EVALUATING THE ORNAMENTAL POTENTIALS OF FOUR NATIVE TEXAS COASTAL SPECIES

A Dissertation

by

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ABSTRACT

With increasing demand for high quality irrigation water and coastal development, new plants need to be developed that thrive with the use of saline irrigation and provide an alternative to invasive exotic landscape plants. Accessions of *Erigeron procumbens* (Houst. *ex* Mill.) G.L. Nesom, *Borrichia frutescens* (L.) DC., *Sesuvium portulacastrum* (L.) L., and *Oenothera drummondii* Hook. were collected along the Texas coast from Port Isabel to Port Arthur. Then taxa were screened for phenotypic variability, responses to plant growth regulators, and tolerances to saline irrigation water. Phenotypic variability appeared most promising in *O. drummondii* and *B. frutescens* for future breeding efforts.

There were differences among accessions for all four species and there were regional differences in flowering and height for *B. frutescens* and *O. drummondii*. For *B. frutescens* height was reduced 54.9% by paclobutrazol at 40 mg a.i.·pot^{-1} and 34.9% by uniconazole at 2 mg a.i.·pot^{-1} when applied as a drench. Height and plant growth in *O. drummondii* was controlled with paclobutrazol drenches of 30 mg a.i.·pot^{-1} and uniconazole drenches of 1.5 mg a.i.·pot^{-1}. Similar results were found in *E. procumbens* and *S. portulacastrum*, with spray applications of PGRs being generally ineffective on all four taxa.

All four taxa were irrigated with four concentrations of saline water, with electrical conductivities (EC) of 0.8, 15.1, 23.8, 51.3, and 92.5 mS·cm^{-1}, either applied sub-canopy or over the foliage. Concentrations above the control roughly represented the

salinity of quarter, half, full, and double the salinity of seawater, respectively. All four species survived irrigation water with an EC of 23.8 $mS \cdot cm^{-1}$ and showed minimal damage with 15.1 $mS \cdot cm^{-1}$. Growth responses, mineral nutrient content, and K/Na ratios were consistent with reports of the halophytic nature of *S. portulacastrum*, which tolerated the greatest salinity exposure.

Due to commercial interest, mating system and pollen storage studies were carried out on *O. drummondii*. In mating system experiments, out crossed flowers had a mean seed count of 240.5 (± 17.5) $seed \cdot fruit^{-1}$ and selfed flowers across all dates and treatments had a mean seed count of 285.0 (± 14.1) $seeds \cdot fruit^{-1}$ after 10 d on filter paper. This indicates a facultative out-crossing species and emasculation of flowers is not necessary in pollinator excluded environments.

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CHAPTER I

INTRODUCTION

With the decreasing availability of high quality irrigation water in urban areas new ornamental horticulture crops need to be developed for landscapes that will thrive with use of lower quality more saline irrigation water (Niu and Rodriguez, 2008). The purpose of this dissertation is to begin the screening process for ornamental traits and the nursery production potential of four Texas coastal natives, *Sesuvium portulacastrum* (L.) L., *Borrichia frutescens* (L.) DC., *Oenothera drummondii* Hook. and *Erigeron procumbens* (Houst. *ex* Mill.) G.L. Nesom. These plant species were selected from Texas coastal regions because given their proximity to the ocean they are thought to have natural tolerance to salt exposure, especially in the form of sodium and chlorine ions. Native plants were also selected because of the growing trend toward use of natives in ornamental landscapes for their adaptability to their endemic region and low potential to become an invasive species. Examples of introduced exotics that are now major invasive species in Florida and Texas are *Schinus terebinthifolia* Raddi (Donnelly et al., 2008) and *Eichhornia crassipes* (Mart.) Solms (Villamagna and Murphy, 2010).

Not all native plants may be suitable for general landscape use. Plants selected must be able to adapt to commercial container nursery production techniques, tolerate low quality irrigation water, salt spray, and have some form of regional variation to provide a basis for the future improvement of cultivated selections. In this study the four species will be subjected to four sets of experiments. The first experiments will sample

regional phenotypic variation in the four species, as the basis for the beginning of selective improvement. The second group of experiments will test the ability of the four species to maintain ornamental viability under salt stress applied in the form of subcanopy saline irrigation. The third set will test the four species' ability to maintain ornamental characteristics (aesthetically appealing flowers, foliage, and growth habit) when foliar salts are applied, as in sprinkler irrigation or salt spray in coastal landscapes. The fourth group of experiments will treat the plants with commercial plant growth regulators to determine if the species are responsive to treatments aimed at producing more compact plants via inhibition of internode extension, and determine the rates with the best efficacy.

The four species selected are all native to the Texas Gulf Coast (Correll and Johnston, 1970; USDA Plants Database, 2009). Plants native to coastal environments are exposed to more saline conditions near seashores and tidal estuaries (Taiz and Zeiger, 2006). Therefore it can be extrapolated that plants native to coastal environments should be able to tolerate greater salt loads in low quality irrigation water and maintain an attractive ornamental state. Other elements, such as heavy metals, are rarely found at phytotoxic levels in irrigation water and treated waste water effluents from urban sources (Wu et al., 2001). Texas native plants were selected to prevent the release of potentially invasive noxious weeds, as opposed to the case of *Schinus terebinthifolia* Raddi, an ornamental used in coastal environments that has become invasive (Donnelly et al., 2008). Results of these studies will reveal whether *Sesuvium portulacastrum* (L.) L., *Borrichia frutescens* (L.) DC., *Oenothera drummondii* Hook., or *Erigeron*

procumbens (Houst. *ex* Mill.) G.L. Nesom are suitable for development of releases for the commercial ornamental nursery and landscape trades. To be considered suitable the four species must possess the ability to remain attractive under both acute and chronic foliar and soil salt exposure, be responsive to commercially acceptable nursery production technology, and have some geographical variation to provide the basis for future and ongoing improvement.

Botanical Descriptions

Botanical Description of Sesuvium portulacastrum (L.) L.

According to Correll and Johnston (1970) *S. portulacastrum* can be described as a "Glabrous fleshy perennial herb; stems trailing, much-branched, often rooting at the nodes, sometimes forming patches 2 m across; leaves narrowly oblong to oblanceolate or elliptic-obovate, to 6 cm long and 25 mm broad, obtuse- rounded to abruptly acute at apex, tapered into a clasping base (with these commonly overlapping); flowers pedicelled, solitary in the leaf axils; calyx lobes broadly ovate-lanceolate to lanceolate, to 1 cm long and 6 mm broad, hooded, pink-purple within, often strongly veined, with a subapical dorsal appendage; stamens numerous, the filaments about 5 mm long, the oblong-elliptic anthers about 1 mm long; ovary ovoid-globose about 3 mm long and thick; styles sometimes distinct to base; capsule conic, about 1 cm long and 5-6 mm in diameter; seeds black, smooth and lustrous, 1.2-1.5 mm long". *Sesuvium* L. is a pan tropical genus that grows on beaches and in brackish environments (USDA Plants Database, 2009). It can be found growing in sand dunes to the edges of bays with clay soils and appears to flower year round (USDA Plants Database, 2009). It is native in the

United States from Texas to Pennsylvania and also to Hawaii and Puerto Rico (USDA Plants Database, 2009).

Botanical Description of Borrichia frutescens (L.) DC.

According to Correll and Johnston (1970) *B. frutescens* can be described as a "Rhizomatous subshrub (2-) 4-8 (-12) dm tall, much branched but the branches all rather stiffly ascending; leaves opposite, variable in size and shape, obovate to oblanceolate to spatulate, 2-6 cm long, sessile or narrowed to a subpetiolar base, acutish or obtuse, entire or spinulose-dentate or even with small lobe like teeth on the sides near the base, thick and somewhat fleshy, gray-green, densely but minutely pubescent; heads terminating the branches on upwardly slightly expanded peduncles 1-3 cm long; involucres hemispheric, about 5 mm high; phyllaries rather indurate, in roughly 2 series; outer phyllaries about half to two thirds as long as the inner, acute, in texture and pubescence and color much like the leaves; inner phyllaries spinose-squarrose, nervate, less pubescent than the outer; receptacle flat or very slightly convex, chaffy throughout; pales firm or indurate, nearly linear but with a stout noxious spine tip; ray flowers 15 to 30, pistillate, fertile; rays 5-10 mm long, yellow or orangish, 3-toothed apically; disk flowers numerous, perfect, fertile, the corolla yellow and 5-toothed terminally; achenes prismatic, those of the ray flowers trigonous, of the disk flowers tetragonus; pappus a low crown of persistent brown scales, one over each angle of the achene". It is common in coastal areas and in inland areas with poor drainage and salt accumulation (Correll and Johnston, 1970). It is native from Texas to Maryland and there are some discontinuous populations in West Texas (USDA Plants Database, 2009).

Botanical Description of Oenothera drummondii Hook.

According to Correll and Johnston (1970) *O. drummondii* can be described as a "Suffrutescent densely canescent-villous perennial with many woody stems to 1m long from the base; leaves oblanceolate to obovate, 1-7 cm long, 5-15 mm wide, entire or remotely sinuate-dentate (especially basal ones), densely canescent-villous throughout; petioles 0-3 cm long; flowers opening near sunset; mature buds erect; hypanthium 2-4 cm long; sepals 2-3 cm long, with free tips 2-3 mm long; petals yellow, fading reddish, 2-3.5 cm long; capsule cylindrical, 2.5-4 cm long; seeds in 2 rows in locule, 1-1.2 mm long; n=7". *Oenothera drummondii* grows on sandy beaches and along the Gulf Coast from Texas to North Carolina (Correll and Johnston, 1970; USDA Plants Database, 2009).

Botanical Description of Erigeron procumbens (Houst. ex Mill.) G.L. Nesom

Erigeron procumbens is listed in Correll and Johnston (1970) as *Erigeron myrionactis* Small. *Erigeron myrionactis* is listed as a synonym of *Erigeron procumbens* (Houst. *ex* Mill.) G.L. Nesom in the USDA Plants Database, and is seen more commonly than *E. myrionactis*. "Perennial with stems stoloniferous or subrhizomatous in coastal sands, rooting at the nodes, prostrate; stems often to 1m long; herbage with spreading hairs; leaves obovate to spatulate or cuneate, 2-8 cm long, 5-25 mm broad, coarsely few-toothed near the end; heads solitary, borne about 1 dm above ground; rays numerous, white, 5-7 mm long, about 0.3mm broad; disk corollas 3.5-4.5 mm long; pappus of ray and disk essentially similar, simple, of about 20 to 25 fragile

capillary bristles" (Correll and Johnston, 1970). *Erigeron procumbens* grows in sandy soils along the coast from Texas to Mississippi (USDA Plants Database 2009).

Geographic Variation

Documenting the amount and kind of variation within a species is important for the success of a plant improvement program (Zobel and Talbert, 1984). Variation that is present due to geographic differences should be documented first, then followed by variation that occurs in other categories (Zobel and Talbert, 1984). "Ecotypic variation is a distinct morphological or physiological form, or population, resulting from selection by a distinct ecological condition" and "is the whole basis of provenance studies" (Arnold, 2008). Provenance studies should provide the foundation for genetic improvement of plant species (Morganstern, 1996). It appears that most adaptability traits are additive in nature and gains in improvement programs can be made by selecting individuals that already possess traits to grow in suboptimal conditions (Zobel and Talbert, 1984). Nooryazdan et al. (2010) found large variation of seed weight, leaf petiole, plant height, composite inflorescence (flower) diameter, leaf length, and sowing to flowering duration in 77 accessions, collections from native or naturalized populations, of wild *Helianthus annus* L. grown in the same field. Nooryazdan et al. (2010) performed correlation analysis between climate characteristics and morphology of the accessions and found sowing to flowering duration and plant height linked to temperature and rainfall. Accessions from arid regions were the most branched (Nooryazdan et al., 2010).

Wood et al. (1998) tested variation of plant height, trunk cross sectional area, limb angle, leaflet droop and tilt angle, winter cold injury, foliar bud break, leaf drop, and foliar coloration, and foliar susceptibility to zinc deficiency and black pecan aphid in *19 Carya illinoinensis* (Wangenh) K. Koch. accessions. Nuts were collected from five trees creating five half-sib families from each of 19 distinct provenances from Oaxaca, Mexico to Northern Missouri. Southern provenances as compared to the northern provenances tended to retain foliage longer in the winter season ($r^2 = 0.82$), tended to exhibit less red coloration of rachis, had taller trees ($r^2 = -0.61$), and generally exhibited more cold injury.

