seeds were tested for viability using a TZ solution and germinated in four alternating temperature regimes to mimic seasons in Florida. From these experiments it was determined that seeds of both species are fairly viable, but also dormant. To determine the type of dormancy, seeds were subjected to various germination experiments. By monitoring fresh weight gain over time it was determined that intact or scarified seeds imbibed water regularly. Moreover, using light microscopy, seeds and fruit coats were examined for the presence of anatomy typical of tissues that do not imbibe water; for example, multiple lignified cell layers, palisade layers of thick-walled sclerids, and water gaps. None of these features were found. Therefore, it was determined that neither species possessed physical dormancy. By measuring the embryo to seed ratios of histological sections it was found that both species have fully developed embryos, and are therefore not morphologically dormant. Physiological dormancy was confirmed using a move-along experiment, where germination was highest when first treated with warmer temperatures, then moved to cooler ones. Additionally, gibberellic acid, known

to improve germination in seeds with physiological dormancy, showed a linear increase in germination with the increase in GA_3 concentration for both species.

To reflect more practical conditions found in a nursery setting (as opposed to germination in petri dishes placed in controlled incubators), *P. polygama seeds* were sown on various substrates and placed in a closed greenhouse with overhead mist (Chapter 2). Greatest germination was achieved with sand as the substrate rather than the standard commercial mix or even the Florida native mix. Physiological dormancy was alleviated in some seeds of the population when seeds were moist stratified for two weeks prior to sowing. Contrary to the laboratory experiments, the application of GA did not improve germination. In each of the greenhouse experiments, seeds were sterilized with bleach or not sterilized prior to sowing; however, sterilization had no effect on percent emergence.

In Chapter 3 asexual propagation methods were investigated. Softwood cuttings were collected from natural populations in south and central Florida. Cuttings were subjected to nine auxin treatments and placed in overhead mist for 6-8 weeks. Visual root quality, root initiation, root length, and root number were assessed. Regardless of species, no auxin treatment proved to be most effective in promoting adventitious root formation. *P. robusta* cuttings appeared to have a greater response to auxin, with greater root indices and rooting percentages, than *P. polygama*. It was observed that the location from which cuttings were collected also affected root initiation and quality.

Germination rates were found to increase with cold stratification and use of sand while rooting of cuttings was variable potentially due to auxin concentrations, cutting type, media, and light. Therefore, it is concluded that due to the results of this thesis

these species can be successfully propagated from seed. However, more research is needed on the germination biology of these species; especially with seeds collected at the shedding stage. Furthermore, landscape trials including both species, transplanted from seedlings and cuttings, are currently being held. Preliminary observations indicate that plants grown from seed appear healthier and with better form then those grown from cuttings.

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BIOGRAPHICAL SKETCH

Alison graduated from the University of Florida in 2007 with a Bachelor of Science degree in Environmental Horticulture. She obtained her Master of Science degree in December of 2009 in the same department with a focus on Plant Restoration and Conservation.