Hester et al. (1996) collected 19 clones of *Spartina patens* (Aiton) Muhl. from coastal marsh locations in Texas, Louisiana, and Florida with varying levels of salinity. These 19 accessions varied in plant height (88 – 119 cm), internode distance (3.98 – 7.88 cm), and leaf length (25 – 62 cm). There were significant differences among the genotypes in lethal salinity levels which ranged from 63.0% to 93.0% (grams salt/ kilogram solution) in Hoagland's nutrient solution (Hester et al., 1996). Lokhande et al. (2009) collected 14 clones of *S. portulacastrum* from coastal regions all over India and characterized the material based on stem color, internode length, stem diameter, leaf length, leaf width, leaf diameter, and leaf area. These 14 clones varied in leaf length (29.60 - 69.60 mm), leaf width (5.86 - 15.93 mm), leaf diameter (2.46 - 4.90 mm), leaf area (14.30 - 59.73 mm^2), internodal distance (24.80 - 129.60 mm), and stem diameter (3.73 - 8.86 mm). Morphologically these clones clustered into three groups based on the pattern of secondary branching (Lokhande et al., 2009).

Salinity Issues

Demand for high quality water for human consumption may make the use of recycled water to irrigate urban landscapes inevitable (Niu and Rodriguez, 2006). The composition of treated waste water varies among communities and depends on composition of the original source of water, and type and number of industrial, commercial, and residential users that are contributing to the source of treated waste water (Wu et al, 2001). With recycled water, such as treated effluent, the major concern is elevated salinity which can be as much as two to three times the level of potable water (Khurram and Miyamoto, 2005). "Generally, potable water picks up large quantities of inorganic salts in one cycle of use" (Wu et al, 2001) and after most recycled water treatment processes, sodium chloride is the most potentially deleterious chemical remaining (Wu et al., 2001). "Foliar burn or chlorosis and even stunting and plant mortality can be caused by long term irrigation with treated effluent which leads to salt build up in the soils" from the elevated salinity of most treated effluents (Fox et al., 2005).

Salts can induce a number of stress responses in plants: salts can affect general water balance, ion toxicity especially Na^+, Cl^-, or SO_4^{2-}, and inhibit nutrient uptake (Taiz and Zeiger, 2006). Water balance in the plant is affected by the dissolved solutes in the root zone causing a low osmotic potential that reduces soil water potential (Taiz and Zeiger, 2006). This results in a situation similar to water deficit even when an otherwise sufficient amount of water is available (Taiz and Zeiger, 2006). Plants can adjust to some extent to prevent a loss of turgor pressure through a lower water potential, however

growth is still slowed and plants exhibit responses similar to adjustment to water deficit (Taiz and Zeiger, 2006). Ions of Na^+, Cl^-, or SO_4^{2-} can accumulate to toxic levels in plants in saline environments (Taiz and Zeiger, 2006). The accumulation of ions especially a high ratio of Na^+ to K^+ can inhibit protein synthesis and inactivate enzymes and lead to cell death (Taiz and Zeiger, 2006).

All of these responses to salt stress can lead to a loss of ornamental appeal. A buildup of ions from the transpirational stream can lead to leaves with necrotic edges or leaf abscission giving the plant a general unhealthy appearance that is unacceptable for ornamental use (Maas, 1993; Marschner, 1995; Sykes, 1993). Salt ions interfering or interacting with nutrient absorption can lead to chlorotic plants (plants yellow from degradation or failure of chlorophyll to form) that are unacceptable in ornamental applications (Arnold, 2008; Maas, 1993; Marschner, 1995; Sykes, 1993).

Salt tolerance can be achieved by salt exclusion or by salt inclusion. Salt exclusion requires mechanisms to avoid internal water deficit and salt inclusion requires either tissue tolerance to high levels of Na^+ or Cl^- or avoidance of high tissue concentrations (Marschner, 1995). Salt excluders whether sensitive to salts or tolerant may translocate Na^+ to the roots, effectively lowering shoot Na^+ levels (Marschner, 1995). Halophytes are plants tolerant to high sodium levels and/or relatively high levels of sodium may stimulate their growth (Marschner, 1995). In many halophytes, barriers in the roots control passive influx of salts, for example *Avicennia marina* (Forssk.) Vierh. when exposed to saline water, can exclude 80% of the salts from uptake (Marschner, 1995; Waisel et al., 1986). Selective permeability and efflux pumps in root

membranes are the main mechanisms that glycophytes (non-halophytes) employ to control passive uptake of Na^+ and Cl^- (Marschner, 1995). The partitioning of Na^+ and Cl^- between old and young leaves and reproductive structures, and the restricted import of Na^+ and Cl^- into young leaves are characteristics of salt tolerant species such as *Kosteletzkya virginica* (L.) C. Presl. *ex* A. Gray which can maintain a concentration gradient from the oldest to youngest leaf of 230 mM to 25 mM of Na^+, respectively (Marschner, 1995; Blits and Gallagher, 1990).

In saline substrates caused by salty irrigation, plants must adjust to decreased substrate water potential to avoid foliar damage induced by water stress. Plants that are salt tolerant via salt exclusion must increase osmotically active solutes such as organic solutes and amino acids or the uptake of K^+, Ca^{2+},or NO^{3-} (Gorham et al., 1985; Marschner, 1995). Plants that are salt includers accumulate salts in the leaf tissues (Marschner, 1995). Under saline conditions salt tolerant includers, such as members of the Chenopodiaceae Vent., the ability of the mesophyll vacuole to accumulate Na^+ and Cl^- is increased through succulence delaying the accumulation of Na^+ and Cl^- in the leaf apoplasm and cytoplasm (Flowers et al., 1986; Gorham et al., 1985; Marschner, 1995). To protect enzymes in the cytoplasm includer species must sequester Na^+ and Cl^- in the vacuole and maintain osmotic potential of the cytoplasm and organelles through non-toxic solutes, such as gylcine betaine (Marschner, 1995). For example spinach (*Spinacia oleracea* L.) leaves exposed to 300 mM of NaCl the Na^+ and Cl^- were mainly sequestered in the vacuole and the K^+ was still adequate to maintain photosynthesis (Marschner, 1995).

Halophytes, salt tolerant plants, can reduce salt loads on photosynthetic tissue by storing salt ions in bladder hairs, secretion, shedding old leaves, or retranslocation to other organs (Marschner, 1995, Waisel et al., 1986). The link of salt tolerance to salt excretion by many halophytes can be shown in halophytes' intolerance to salt during *in vitro* callus culture and tolerance of salt as a whole plant *in vivo* (Marschner, 1995, Smith and McComb, 1981).

"Visual appearance of landscape plants is very important to landscape designers, managers, and to the public" (Fox et al., 2005). The use of salt tolerant or halophyte species could potentially reduce the ornamental liabilities of salt stress, allowing for lower quality irrigation water to be used in production of the plant materials and landscape irrigation without a loss of ornamental function. Currently there is a general absence of literature on the four species described herein with respect to ornamental horticulture and salt studies.

Growth Regulation in Production

According to Basra (2000), "Growth retardant chemicals are used primarily to control stem elongation of ornamental plants while they are growing in containers rather than after the plants are transplanted into the landscape. In production of horticultural crops, high fertility and unlimited supply of water are used to enhance plant growth. This often leads to a plant that is taller than desired". Plant growth retardants, such as triazole compounds, onium compounds, and daminozide, can be used to reduce plant height to produce more marketable plants (Basra 2000; Niu et al., 2002; Pallez et al.,,

2002; Whipker and McCall, 2000), extend postharvest life (Keever and Kessler, 2008) and increase tolerance to salinity stress (Dubey et al., 2008).

Triazoles' main mode of action is to inhibit the biosynthesis of gibberellins and sterols in the isopreniod pathway (Fletcher and Hofstra, 1985), thus reducing plant stem elongation (Basra, 2000). With triazoles this is achieved by binding to cytochrome P-450 systems, displacing oxygen and disrupting the cytochrome P-450 system, thus reducing the synthesis of ent-kaurene to ent-kaurenoic acid blocking GA production (Basra, 2000; Rademacher, 1997). This reduction in GA synthesis leads to reduced stem internode elongation and more compact plants (Basra, 2000; Fletcher and Hofstra, 1985; Taiz and Zeiger, 2006). Triazoles also affect the same biosynthetic pathways in fungi resulting in limited fungicidal properties (Basra, 2000; Benton and Cobb, 1997; Fletcher and Hofstra, 1985; Rademacher, 1991). In addition to fungicidal properties triazoles are known to affect levels of ABA (Mackay et al., 1990), cytokinins, and ethylene production in plants (Basra, 2000; Luo et al., 1987; Sauerbrey et al., 1988). Many species of plants treated with triazoles develop darker green leaves from elevated chlorophyll levels (Basra, 2000; Fletcher et al., 1986). These responses may lead to increased tolerances to other biotic and abiotic stresses (Basra, 2000; Fletcher and Hofstra, 1985).

Daminozide acts either as a blocker of gibberellin translocation or as a promoter of gibberellin degradation (Basra, 2000; Rademacher, 1991). Daminozide appears to function in a similar manner has prohexadione to block late stages of GA synthesis (Brown et al., 1997). The effective dose of daminozide is often much larger than

effective doses of triazoles to gain the same amount of height control (Banko and Stefani, 1988; Basra, 2000; Burnett et al., 2000; Whipker and Dasoju, 1998).

The family of triazole plant growth regulators (PGRs) are known plant growth retardants. However different members of the triazole PGRs can vary in efficacy on the same species (Burnett et al., 2000; Whipker and Dasoju, 1998), among species (Banko and Stefani, 1988), and among cultivars of the same species (Whipker and McCall, 2000). Whipker and Dasoju (1998) reported, in *Helianthus annuus* L., that uniconazole ([(E)-(p-chlorophenyl)-4,4-dimethyl-2-(1,2,4-triazol- 1-yl)- 1-penten-3-ol]) applied at 32 $mg \cdot L^{-1}$ as a foliar spray provided a 17% reduction in height over controls as compared to paclobutrazol ((±)-(R*,R*)-β-[(4-Chlorophenyl)methyl]-α-(1,1-dimethylethyl)-IH-1,2,4-triazole-1-ethanol) which when applied at 40 $mg \cdot L^{-1}$ or 80 $mg \cdot L^{-1}$ as a foliar spray reduced height only 6%. Daminozide ([butanedioic acid mono (2,2-dimethylhydrazide)]) in concentrations greater than 4,000 $mg \cdot L^{-1}$ as a foliar spray resulted in a 17% or greater reduction in height (Whipker and Dasoju, 1998). Uniconazole applied at 32 $mg \cdot L^{-1}$ as a foliar spray and daminozide at 4,000 to 16,000 $mg \cdot L^{-1}$ as a foliar spray reduced inflorescence diameter, while paclobutrazol had no effect on inflorescence diameter.

Daminozide, paclobutrazol, and uniconazole flowering was 2 to 3 days later than the control (Whipker and Dasoju, 1998). Burnett et al. (2000) reported a similar 2 to 3 day delay of flowering in paclobutrazol, uniconazole, and daminozide in *Coreopsis rosea* Nutt. 'American Dream'. Paclobutrazol (applied at rates 12 to 60 $mg \cdot L^{-1}$ as a foliar spray) did not significantly control height of *C. rosea* Nutt. 'American Dream',

however uniconazole (applied at rates of 10 to 40 mg·L^{-1} as a foliar spray) reduced shoot height by 25-29% and daminozide (applied at rates of 2,500 to 7,500 mg·L^{-1} as a foliar spray) reduced shoot height 17-29% (Burnett et al., 2000). In *Oenothera fruticosa* L. 'Youngii-lapsley', three foliar applications of daminozide and paclobutrazol at 5000 mg·L^{-1} and 30 mg·L^{-1}, respectively, had no effect on plant height, however uniconazole at 15 mg·L^{-1} reduced height by 31% (Clough et al., 2001). Uniconazole also reduced flower diameter of *O. fruticosa* by 36% (Clough et al., 2001). Uniconazole and daminozide treatments also reduced lateral branch length altering the plants' form (Clough et al., 2001). Because of the shortage of literature regarding the four species in this study, experimental rates will be derived from responses of related species such as *Helianthus annuus* L. for *B. frutescens* and *E. procumbens*, *Oenothera fruticosa* for *O. drummondii*, and *Portulaca grandiflora* Hook. for *S. portulacastrum*.

Whipker and McCall (2000) reported that paclobutrazol drenches at 2 mg/pot and 4 mg/pot controlled height of sunflower by 24% and 33%, respectively. Gibson and Whipker (2003) found that paclobutrazol sprays ≤ 80 mg·L^{-1} and uniconazole sprays ≤ 24 mg· L^{-1} were ineffective at controlling height in *Osteospermum ecklonis* (DC.) Norl., however paclobutrazol drenches of 16 mg/pot reduced height 16%, and uniconazole drenches of 0.5 mg·pot^{-1} reduced height 35% and 1.0 mg·pot^{-1} caused severe plant stunting. However, paclobutrazol was ineffective at controlling plant height when applied as a foliar spray to *O. fruticosa* 'Youngii-lapsley' , *Coreopsis rosea* Nutt. 'American Dream' and *Helianthus annuus* L. (Clough et al., 2001; Burnett et al., 2000; Whipker and Dasoju, 1998), but was effective when applied as a drench on *H. annuus*

(Whipker and McCall 2000; Pallez et al., 2002) and *O. ecklonis* (Gibson and Whipker, 2003). The differences in efficacy between the modes of application suggests that paclobutrazol and uniconazole both need to be tested as both foliar sprays and media drenches to control height of *S. portulacastrum*., *O. drummondii*, *E. procumbens*, and *B. frutescens*.

On the basis of work performed in the first portion of the proposal and interest from the commercial marketing firms in the ornamental horticulture industry *O. drummondii* was chosen for advancement to further studies.

Mating System

The breeding system of *O. drummondii* needs to be documented to ease the process of controlled pollination under greenhouse conditions. Flowers of *O. drummondii* have approach herkogamy (separation of the stigma above the anthers), this in combination with heavy viscin threads on the pollen may make emasculation of flowers unnecessary where pollinators are excluded (Wagner et al. 2007; Theiss et al., 2010).

Theiss et al. (2010) used pollination tests to determine if 10 species of *Oenothera* L. were self-compatible or self-incompatible. Most of the species in the study exhibited herkogamy. The authors grew plants under greenhouse conditions and allocated equal number of flowers to each pollination treatment. Pollination treatments were pollination from pollen of the same plant as the flower (selfing) or pollen from another plant of the same species (outcrossing). Flowers were not emasculated, but stigmas were visually checked for contamination by unwanted pollen. Swollen fruit with seed were considered

successful and recorded. One thing they may have over looked is the number of seed set to determine the preference to one type of pollen treatment. Also, if they had added an un-emasculated un-pollinated flower with an emasculated un-pollinated flower the percent of self-fertilization and agamospermy could have been determined.

Wolin et al. (1984) performed a similar experiment on *Oenothera speciosa* Nutt. Plants were enclosed in wire mesh to exclude pollinators. Flowers were either manually self-pollinated, crossed with pollen from a different genotype (out crossed), flowers were enclosed for self-pollination, or emasculated or left open for insect pollination. Then fruit set and seed per fruit were recorded. The emasculated and enclosed flowers did not set fruit. Self-pollination, intra-clonal pollination, emasculation (removal of anthers) and bagging (excluding pollen from the stigma), and bagging the flowers resulted in lower fruit set than open pollinated controls. Out crossed flowers set fruit at 100 % and set more seed than the other treatments. From the study Wolin et al. (1984) concluded that *O. speciosa* is partially self-incompatible and is not apomictic.

The purpose of the work described in this dissertation is to determine if four Texas coastal natives hold potential as alternatives to potentially invasive exotic species. They will be screened for naturally occurring phenotypic variation for the future improvement in selection and breeding programs and screened for responsiveness to PGR's which is often necessary in commercial nursery production. They will also be tested for tolerance to saline irrigation to represent non-traditional sources of irrigation and harsh saline environments. On the basis of interest from commercial entities, methods to more easily facilitate the controlled crossing of *O. drummondii* are

investigated through pollen storage and mating system studies as a prelude to initiation of subsequent breeding efforts.

CHAPTER II

VARIATION IN GROWTH HABIT AND PROPENSITY TO FLOWER

With the decreasing availability of high quality irrigation water in urban areas, new ornamental horticulture crops need to be developed for landscapes that will thrive with use of lower quality irrigation water. When introducing plants to the landscape or nursery trade it is important to determine the extent of variation present in native populations for ornamental traits. *Oenothera drummondii* Hook., *Sesuvium portulacastrum* (L.) L., *Borrichia frutescens* (L.) DC., and *Erigeron procumbens* (Houst. *ex* Mill.) G.L. Nesom were selected from Texas coastal regions based on their close proximity to the coast (Correll and Johnston, 1970; USDA Plants Database, 2009). This proximity to the coast should provide natural tolerance to salt exposure, especially in the form of sodium and chlorine ions because in these habitats plants are exposed to saline conditions (Taiz and Zeiger, 2006) for the four species. Regional native plants were also selected because of the growing trend toward use of natives in built landscapes for their adaptability to their endemic region and low potential to become invasive species. Sensitive coastal ecosystems can be threatened by invasive exotics such as *Schinus terebinthifolius* Raddi, *Melaleuca quinquenervia* (Cav.) S.F. Blake, and *Eichhornia crassipes* (Mart.) Solms (Ewe and Sternberg, 2002; Turner et al., 1998; Villamagna and Murphy, 2010).

Not all native plants may be suitable for general landscape use in built environments, particularly in coastal locations. Plants selected must be able to adapt to

commercial container nursery production techniques, tolerate low quality irrigation water, salt exposure, and have some form of regional and/or genetic variation to provide a basis for the future improvement of cultivated selections.

Documenting the amount and kind of variation within a species is important for the success of a plant improvement program (Zobel and Talbert, 1984). Variation that is present due to geographic differences should be documented first, then followed by variation that occurs from other sources (Zobel and Talbert, 1984). “Ecotypic variation is a distinct morphological or physiological form, or population, resulting from selection by a distinct ecological condition” and “is the whole basis of provenance studies” (Arnold, 2008). Provenance studies should provide the foundation for genetic improvement of plant species (Morganstern, 1996). It appears that most adaptability traits are additive in nature and gains in improvement programs can be made by selecting individuals that already possess traits to grow in suboptimal conditions (Zobel and Talbert, 1984).

Nooryazdan et al. (2010) found large variation of seed weight, leaf petiole length, plant height, composite inflorescence (flower) diameter, leaf length, and sowing to flowering duration in 77 accessions, collections from native or naturalized populations, of wild *Helianthus annuus* L. grown in the same field. Nooryazdan et al. (2010) performed correlation analysis among climate characteristics and morphology of the accessions and found sowing to flowering duration and plant height linked to temperature and rainfall. Accessions from arid regions were the most branched (Nooryazdan et al., 2010).

Wood et al. (1998) tested variation of plant height, trunk cross sectional area, limb angle, leaflet droop and tilt angle, winter cold injury, foliar bud break, leaf drop, and foliar coloration, and foliar susceptibility to zinc deficiency and black pecan aphid in 19 *Carya illinoinensis* (Wangenh) K. Koch. accessions. Nuts were collected from five trees creating five half-sib families from each of 19 distinct provenances from Oaxaca, Mexico to Northern Missouri. Southern provenances as compared to the northern provenances tended to retain foliage longer in the winter season, tended to exhibit less red coloration of the rachis, had taller trees, and generally exhibited more cold injury.

Hester et al. (1996) collected 19 clones of *Spartina patens* (Aiton) Muhl. from coastal marsh locations in Texas, Louisiana, and Florida with varying levels of salinity. These 19 accessions varied in plant height (88 – 119 cm), internode distance (3.98 – 7.88 cm), and leaf length (25 – 62 cm). There were significant differences among the genotypes in lethal salinity levels which ranged from 63.0% to 93.0% (grams salt/kilogram solution) in Hoagland's nutrient solution (Hester et al., 1996). Lokhande et al. (2009) collected 14 clones of *S. portulacastrum* from coastal regions all over India and characterized the material based on stem color, internode length, stem diameter, leaf length, leaf width, leaf diameter, and leaf area. These 14 clones varied in leaf length (29.60 - 69.60 mm), leaf width (5.86 - 15.93 mm), leaf diameter (2.46 - 4.90 mm), leaf area (14.30 - 59.73 mm2), internodal distance (24.80 - 129.60 mm), and stem diameter (3.73 - 8.86 mm). Morphologically these clones clustered into three groups based on the pattern of secondary branching (Lokhande et al., 2009).

The objectives of experiments described herein were to begin to characterize the variation in traits of ornamental interest in Texas' coastal populations of *O. drummondii*, *B. frutescens*, *E. procumbens*, and *S. portulacastrum* in a common field location and under container nursery conditions.

Materials and Methods

Clonal material of *B. frutescens*, *E. procumbens*, *S. portulacastrum*, and *O. drummondii* were collected from locations along the Texas coast from South Padre Island, Texas to Port Arthur, Texas. Global positioning system (GPS) data and physical location data were recorded (see appendix). Stock plants were generated from the collected material and used to conduct this provenance study in College Station, Texas.

Tip cuttings, 4-6 cm long, were taken on 17 April 2010, from containerized stock plants maintained in a gravel bottom nursery in College Station, TX (30° 37' 24.24", -97° 22' 0.17"). Basal ends of cuttings were dipped in talc based IBA at the concentration of 1 $g \cdot kg^{-1}$ (Hormodin® 1, OHP, Inc., Mainland, Pa.). Cuttings were placed in 36 cm x 51 cm x 10 cm deep flats (Kadon Corp., Dayton, OH) filled with coarse perlite (Sun Gro Horticulture Canada Ltd., Seba Beach, Alta.). Intermitted mist was applied at 16 min intervals for a 15 sec duration using reverse osmosis water from 1 h before sunrise to 1 h after sunset. On 13 May 2010, rooted cuttings were potted in 0.47 L black plastic pots (Dillen Products, Middlefield, Ohio) containing Metro-Mix 700 media (Sun Gro Horticulture Canada Ltd., Vancouver, B.C.).

Container responses: rooted cuttings generated as described above, from each accession collected, were potted into 2.3-L black plastic containers (C400, Nursery

Supplies Inc., Kissimmee, Fla.) containing Metro-Mix 700 media (Sun Gro Horticulture Canada Ltd, Vancouver, B.C.) with 6.53 $kg \cdot m^{-3}$ 15N-3.9P-9.9K controlled release fertilizer (3-4 month Osmocote® Plus, Scotts Co., Marysville, OH) on 3 June 2010. Plants were placed in an outdoor gravel bottom nursery with full sun exposure in a completely randomized design with three replicates of each genotype collected (N=3). Plants were irrigated as needed by hand using tap water with constant fertilizer injection (300 $mg \cdot L^{-1}$ of N, Peters Professional 20N-8.74P-16.6K, Scotts Co., Marysville, Ohio). On 10 July 2010 plant height, leaf lamina length, leaf width, internode length, stem diameter, and flower diameter were recorded for each as was done with other species in prior studies (Hester et al., 1996, Nooryazdan et al., 2010, Wood et al., 1998). Leaf and internode measurements were taken from three fully expanded leaves per plant. Flower data were taken from three open flowers per plant.

Landscape responses: rooted cuttings generated as described in the container nursery experiment were planted in field conditions at the Texas A&M University Horticulture Farm (30° 37' 34.0608", -96° 22' 14.2104") with five replicates of each genotype (N=5) on 1 m in row spacings and 4 m between row spacings on 2 June 2010. The soil is a sandy clay loam (66% sand, 8% silt, 26% clay) with a pH of 6.0. Plants were drip irrigated (T-Tape Model 505, Deere and Company, Moline, IL) as needed to maintain turgidity. Flower counts, plant volume (height x width in the widest direction x width perpendicular to the widest direction), and an ornamental rating were taken at the end of the growing season (1 Nov 2010). Also end of the season plant height, leaf lamina length, leaf width, internode length, and flower diameter were recorded for each

genotype (Hester et al., 1996; Nooryazdan et al., 2010; Wood et al., 1998). Leaf and internode measurements were taken on three fully expanded leaves on each plant. Flower width at the widest point was collected from three open flowers on each plant.

An ornamental rating of 1 to 5 was recorded by the same observer at harvest, with 1) representing a dead plant or plant near death (unacceptable for ornamental use), 2) plant with severe damage to the canopy but surviving, 3) plant with open holes in the canopy, erratic growth, and general lack of flowers, 4) canopy was full with uniform growth throughout, with or without flowers (acceptable ornamental landscape plant), and 5) canopy is full with uniform growth throughout with flowers covering at least 10 % of the canopy(acceptable ornamental landscape plant).

The accessions were separated into large regional groupings based on collection site along the Texas coast (Fig. 2.1.), then statistically analyzed using ANOVA in JMP (SAS Institute Inc., Cary, N.C.). Effects were considered significant at $P \leq 0.05$. Hierarchical cluster analysis with Wards distance was performed. All non-normal data was analyzed using permutations in the lmPerm package (Wheeler, 2010) in R (R Core Team, 2013), set to defaults.

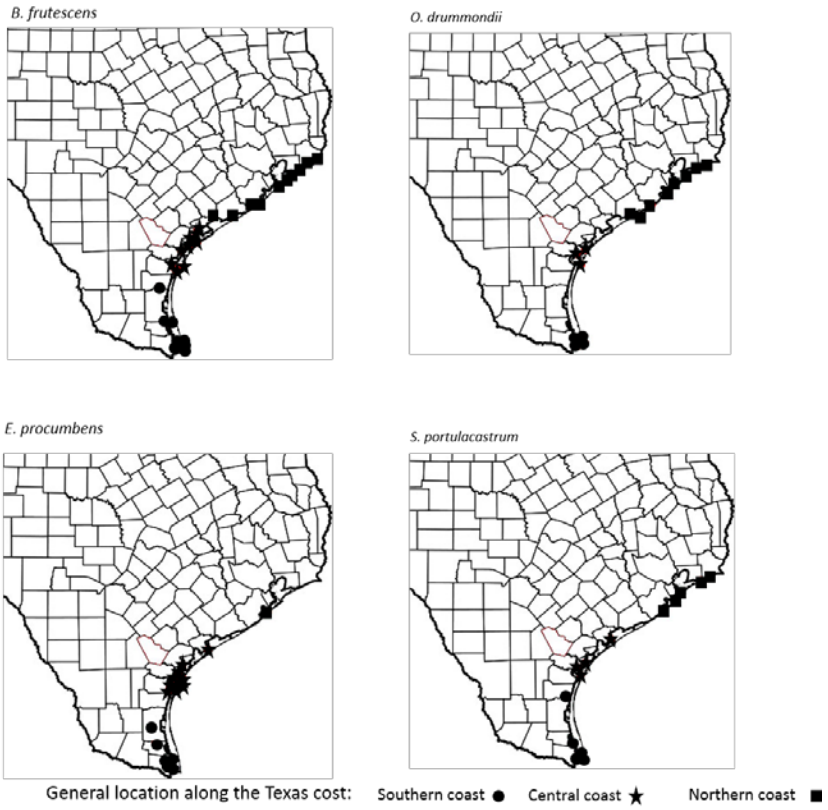

Fig. 2.1. Collection regions for accessions of *B. frutescens, O. drummondii, E. procumbens*, and *S. portulacastrum*.

Results and Discussion

Oenothera drummondii

There were differences amongst the accessions for height, height/width ratio, flower count at harvest, flower diameter, leaf length, leaf width, petiole length and the number of serrations present on each leaf (Tables 2.1 & 2.2). When accessions were allocated to regional groups along the Texas Coast (South, Central, and Northern) based on original of collection location there were differences among regional groups and accessions for height, height/width ratio, flower count, leaf length leaf width, and petiole

length. Internode length was only significant for environment but not for accession or collection region.

Height varied from 68 cm to 8 cm with a mean across all accessions of 25.2 cm and height/width ratio ranged from 0.67 to 0.05 with a mean of 0.30 (Table 2.3). Larger height width ratios are indicative of upright plants and lower ratios are indicative of a spreading habit. In general accessions from the southern coast were taller, in field conditions almost 100 % taller than the mean of plants from either the central or northern Texas coast (Table 2.2). This would explain the negative correlation between height and latitude of original collection site of $r = 0.56$ and the negative correlation between height/width ratio and latitude of originally collection site of $r = 0.49$ (Table 2.4). All accessions, except 010, were not as tall in the nursery environment as they were in the field environment. There was an interaction for environment by accession for height (Table 2.1). In the field environment, 010 had a mean height of 12.8 cm and in the nursery environment 010 had a mean height of 13.7 cm. All other accessions had reduced height in in the nursery compared to the field environment.

Table 2.1. Means of growth measures by accession and regional grouping along the Texas coast of *Oenothera drummondii* grown in field and nursery conditions.

	Height (cm)		Height/width ratio (cm·cm^{-1})		Internode length (mm)		Flower Count (No./plant)		Flower Diameter (mm)	
Accession	Field	Nursery	Field	Nursery	Field	Nursery	Field	Nursery	Field	Nursery
1	31.0 ± 5.8[T]	21.7 ± 5.2	0.27 ± 0.04	0.31 ± 0.07	8.7 ± 1.0[V]	34.3 ± 7.0	0.6 ± 0.4	0.0 ± 0.0	51.4 ± 1.4	-
2	42.8 ± 3.0	22.0 ± 2.3	0.31 ± 0.03	0.31 ± 0.03	13.7 ± 1.8	18.1 ± 3.0	17.6 ± 4.8	1.0 ± 1.0	62.3 ± 1.4	60.6 ± 2.8
3	47.6 ± 4.6	22.7 ± 3.5	0.30 ± 0.03	0.33 ± 0.10	11.6 ± 1.4	17.6 ± 2.7	4.8 ± 1.0	0.0 ± 0.0	55.1 ± 1.7	59.7 ± 2.7
4	52.75 ± 4.4	23.0 ± 2.6	0.33 ± 0.04	0.39 ± 0.06	14.3 ± 2.4	21.9 ± 2.9	11.8 ± 3.6	0.3 ± 0.3	59.4 ± 1.4	-
5	51.0 ± 17.0	24.7 ± 2.0	0.39 ± 0.09	0.40 ± 0.06	11.0 ± 1.0	17.2 ± 2.6	17.0 ± 16.0	0.0 ± 0.0	54.6 ± 1.5	-
6	49.6 ± 5.0	23.0 ± 2.6	0.36 ± 0.08	0.37 ± 0.02	11.6 ± 1.1	24.4 ± 1.5	0.0 ± 0.0	0.0 ± 0.0	47.9 ± 1.2	-
7	23.6 ± 4.0	14.0 ± 1.5	0.35 ± 0.08	0.24 ± 0.03	11.2 ± 0.9	19.6 ± 2.5	4.2 ± 0.8	0.7 ± 0.7	55.0 ± 2.4	59.8 ± 3.3
8	14.2 ± 2.3	13.3 ± 1.8	0.13 ± 0.03	0.20 ± 0.03	11.4 ± 1.3	24.2 ± 3.5	7.2 ± 2.9	1.7 ± 0.3	62.5 ± 1.7	58.3 ± 5.1
9	23.0 ± 4.1	14.7 ± 3.3	0.20 ± 0.04	0.18 ± 0.04	13.7 ± 1.4	24.1 ± 1.9	11.4 ± 3.9	3.3 ± 1.3	65.0 ± 2.4	51.4 ± 6.8
10	12.8 ± 1.5	13.7 ± 2.4	0.12 ± 0.02	0.18 ± 0.03	10.5 ± 0.6	22.7 ± 2.9	9.4 ± 2.7	3.7 ± 0.6	51.8 ± 1.8	56.4 ± 2.3
11	20.0 ± 4.5	15.0 ± 2.0	0.15 ± 0.04	0.20 ± 0.03	10.5 ± 0.7	19.8 ± 1.8	34.0 ± 11.7	3.3 ± 1.3	53.5 ± 1.2	57.1 ± 0.8
12	25.6 ± 3.7	22.0 ± 2.5	0.19 ± 0.02	0.35 ± 0.04	12.7 ± 1.1	34.0 ± 4.8	33.4 ± 9.1	0.3 ± 0.3	60.3 ± 2.0	63.6 ± 3.6
13	17.8 ± 2.8	15.0 ± 1.5	0.29 ± 0.03	0.27 ± 0.01	8.0 ± 0.6	14.6 ± 1.1	4.0 ± 1.4	2.0 ± 1.0	55.0 ± 1.8	53.7 ± 3.3
14	28.2 ± 2.6	13.7 ± 1.2	0.19 ± 0.02	0.18 ± 0.01	10.6 ± 1.3	24.9 ± 3.7	20.2 ± 3.3	0.3 ± 0.3	57.8 ± 2.9	60.6 ± 1.4
15	24.0 ± 3.3	20.3 ± 5.4	0.23 ± 0.03	0.29 ± 0.04	13.4 ± 1.5	28.11 ± 3.8	9.0 ± 2.6	2.7 ± 0.9	50.0 ± 1.4	55.1 ± 0.9
16	24.4 ± 2.2	16.0 ± 1.2	0.13 ± 0.02	0.22 ± 0.02	11.3 ± 1.1	27.11 ± 4.7	52.2 ± 8.4	1.0 ± 0.6	49.3 ± 0.7	48.4 ± 3.6
ANOVA										
Environment	***[W]		NS		***		***		*	
Accession	***		***		NS		***		*	
Environment x Accession	***		***		NS		***		NS	
Accessions grouped by region										
Location	Field	Nursery	Combined		Field	Nursery	Field	Nursery	Combined	
South	45.4 ± 3.0[X]	23.0 ± 1.2	0.34 ± 0.02		11.3 ± 0.7[Y]	23.1 ± 1.9	5.1 ± 1.8	0.06 ± 0.06	53.6 ± 0.8[Z]	
Central	25.8 ± 2.3	17.1 ± 1.4	0.29 ± 0.02		12.2 ± 0.6	23.4 ± 1.6	18.0 ± 4.1	1.4 ± 0.3	56.2 ± 0.8	
North	21.2 ± 1.6	15.7 ± 1.0	0.20 ± 0.01		11.0 ± 0.7	23.3 ± 1.4	18.7 ± 3.2	2.2 ± 0.5	57.1 ± 0.9	
ANOVA										
Environment	***[z]		NS		***		***		NS	
Location	***		***		NS		*		NS	
Environment x Location	***		NS		NS		NS		NS	

[T]Values represent mean (± standard errors) of 5 observations for the field environment and 3 observations for the nursery environment.
[U]Environments combined when not significant to $P \leq 0.05$.
[V]Values represent mean (± standard errors) internode extension of 15 observations for field environment and 9 observations for nursery environment.
[W]NS,*,**,***Non-significant or significant at $P \leq 0.05$, 0.01, or 0.001, respectively.
[X]Values represent means (± standard errors) of 21, 25, and 30 observation for south, central, and northern coast, respectively for field environment and observation of 15, 15, and 18 observation for south, central, and northern coast, respectively for nursery environment.
[Y]Values represent means (± standard errors) internode extension of 108, 120, and 144 observation for south, central, and northern coast, respectively.
[Z]Values represent means (± standard errors) flower diameter of 59, 102, and 107 observation for south, central, and northern coast, respectively.

Table 2.2. Means of leaf measures by accession of *Oenothera drummondii* grown in both field and nursery conditions.

	Lamina length (mm)		Lamina width (mm)		Petiole length (mm)		Serrations (No./leaf)	
Accession	Field	Nursery	Field	Nursery	Field	Nursery	Field	Nursery
1	25.8 ± 0.8[x]	46.3 ± 1.3	10.7 ± 0.3[y]	18.2 ± 0.6	4 ± 0.5	5.7 ± 0.2	1.9 ± 0.3	7.2 ± 0.5
2	23.7 ± 1.4	28.9 ± 0.7	10.7 ± 0.2	13.0 ± 0.3	2.8 ± 0.3	3.9 ± 0.4	2.4 ± 0.4	5.1 ± 0.5
3	29.8 ± 1.0	45.9 ± 1.6	11.2 ± 0.3	16.7 ± 0.7	4.5 ± 0.2	7.3 ± 0.4	4.9 ± 0.3	6.3 ± 0.5
4	27.3 ± 1.0	50.2 ± 1.2	10.1 ± 0.4	15.9 ± 0.4	3.6 ± 0.3	4.6 ± 0.4	2.6 ± 0.5	4.3 ± 0.3
5	24.9 ± 1.3	41.7 ± 0.9	7.9 ± 0.7	10.7 ± 0.4	2.3 ± 0.3	3.6 ± 0.3	2.3 ± 0.6	4.6 ± 0.5
6	24.3 ± 1.3	34.2 ± 0.5	10.7 ± 0.3	13.2 ± 0.5	2.3 ± 0.2	2.1 ± 0.3	1.2 ± 0.1	2.4 ± 0.3
7	32.6 ± 1.5	34.6 ± 0.6	13.4 ± 0.5	15.2 ± 0.7	3.5 ± 0.4	3.1 ± 0.3	5.9 ± 0.5	6.3 ± 0.5
8	39.7 ± 2.1	46.1 ± 0.5	12.4 ± 0.6	16.8 ± 0.4	3.8 ± 0.4	4.7 ± 0.3	8.7 ± 0.8	8.2 ± 0.4
9	36.3 ± 1.2	38.7 ± 1.8	11.3 ± 0.8	14.8 ± 0.4	2.5 ± 0.2	2.9 ± 0.2	8.6 ± 0.5	9.0 ± 0.6
10	31.0 ± 1.0	37.2 ± 1.6	10.7 ± 0.3	12.7 ± 0.8	2.6 ± 0.3	2.7 ± 0.4	8.7 ± 0.6	9.1 ± 0.6
11	24.8 ± 1.0	29.9 ± 0.7	10.0 ± 0.4	11.2 ± 0.3	2.3 ± 0.3	2.4 ± 0.2	10.9 ± 0.3	10.1 ± 0.5
12	31.7 ± 0.8	34.4 ± 1.0	10.8 ± 0.2	12.1 ± 0.4	3.1 ± 0.3	3.3 ± 0.2	10.0 ± 0.6	11.0 ± 0.9
13	37.9 ± 1.1	35.2 ± 1.6	11.1 ± 0.4	11.1 ± 0.8	3.7 ± 0.3	2.1 ± 0.4	5.7 ± 0.5	4.0 ± 0.7
14	25.7 ± 0.9	35.9 ± 1.7	10.4 ± 0.3	13.7 ± 0.4	2.6 ± 0.2	2.1 ± 0.2	4.9 ± 0.6	8.3 ± 0.3
15	29.2 ± 1.2	33.4 ± 1.2	9.5 ± 0.4	15.6 ± 0.5	2.1 ± 0.2	2.8 ± 0.2	4.4 ± 0.6	6.4 ± 0.5
16	26.3 ± 1.0	36.8 ± 1.7	10.2 ± 0.5	15.8 ± 0.3	2.3 ± 0.2	3.2 ± 0.4	6.3 ± 0.5	7.8 ± 0.4
ANOVA								
Environment	***		***		***		***	
Accession	***		***		***		***	
Environment x Accession	***		***		***		***	

Location	Field	Nursery	Field	Nursery	Field	Nursery	Field	Nursery
South	26.6 ± 0.5[y]	43.7 ± 1	10.4 ± 0.2	14.9 ± 0.5	3.5 ± 0.2	4.6 ± 0.3	2.6 ± 0.2	5.0 ± 0.3
Central	30.3 ± 0.9	36.0 ± 1	11.2 ± 0.3	15.3 ± 0.3	2.9 ± 0.1	3.5 ± 0.2	5.5 ± 0.3	6.8 ± 0.3
North	31.2 ± 0.7	35.2 ± 0.7	10.7 ± 0.2	12.6 ± 0.3	2.8 ± 0.1	2.6 ± 0.1	8.2 ± 0.3	8.6 ± 0.4
ANOVA								
Environment	***[z]		***		***		***	
Location	*		***		***		***	
Environment x Location	***		***		***		***	

[x]Values represent mean (± standard errors) of 15 observations for field environment and 9 observations for nursery environment.
[y]Values represent means (± standard errors) of 63, 75, and 90 observation for south, central, and northern coast, respectively for field environment and observation of 45, 45, and 54 observation for south, central, and northern coast, respectively for nursery environment.
[z] NS,*,**,***Non-significant or significant at $P \leq 0.05$, 0.01, or 0.001, respectively.

Table 2.3. Maximum, minimum, mean, standard deviation, and coefficient of variation of growth measures combined for all accessions of *Oenothera drummondii* across environments of field and nursery.

Growth characteristic	Max	Min	Mean	Standard Deviation	CV[x]
Plant height (cm)	68	8	25.2[y]	13.3	53.0
Flower count	85	0	9.5	15.3	160.1
Height/width ratio	0.67	0.05	0.3	0.1	44.3
Ornamental rating	5	2	3.1	0.7	22.4
Internode length (mm)	52	1	16.1[z]	9.1	57.3
Flower diameter (mm)	79	35	56.0	8.2	14.7
Lamina length (mm)	56	17	32.9	7.9	24.1
Lamina width (mm)	21	2	12.1	2.8	22.9
Petiole length (mm)	11	1	3.2	1.4	45.0
Number of Teeth	15	1	6.2	3.3	52.9

[x]Coefficient of variation.
[y]Means combined across all accessions and environments, n=124.
[z]Means combined across all accession and environments N=372 for internode mean and N=268 for floral data.

Flower count was different among the individual accessions, dependent on the environment and there was an accession by environment interaction as shown in Table 2.1. The accessions from central and northern collection sites tended to have more flowers in both the nursery and field environments (Table 2.1). All groups did not flower as freely in the nursery environment as they did in the field conditions. Some accessions came into flower sooner such as O13 in the nursery environments and O16 in the field environment (Fig. 2.2). Early flowering accessions were not consistent between the environments and some accessions came into heavier flower later during the experiment (Fig 2.2).

Table 2.4. Correlations coefficients between morphological characteristics and collection location coordinates of *Oenothera drummondii* accessions from the Texas coast.

	Height	Flower count	Ht/W	Leaf length	Leaf width	Petiole length	Number of leaf serrations	Ornamental rating	Lat.	Long.
Height	1	0.05	0.56	-0.44	-0.33	-0.02	-0.55	0.28	-0.56	0.45
Flower count	0.05	1	-0.38	-0.39	-0.41	-0.23	0.17	0.3	0.25	-0.13
Height/width	0.56	-0.38	1	0.07	0.12	0.14	-0.44	-0.05	-0.5	0.38
Leaf length	-0.44	-0.39	0.07	1	0.74	0.53	0.29	-0.18	-0.03	-0.03
Leaf width	-0.33	-0.41	0.12	0.74	1	0.51	0.19	-0.01	-0.11	0.14
Petiole length	-0.02	-0.23	0.14	0.53	0.51	1	-0.01	0.06	-0.39	0.27
Number of leaf serrations	-0.55	0.17	-0.44	0.29	0.19	-0.01	1	-0.04	0.67	-0.62
Ornamental rating	0.28	0.3	-0.05	-0.18	-0.01	0.06	-0.04	1	-0.23	0.14
Latitude	-0.56	-0.25	-0.5	-0.03	-0.11	-0.39	0.67	-0.23	1	-0.87
Longitude	0.45	-0.13	0.38	-0.03	0.14	0.27	-0.62	0.14	-0.87	1

This could be due to the photoperiod at time of harvest and a more constricted root zone when the plants are grown in containers and the smaller size of the container-grown plants. Plants were smaller across genotypes in containers. The mean growth indices (height x width at widest point x width perpendicular to widest point) was 63,958 cm^3 in the nursery compared to 551,452 cm^3 in the field, nearly a nine fold difference in size. Several accessions from the southern collection region might be photoperiod sensitive (Tables 2.2 & 2.5). Nursery grown plants were harvested in late summer (7 July 2010) and field grown plants were harvested at the end of the season (1 Nov 2010) allowing

these possibly day length sensitive accessions to be exposed short days. There are many reports of members of the genus *Oenothera* L. being sensitive to day length so the presence of day length sensitivity in some accessions would not be surprising (Clough et al., 2001; Gimenez et al., 2013; Kachi and Hirose, 1983). However further studies need to be performed to determine if it is indeed photoperiodicity or other factors such as plant size, temperature, or general reluctance to flower. The further testing is needed because of a lack of sampling dates could have resulted in the appearance of a reduction in flower number for some accessions and an increase in flowering for some accessions through time at both the nursery and field locations (Fig. 2.2). They had lower flower counts but, as far as ornamental horticulture is concerned, better growth habits with fewer defoliated sections in the canopy as shown in figure 2.3.

This is also similar to results found by Gratani et al. (2003) when growing several provenances of *Quercus ilex* L. from varying mesic and xeric climates, who found leaf morphology was related to provenance. This could explain the smaller leaves in the field on accessions from the southern coast. Rainfall along the Texas coasts varies from 61 – 71 cm in the southern region, 91-101 cm in the central coast to 132- 142 cm in the northern coast (Texas Water Development Board, 2014).

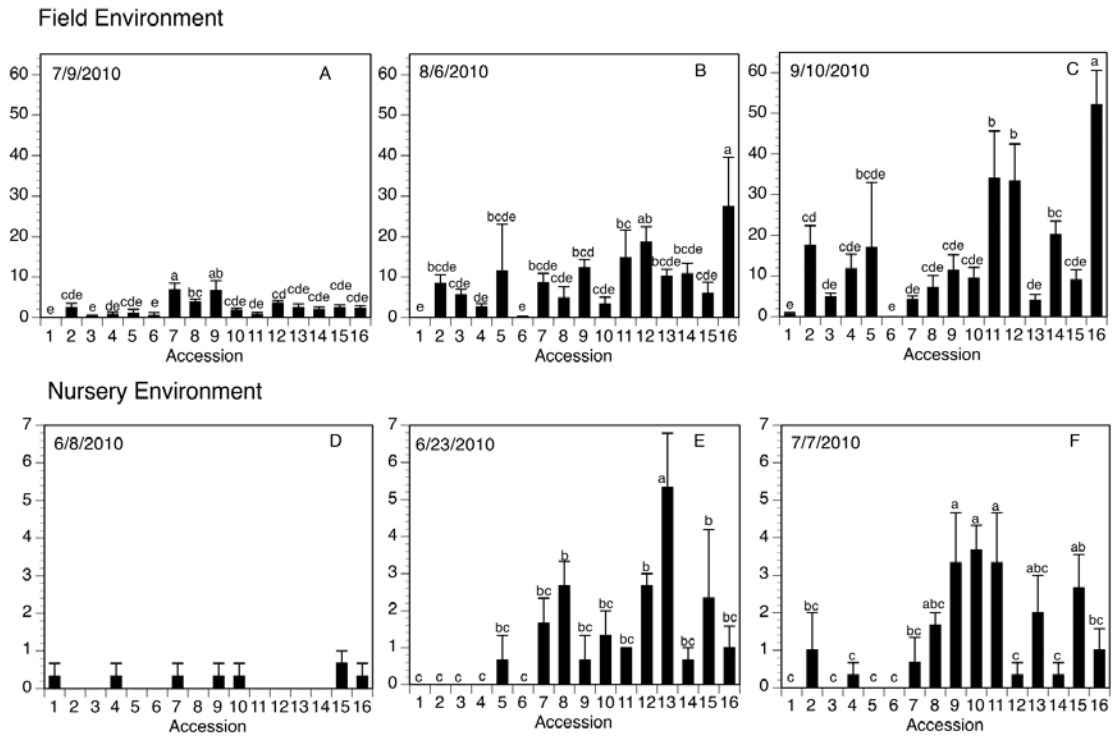

Fig. 2.2. Mean flower count of *Oenothera drummondii* on three sampling dates planted in field conditions or grown in 2.3 L containers in a nursery. Values represent mean (± standard errors) of 5 observations for the field environment and 3 observations for the nursery environment. There were no significant differences among accessions ($P \leq 0.05$) for sampling date 6 Jun 2010 (A). Any two means within a sampling date not followed by the same letter are significantly different at $P \leq 0.05$ using LSD mean separation.

Fig. 2.3. Example of *Oenothera drummondii* accessions exhibiting green foliage, blue foliage intact canopies and defoliated holes in in canopy. Example of an *O. drummondii* exhibiting green foliage and defoliated holes in the canopy (A) and an example of an *O. drummondii* accession exhibiting blue foliage and an intact canopy (B).

In general all leaf measures increased in the nursery environment, most likely from more favorable cultural conditions in the form of ample water and nitrogen fertilizer. When accessions are grouped by collection region, accessions from the south had shorter leaves, than either plants from the central or northern collection zones in the field, however when grown in nursery conditions plants from the southern region had larger leaves than plants from either the northern or central regions (Table 2.2). This suggests leaf morphology is more plastic in accessions from the southern Texas coast and may provide some form of adaptability to harsher environments as was reported for other taxa (Sultan, 1987; Wood et al., 1998; Gratani et al., 2003). The number of leaf serrations is also reduced in the southern region accessions, with plants from the northern regions having more leaf serrations on average. In addition to reduced leaf serrations, accessions from the southern collection region tended to have blue foliage, (Chi Square $P = 0.0001$) whereas the other collection locations tended to have green foliage. Sixty-six percent of blue observations were collected from the southern location. The blue foliage color is brought on by the increased presence of pubescence on the leaves, another drought adaptation strategy employed by many plants (Sandquist and Ehleringer, 1998; Ehleringer and Mooney, 1978), and reflecting the reduced rainfall in the southern collection region.

Leaf length and height, and number of leaf serrations and height were both negatively correlated -0.45 and -0.54, respectively. Wood et al. (1998) also found correlations among height and latitude and other leaf characteristics such as leaflet droop angle and leaflet tilt angle and latitude in pecan. Pecan tree height and latitude were

negatively correlated with increasing height and decreasing latitude (Wood et al., 1998), very similar to what was found in *O. drummondii* in this study. Number of leaf serrations and height were also correlated to the longitude of the original collection site (Table 2.4). Flower count was weakly correlated to leaf width and length, but not to latitude of collection site (Table 2.4). This suggests that in each group there might be free-flowering and not free-flowering accessions.

Based on hierarchical cluster analysis using only morphologic measures accessions clustered into two large groups (Fig. 2.4). This is different than the expected three clusters based on location of collection. Accessions collected from central and northern locations formed one large cluster and accessions from the southern collection locations formed a separate cluster. This is in line with Nooryazdan et al. (2010) who also found that sunflowers from similar climatic zones clustered together. One accession collected from the central coast (O2) clustered in the southern group as did one accession (O1) from the southern region which clustered with the northern accessions. Neither accession O1 or O2 were from transition zones. These clustering patterns were also supported by LSD means separation performed on the means of the three regional groups for height, flower count and height/width ratio (Table 2.5). For these measures only plants from the southern region were significantly different from the other collection locations.

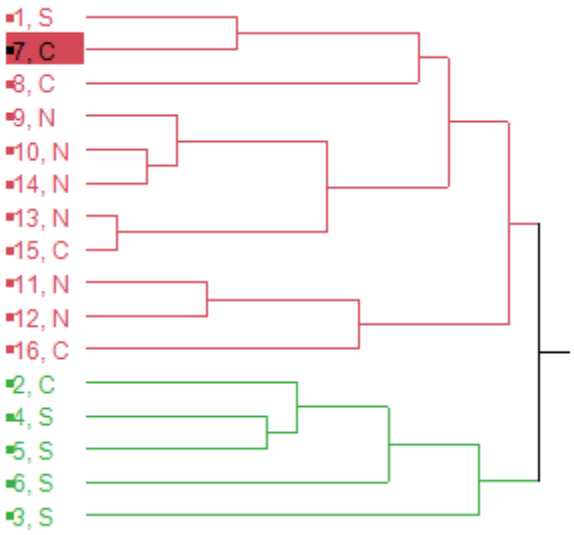

Fig. 2.4. Hierarchical Cluster analysis using Wards distance of *Oenothera drummondii* accessions based on morphological traits. Numbers represent accession numbers of *O. drummondii* and letters represent accessions' collection region along the coast S=Southern coast, C= Central coast, and N= Northern coast. Clusters separated by color.

Table 2.5. Means of growth measures separated by origin of *Oenothera drummondii* accession along Texas coast combined across both field and nursery environments.

Location	Height (cm)	Flower (No./plant)	Height/width ratio ($cm \cdot cm^{-1}$)
South	36.08a[v]	3.03b	0.34a
Central	22.56b	11.80a	0.24b
North	19.15b	12.52a	0.20b
ANOVA Location	***	***	***

[v] Values represent means of 21, 25, and 30 observation for south, central, and northern coast, respectively for field environment and observation of 15, 15, and 18 observation for south, central, and northern coast, respectively for nursery environment. Any two means within a column not followed by the same letter are significantly different at $P \leq 0.05$ using LSD mean separation.
[z] NS,*,**,***Non significant or significant at $P \leq 0.05$, 0.01, or 0.001, respectively.

There is variation among accessions of *O. drummondii* when sampled from the southern, central, and northern coast of Texas for height, propensity to flower, growth form, leaf length and width, as well as the number of serrations on the margin of the leaf. We did not find variation in flower diameter based on the region of collection but it was present amongst the accessions as a whole. There was no variation in internode length associated with region of collection or accession. There also was no variation found in flower color based on visual observation (data not presented) all were of a similar shade of yellow, however plants from the southern collection region tended to have blue foliage.

In general the southern forms have a more upright and less spreading subshrub habit, whereas the plants from the central and northern areas have a shorter more spreading groundcover growth form and a greater tendency to be free flowering. This will allow one to target their collection efforts to regions based on the characteristics of material in which they are interested and to potentially combine desirable traits via controlled crosses.

Borrichia frutescens

There were significant ($P \leq 0.05$) differences among the accessions of *B. frutescens* for height, height width ratio, internode extension, flower count, flower diameter, leaf length leaf width, petiole length and number of serrations along the margin of the leaf (Table 2.6 and 2.7). When the accessions are grouped by their region of collection along the Texas coasts there were differences in height, flower count,

flower diameter, internode extension, leaf length leaf width, petiole length and the number serrations along the leaf margin (Tables 2.8 and 2.9).

Plant height ranged from a maximum of 78 cm to a minimum of 17 cm and coefficient of variation of 24.1%. Plants collected from the southern and central regions were on average shorter than plants collected from the northern coast. Environment did affect the mean plant height when the accessions are grouped, based on collection location mean height for plants grown in the field was 42 ± 0.9 cm and mean height for plants grown in the nursery was 45 ± 1.1 cm. When analyzed as individual accessions environment did play a role, plants could have been taller in the nursery due to ample water and nitrogen fertilizer.

Flower count was variable among accessions, and highly significant for accession but not for environment (Table 2.6). When grouped in collection areas plants from the southern sites had a larger mean flower count of 5.2 flowers per plant compared to northern sites with 3.0 flowers per plant. Southern collection sites had larger flowers with a mean of 31.1mm compared to 28.1 mm for plants collected from northern locations when planted in the field, but plants collected from northern locations had larger flowers than southern accessions when grown in the nursery (Table 2.6). Flower count was much more variable with a CV of 106.4 than flower diameter with a CV of 14.2 (Table 2.10).

Table 2.6. Means of *Borrichia frutescens* growth measures by accession when grown in 2.3 L containers in the nursery or planted to the field.

	Height (cm)		Height/width ratio ($cm \cdot cm^{-1}$)		Internode length (mm)		Flower count (No./plant)	Flower Diameter (mm)	
Accession	Field	Nursery	Field	Nursery	Field	Nursery	Combined[x]	Field	Nursery
1	43.0 ± 4.0[w]	50.0 ± 10.5	1.0 ± 0.2	1.1 ± 0.3	16.9 ± 1.0[y]	50.3 ± 3.2	2.0 ± 0.5	34.4 ± 0.0	33.2 ± 1.7
2	37.6 ± 2.8	37.0 ± 7.0	0.6 ± 0.1	0.9 ± 0.2	24.1 ± 1.6	52.4 ± 1.9	4.4 ± 1.0	30.7 ± 1.2	33.3 ± 2.3
3	56.6 ± 3.6	46.0 ± 6.8	1.4 ± 0.1	1.3 ± 0.3	21.4 ± 1.4	59.4 ± 3.4	3.4 ± 1.4	37.2 ± 1.0	35.8 ± 0.9
4	40.6 ± 1.3	47.7 ± 3.7	0.7 ± 0.0	1.1 ± 0.1	26.4 ± 1.4	46.9 ± 3.0	14.9 ± 3.4	28.9 ± 0.5	28.3 ± 0.6
5	36.6 ± 1.8	49.0 ± 1.2	1.0 ± 0.1	1.4 ± 0.1	20.4 ± 1.7	40.3 ± 1.3	2.9 ± 0.7	26.6 ± 0.7	26.5 ± 0.5
6	46.6 ± 1.7	42.0 ± 2.1	1.0 ± 0.1	1.4 ± 0.1	17.5 ± 0.7	44.6 ± 2.5	3.0 ± 0.7	29.3 ± 0.4	35.6 ± 0.5
7	37.8 ± 2.1	45.7 ± 4.3	1.1 ± 0.1	1.3 ± 0.2	20.7 ± 1.3	43.8 ± 1.3	3.8 ± 1.4	30.6 ± 0.0	29.3 ± 0.7
8	36.4 ± 2.4	45.3 ± 3.0	0.7 ± 0.1	1.2 ± 0.2	15.2 ± 2.0	41.3 ± 2.6	6.3 ± 2.4	0.0 ± 0.0	27.6 ± 0.7
9	44.4 ± 4.0	42.3 ± 5.4	0.8 ± 0.1	1.0 ± 0.2	17.8 ± 0.9	45.4 ± 2.6	4.0 ± 1.0	28.6 ± 1.2	29.7 ± 1.2
10	50.2 ± 2.9	46.7 ± 0.9	1.0 ± 0.1	1.5 ± 0.2	20.3 ± 1.7	43.3 ± 3.1	5.5 ± 2.1	34.0 ± 1.7	28.5 ± 0.6
11	43.2 ± 3.5	39.7 ± 5.9	0.8 ± 0.1	1.1 ± 0.2	20.3 ± 1.6	40.8 ± 2.5	8.0 ± 1.7	30.0 ± 0.7	34.0 ± 0.8
12	26.2 ± 3.1	31.3 ± 2.7	0.6 ± 0.1	0.7 ± 0.1	20.7 ± 1.5	49.7 ± 3.1	2.8 ± 0.6	31.3 ± 1.2	29.4 ± 0.5
13	36.2 ± 2.2	33.0 ± 2.1	0.6 ± 0.0	0.6 ± 0.1	18.3 ± 1.7	50.4 ± 3.0	3.1 ± 1.0	27.0 ± 0.6	0.0 ± 0.0
14	40.2 ± 2.1	45.0 ± 1.2	0.9 ± 0.1	1.1 ± 0.1	17.3 ± 1.4	39.9 ± 2.0	1.5 ± 0.3	27.6 ± 2.1	32.3 ± 1.8
15	44.4 ± 2.3	56.7 ± 0.9	0.8 ± 0.1	1.4 ± 0.1	17.9 ± 1.4	40.3 ± 1.5	3.4 ± 0.5	21.8 ± 0.6	30.0 ± 1.1
16	32.0 ± 2.3	38.7 ± 2.9	0.7 ± 0.1	1.1 ± 0.0	15.2 ± 1.5	46.7 ± 2.8	5.8 ± 1.2	26.0 ± 1.0	27.2 ± 0.9
17	40.2 ± 3.8	52.0 ± 8.6	0.9 ± 0.1	1.3 ± 0.3	15.3 ± 1.7	47.2 ± 3.3	2.4 ± 0.3	27.3 ± 1.5	26.7 ± 0.9
18	49.4 ± 2.1	48.3 ± 0.7	0.9 ± 0.0	1.6 ± 0.2	18.6 ± 1.0	39.9 ± 1.5	6.8 ± 1.4	27.5 ± 0.7	27.1 ± 0.7
19	48.6 ± 1.1	51.7 ± 4.9	0.9 ± 0.1	1.3 ± 0.1	16.3 ± 0.9	44.1 ± 1.2	3.9 ± 1.0	27.4 ± 0.6	34.2 ± 1.5
20	69.8 ± 2.9	63.3 ± 0.3	0.9 ± 0.0	1.7 ± 0.1	18.0 ± 1.2	39.2 ± 2.0	2.1 ± 1.0	28.6 ± 1.5	0.0 ± 0.0
21	37.0 ± 3.4	43.7 ± 4.7	0.6 ± 0.1	1.0 ± 0.1	11.8 ± 1.3	48.2 ± 2.2	0.8 ± 0.3	0.0 ± 0.0	33.3 ± 2.7
22	41.6 ± 3.4	45.0 ± 5.3	0.8 ± 0.1	0.9 ± 0.2	18.6 ± 2.3	52.2 ± 2.7	4.1 ± 1.0	27 ± 1.3	32.0 ± 1.1
23	58.2 ± 1.9	63.3 ± 1.7	1.3 ± 0.1	1.7 ± 0.2	16.8 ± 1.6	48.7 ± 3.0	0.9 ± 0.4	28.4 ± 3.2	0.0 ± 0.0
24	34.6 ± 5.0	29.7 ± 4.2	1.0 ± 0.1	1.0 ± 0.1	14.3 ± 1.4	37.4 ± 2.7	6.6 ± 2.0	25.4 ± 0.8	30.3 ± 1.2
26	43.4 ± 3.7	49.3 ± 2.3	0.8 ± 0.1	1.2 ± 0.1	18.6 ± 0.9	57.0 ± 3.3	2.0 ± 0.8	32.6 ± 1.6	37.0 ± 0.6
27	39.0 ± 2.2	42.3 ± 1.2	1.0 ± 0.1	1.2 ± 0.0	13.7 ± 1.0	43.0 ± 2.9	4.3 ± 0.8	29.2 ± 0.7	30.6 ± 1.3
28	33.2 ± 2.9	40.7 ± 2.8	0.7 ± 0.0	1.1 ± 0.1	12.6 ± 1.8	44.9 ± 1.6	6.8 ± 1.9	29.1 ± 0.9	28.5 ± 0.5
ANOVA									
Environment	**[z]		***		***		NS	***	
Accession	***		***		***		***	***	
Environment x Accession	NS		**		***		NS	***	

[w]Values represent mean (± standard errors) of 5 observations for field environment and 3 observations for nursery environment.
[x]Environments combined when not significant to $P \leq 0.05$.
[y]Values represent mean (± standard errors) internode extension of 15 observations for field environment and 9 observations for nursery environment.
[z] NS,*,**,***Non significant or significant at $P \leq 0.05$, 0.01, or 0.001, respectively.

Table 2.7. Mean of leaf measures by accession for *Borrichia frutescens* when grown in 2.3 L containers in the nursery or planted to the field

	Lamina length (mm)		Lamina width (mm)		Petiole length (mm)		Teeth (No./leaf)	
Accession	Field	Nursery	Field	Nursery	Field	Nursery	Field	Nursery
1	36.8 ± 0.9[y]	41.3 ± 1.8	13.5 ± 0.7	23.6 ± 1.7	4 ± 0.2	6.1 ± 0.4	12.1 ± 1	19.7 ± 1.8
2	38.8 ± 1.6	39.4 ± 1.6	18.6 ± 1.3	24 ± 2.3	6.3 ± 0.3	6.6 ± 0.4	6 ± 1.4	15.1 ± 1.8
3	36.6 ± 0.7	39.6 ± 1.1	16.9 ± 0.6	29 ± 1.4	6.1 ± 0.3	6.2 ± 0.3	6.1 ± 0.9	17 ± 2.1
4	31.2 ± 1.1	41.1 ± 1.1	9.8 ± 0.4	19.2 ± 1.3	4.4 ± 0.2	5.9 ± 0.4	1.7 ± 0.5	10.9 ± 2.2
5	27 ± 0.7	29.8 ± 0.7	11.6 ± 0.5	16.2 ± 1.1	3.9 ± 0.2	5.1 ± 0.3	13.5 ± 1.1	18 ± 0.9
6	37.9 ± 1.5	40.4 ± 0.9	15.6 ± 0.9	28 ± 1.3	5.6 ± 0.2	6.8 ± 0.3	5.4 ± 0.9	15.9 ± 1.6
7	31 ± 1.3	36.3 ± 2.3	8.9 ± 0.5	16.9 ± 1.3	4.3 ± 0.2	6 ± 0.3	9.5 ± 1.2	19.3 ± 0.7
8	23.7 ± 0.8	26.8 ± 0.7	10.1 ± 0.5	15.1 ± 1	3.9 ± 0.2	5.7 ± 0.4	11.7 ± 1.2	16.9 ± 1.3
9	35.4 ± 1.2	36.2 ± 2	14.8 ± 0.6	19.1 ± 1.7	4.5 ± 0.2	6.4 ± 0.5	14.5 ± 0.8	14.2 ± 1
10	43.7 ± 1.2	35.1 ± 1.4	26.0 ± 1.0	25.4 ± 1.7	7.2 ± 0.5	6.2 ± 0.5	26.7 ± 1.3	23.9 ± 2.5
11	31.4 ± 1.4	38.2 ± 1.3	11.8 ± 0.7	24.4 ± 1.8	5 ± 0.3	6.9 ± 0.3	2.5 ± 0.6	15.6 ± 1.8
12	32.3 ± 1.3	39.3 ± 1.5	10.9 ± 0.7	21.7 ± 1.3	4.5 ± 0.3	7.3 ± 0.5	2.7 ± 0.3	12.8 ± 1.5
13	35.9 ± 0.9	39.3 ± 1.6	10.2 ± 0.3	16.8 ± 1.4	5.5 ± 0.2	8.2 ± 0.5	1.1 ± 0.1	6.8 ± 1.2
14	37.8 ± 1.4	39.6 ± 1.4	16 ± 1.3	24.2 ± 2.3	6 ± 0.4	8 ± 0.4	1.5 ± 0.4	15.1 ± 2.5
15	35.9 ± 1.1	38.4 ± 1.3	11.9 ± 0.8	19.2 ± 1.3	4.3 ± 0.3	5.9 ± 0.3	1.6 ± 0.4	4.2 ± 0.5
16	32 ± 1.4	42.2 ± 2.3	7.6 ± 0.5	20.1 ± 1.6	4.8 ± 0.3	8.4 ± 0.5	2.0 ± 0.6	11.2 ± 1.1
17	29.1 ± 1.9	37.4 ± 1.5	11.7 ± 0.8	19.7 ± 1.4	4.6 ± 0.2	6.8 ± 0.3	2.1 ± 0.7	6.3 ± 1.2
18	37.8 ± 1.2	41.8 ± 1.6	13.5 ± 0.7	23.8 ± 0.6	5.5 ± 0.2	6.8 ± 0.4	5.2 ± 1.3	14.2 ± 1
19	34.4 ± 1.2	39.3 ± 1.7	13.6 ± 0.7	22.6 ± 1.8	4.9 ± 0.3	6.7 ± 0.3	2.3 ± 0.5	14.4 ± 1.4
20	32 ± 1.1	36.3 ± 1.4	15.8 ± 0.7	23.7 ± 0.9	6 ± 0.3	8.1 ± 0.5	1.0 ± 0.0	2 ± 0.3
21	31.6 ± 1.3	42.4 ± 1.2	9.7 ± 0.7	22 ± 1.1	4.6 ± 0.2	7.8 ± 0.3	1.0 ± 0.0	2.3 ± 0.3
22	30.2 ± 1.1	35.3 ± 1.4	12.3 ± 0.6	18.1 ± 1.4	6.6 ± 0.3	8.4 ± 0.5	1.3 ± 0.3	8 ± 1.9
23	36 ± 1.1	43.4 ± 1.9	13.9 ± 0.6	20.1 ± 1.1	5.6 ± 0.3	8.7 ± 0.5	1.0 ± 0.0	5.9 ± 1.2
24	26.7 ± 1	32.7 ± 1	11.1 ± 0.6	20.6 ± 1.6	4.2 ± 0.2	6.9 ± 0.4	1.0 ± 0.0	3.1 ± 0.5
26	42.1 ± 1.7	40.1 ± 0.9	15.9 ± 0.9	20.8 ± 1.3	7 ± 0.4	8.1 ± 0.4	1.1 ± 0.1	1.6 ± 0.2
27	29.2 ± 1	35.9 ± 1.4	11.1 ± 0.6	21.8 ± 1.6	4.8 ± 0.1	6.2 ± 0.4	4.6 ± 0.7	14.9 ± 1.8
28	33.1 ± 1.7	41 ± 1.1	12.2 ± 0.8	21.3 ± 1.3	4.2 ± 0.2	5.9 ± 0.3	1.2 ± 0.2	5.8 ± 1.2
ANOVA								
Environment	***[z]		***		***		***	
Accession	***		***		***		***	
Environment x Accession	***		***		***		***	

[y]Values represent mean (± standard errors) of 15 observations for field environment and 9 observations for nursery environment.
[z] NS,*,**,***Non significant or significant at $P \leq 0.05$, 0.01, or 0.001, respectively.

Table 2.8. Mean of growth measures separated by origin of accession along Texas coast for *Borrichia frutescens* when grown in 2.3 L containers in the nursery or planted to the field.

Location	Height (cm)	Flower count (No./plant)	Height/width ratio ($cm \cdot cm^{-1}$)		Internode length (mm)		Flower diameter (mm)	
	Combined[u]	Combined	Field	Nursery	Field	Nursery	Field	Nursery
South	42.6 ± 1.2b	5.2 ± 0.5a	0.9 ± 0.0 [v]	1.2 ± 0.1	20.0 ± 0.5	46.1 ± 1.0	31.1 ± 0.5[x]	29.8 ± 0.5
Central	42.5 ± 1.0b	4.4 ± 0.4ab	0.8 ± 0.0	1.1 ± 0.1	17.5 ± 0.5	45.2 ± 0.8	27.6 ± 0.5	30.5 ± 0.6
North	46.9 ± 1.3a	3.0 ± 0.6b	0.9 ± 0.0	1.2 ± 0.1	16.0 ± 0.6[x]	46.5 ± 1.1	28.1 ± 0.7	31.8 ± 0.7
ANOVA								
Environment	NS[z]	NS	***		***		***	
Location	*	*	NS		*		**	
Environment x Location	NS	NS	NS		NS		***	

[u]Environments combined when not significant to $P \leq 0.05$. Values represent means (± standard errors) of 72, 88, and 56 observation for south, central, and northern coast, respectively.
[v]Values represent means (± standard errors) of height/ width ratio of 45, 55, and 35 observations for south, central, and northern coast, respectively for field environment and observation of 27, 33, and 21 observations for south, central, and northern coast, respectively for nursery environment.
[x]Values represent means (± standard errors) of internode extension for 135, 164, and 104 observations for south, central, and northern coast, respectively for field environment and observation of 81, 99, and 63 observations for south, central, and northern coast, respectively for nursery environment.
[y]Values represent means (± standard errors) of flower diameter for 57, 73, and 37 observations for south, central, and northern coast, respectively for field environment and observation of 60, 53, and 32 observations for south, central, and northern coast, respectively for nursery environment.
[z] NS,*,**,***Non significant or significant at $P \leq 0.05$, 0.01, or 0.001, respectively.

Table 2.9. Mean of leaf measures separated by origin of accession along Texas coast for *Borrichia frutescens* when grown in 2.3 L containers in the nursery or planted to the field.

Location	Lamina length (mm)		Lamina width (mm)		Petiole length (mm)		Teeth (No./leaf)	
	Field	Nursery	Field	Nursery	Field	Nursery	Field	Nursery
South	33.2 ± 0.6[y]	36.1 ± 0.7	13.8 ± 0.5	21.2 ± 0.7	5.0 ± 0.1	6.2 ± 0.1	10.2 ± 0.7	16.5 ± 0.7
Central	34.9 ± 0.5	39.8 ± 0.5	12.8 ± 0.3	21.8 ± 0.5	5.0 ± 0.1	6.9 ± 0.1	3.4 ± 0.3	11.7 ± 0.6
North	32.5 ± 0.6	38.0 ± 0.7	12.8 ± 0.3	21.0 ± 0.5	5.5 ± 0.1	7.7 ± 0.2	1.6 ± 0.2	5.4 ± 0.7
ANOVA								
Environment	***[z]		***		***		***	
Location	*		***		***		***	
Environment x Location	***		***		***		***	

[y]Values represent mean (± standard errors) of 15 observations for field environment and 9 observations for nursery environment.
[z] NS,*,**,***Non significant or significant at $P \leq 0.05$, 0.01, or 0.001, respectively.

Table 2.10. Maximum, minimum, mean, standard deviation, coefficient of variation of growth measures combined for all accessions across all environments for accessions of *Borrichia frutescens.*

	Max	Min	Mean	Standard Deviation	CV[x]
Plant height (cm)	78	17	43.6[y]	10.5	24.1
Flower count	34	0	4.3	4.5	106.4
Height/width ratio	2.0	0.3	1.0	0.3	33.8
Ornamental rating	5	2	3.1	0.8	24.2
Growth index (cm^3)	507744.0	9500	81276.5	64607.0	79.5
Internode length (mm)	73.0	2.3	28.5[z]	15.4	54.2
Flower diameter (mm)	42.53	18.1	29.7	4.2	14.2
Pedicle length (mm)	76.0	14.0	38.3	11.7	30.5
Lamina length (mm)	57.0	18.4	35.4	6.6	18.6
Lamina width (mm)	37.0	3.9	16.2	6.3	38.8
Petiole length (mm)	12.0	2.6	5.8	1.7	28.6
Number of Teeth	37	1	7.6	7.5	98.6

[x]Coefficient of Variation.
[y]Means combined across all accessions and environments, N=216.
[z]Means combined across all accession and environments N=646 for internode mean and N=312 for floral data.

Leaf width, length, petiole and leaf margin serration were significantly different among groups and among accessions (Table 2.7). Leaves tended to be larger in accessions for the central collection sites, having longer leaf laminas and wider leaf laminas (Table 2.9). The northern plants had longer petioles compared to plants collected from either of the other locations. The size of the leaves was different among field and nursery grown plants, with plants generally producing larger leaves when grown in the nursery (Table 2.7 and 2.9). The larger leaves are most likely the result of more favorable cultural conditions found in the nursery. Plants from the northern Texas coast had more entire margins on their leaves compared to plants collected from either the central or southern locations (Table 2.9).

There was a difference in the ornamental ratings on the accession level ($P<0.0001$) and on the regional level ($P<0.038$) using Chi square analysis. The southern region had 2 individuals scoring a 5, on the ornamental rating scale, whereas the other

collection regions had none. Latitude was only correlated with number of leaf serrations (r=-0.59), all other variables measured had correlation coefficients between 0.25 and -0.18. Longitude was positively correlated with the number of leaf serrations (r=0.46) and petiole length (r=-0.32). Leaf lamina length was strongly correlated with leaf width (r=0.70), petiole length (r=0.65), and internode length (r=0.46). Flower diameter was correlated with both leaf lamina length (r=0.40) and leaf lamina width (r=0.52).

Cluster analysis based on Wards method using all collected growth measures was not aligned with region of collection. Three clusters were developed and accessions from all three collection zones were randomly dispersed throughout.

Flower count was variable and highly significant among accessions. Flower diameter was also correlated with leaf width (r=0.52). Southern accessions had more flowers and larger diameter flowers. This means collections can be targeted for certain traits of interest and there is most likely a source of variation for the creation of improved populations in the wild, however not all morphological measures may be correlated with the region of the Texas coast where plants are collected.

Erigeron procumbens

There were differences in height, height width ratio, internode extension, lamina length, lamina width and petiole length among accessions and regional collection of *E. procumbens* (Table 2.11). There were no differences in this test for flower count. Differences for floral measures could not be determined because of small sample size on the date of data collection.

There was only one accession collected from the northern region of the Texas coast. This is to be expected, encountering *E. procumbens* in this region would be rarer

Table 2.11. Means of *Erigeron procumbens* growth measures by accession when grown in 2.3 L containers in the nursery or planted to the field.

	Height (cm)		Height/width ratio ($cm \cdot cm^{-1}$)		Internode length (mm)	
Accession	Field	Nursery	Field	Nursery	Field	Nursery
1	5.8 ± 0.4	11.0 ± 3.2	0.1 ± 0.0	0.2 ± 0.1	16.2 ± 1.5	28.8 ± 3.7
2	7.0 ± 1.4	8.7 ± 1.2	0.1 ± 0.0	0.2 ± 0.0	17.9 ± 1.2	22.6 ± 1.0
3	5.4 ± 0.5	6.3 ± 0.7	0.1 ± 0.0	0.1 ± 0.0	13.6 ± 0.9	19.4 ± 2.0
4	6.5 ± 1.3	8.7 ± 2.3	0.1 ± 0.0	0.2 ± 0.0	11.4 ± 1.5	18.4 ± 2.6
5	10.8 ± 2.1	11.7 ± 1.9	0.1 ± 0.0	0.2 ± 0.0	15.0 ± 1.3	17.0 ± 1.0
6	4.2 ± 0.9	6.3 ± 0.3	0.1 ± 0.0	0.1 ± 0.0	16.6 ± 1.6	24.2 ± 2.4
7	5.6 ± 0.7	7.3 ± 1.5	0.1 ± 0.0	0.1 ± 0.0	18.0 ± 1.0	24.9 ± 3.1
8	6.8 ± 0.7	9.3 ± 1.5	0.1 ± 0.0	0.2 ± 0.0	15.1 ± 0.9	22.9 ± 2.4
9	6.2 ± 0.4	10.3 ± 1.9	0.1 ± 0.0	0.2 ± 0.0	13.5 ± 0.8	20.1 ± 1.7
10	7.8 ± 1.4	7.7 ± 1.2	0.1 ± 0.0	0.1 ± 0.0	15.5 ± 1.1	23.8 ± 2.8
11	9.0 ± 1.3	14.0 ± 1.5	0.1 ± 0.0	0.3 ± 0.0	14.9 ± 1.5	17.3 ± 1.2
12	4.8 ± 0.7	6.7 ± 0.3	0.1 ± 0.0	0.1 ± 0.0	15.1 ± 1.4	22.8 ± 1.4
13	5.0 ± 0.8	7.0 ± 0.6	0.0 ± 0.0	0.1 ± 0.0	17.7 ± 0.7	23.8 ± 2.1
15	7.4 ± 1.6	5.3 ± 0.9	0.1 ± 0.0	0.1 ± 0.0	21.9 ± 2.2	25.9 ± 2.0
16	4.6 ± 0.5	5.0 ± 0.6	0.1 ± 0.0	0.1 ± 0.0	13.8 ± 1.2	21.7 ± 1.5
17	5.2 ± 0.8	8.7 ± 0.9	0.1 ± 0.0	0.2 ± 0.0	18.7 ± 2.2	25.6 ± 1.8
18	6.8 ± 0.6	7.0 ± 0.6	0.1 ± 0.0	0.1 ± 0.0	11.9 ± 1.0	18.8 ± 1.0
ANOVA						
Environment	***[z]		***		***	
Accession	***		***		***	
Environment x Accession	NS		*		NS	

Values represent mean (± standard errors) of 5 observations for field environment and 3 observations for nursery environment.
[x]Environments combined when not significant to $P \leq 0.05$.
[y]Values represent mean (± standard errors) internode extension of 15 observations for field environment and 9 observations for nursery environment.
[z] NS,*,**,***Non significant or significant at $P \leq 0.05$, 0.01, or 0.001, respectively.

due to this being extreme northern end of its natural range. In statistical analysis with a regional effect, only the southern and central regions were considered because of the small sample size from the northern region.

Accessions from the southern collection region in the vicinity of Brownsville were taller in both the nursery and field environment (Table. 2.12). Plants from the southern collection region also had a larger height to width ratio when grown in containers (Table 2.12) than plants from the other collection area. This greater height to width ratio indicates that the plants were not only taller but also had less of a prostrate habit than the wild accessions collected from the central coast of the Texas. However in general terms the variability of height found in *E. procumbens* was much less than found in *B. frutescens* with a CV of 106.4 (Table 2.13).

All leaf growth measures recorded were different among the accessions and between the collection groups (Tables 2.14 and 2.15). Plants collected from the southern collection region had larger leaves in both length and width of the leaf lamina. There was only an interaction among environments and regions of collection for leaf width, plants collected from the central Texas coast having a much larger increase in leaf width when grown in containers than in the field. On the accession level most plants had larger leaves in terms of width, length, and petiole length when grown in the nursery environment (Table 2.15). This could be explained by the more favorable cultural conditions provided by the nursery compared to the field. The interaction between accession and environment could be explained by not all accessions being as plastic in phenotype. Differences in plasticity are shown by not all accessions having similar

increases in leaf size (Table 2.14). Some accessions (e.g. 12) increased leaf size by 53% when grown in the nursery and other accessions (e.g. 18) only increased leaf size by 4 % when grown in nursery conditions.

Table 2.12. Means of growth measures separated by origin of accession along Texas coast for *E. procumbens* when grown in 2.3 L containers in the nursery or planted to the field.

Location	Height (cm)		Height/width ratio ($cm \cdot cm^{-1}$)		Internode length (mm)	
	Field	Nursery	Field	Nursery	Field	Nursery
South[x]	7.7 ± 0.6[y]	10.1 ± 0.8	0.1 ± 0[y]	0.2 ± 0	15.33 ± 0.47	21 ± 0.95
Central	5.6 ± 0.3	7.4 ± 0.5	0.1 ± 0	0.1 ± 0	16.4 ± 0.51	23.31 ± 0.72
North	6.8 ± 0.6	7 ± 0.6	0.1 ± 0	0.1 ± 0	11.9 ± 0.95	18.78 ± 1
ANOVA						
Environment	***[z]		***		***	
Location	***		***		***	
Environment x Location	NS		*		NS	

[x]Location is for Central and Southern Region only, due to the lack of samples from Northern Region.
[y]Values represent mean (± standard errors) of 5 observations for field environment and 3 observations for nursery environment for height, height /width ratio. Means (± standard errors) for internode extension represent 15 observations for field environment and 9 observations for nursery environment.
[z] NS,*,**,***Non significant or significant at $P \leq 0.05$, 0.01, or 0.001, respectively.

Table 2.13. Maximum, minimum, mean, standard deviation, coefficient of variation of growth measures combined for all accessions across all environments for accession of *Erigeron procumbens*.

	Max	Min	Mean	Standard Deviation	CV[x]
Plant height (cm)	19.0	2.0	7.1[y]	3.0	41.9
Flower count	34	0	8.9	8.7	97.3
Height/width ratio	0.3	0.02	0.1	0.1	62.5
Ornamental rating	5	1	3.13	0.7	21.3
Growth index ($cm^{3)}$	327750.0	580.0	50548.7	47062.4	93.1
Internode length (mm)	53.0	2.1	18.3	6.8	36.9
Flower diameter (mm)	25.0	15.0	19.5	2.2	11.1
Pedicle length (mm)	184	81	121.0	21.6	17.8
Lamina length (mm)	31.0	8.7	19.2	4.3	22.3
Lamina width (mm)	24.0	5.3	12.1	3.0	25.0
Petiole length (mm)	13.4	2.7	6.8	2.0	29.2

[x]Coefficient of variation.
[y]Means combined across all accessions and environments, N=135.
[z]Means combined across all accession and environments N=387 for internode mean and N=59 for floral data.

Ornamental ratings were different among the accessions (Chi square *P*=0.02) and the two regional collection (Chi square *P*=0.01) groups and different among the accessions. The accessions and plants collected from the southern region tended to have a greater ornamental rating than plants from the central Texas coast in both the field and nursery environment.

There were differences in height, height width ratio, ornamental rating, internode extension, lamina length, lamina width and petiole length among accessions and regional collection of *E. procumbens*. However, with the relatively low CV for ornamental characteristics of interest such as height, height width ratio, and ornamental rating (Table 2.13) indicate there is not much variability available for selection, with the exception of flower count. Flower count was not significant among accessions or regional collection

groups in this study. The lack of differences in flower count could be due to the habit of *E. procumbens* to flower in flushes, peaking during the cooler spring temperatures.

Table 2.14. Means of leaf measures by accession of *Erigeron procumbens* when grown in 2.3 L containers in the nursery or planted to the field.

	Lamina length (mm)		Lamina width (mm)		Petiole length (mm)	
Accession	Field	Nursery	Field	Nursery	Field	Nursery
1	18.9 ± 1.8	21.4 ± 1.6	11.1 ± 0.9	15.2 ± 1.0	8.4 ± 0.8	9.3 ± 0.4
2	15.6 ± 0.7	20.8 ± 1.0	10.6 ± 0.5	14.7 ± 0.6	4.5 ± 0.3	7.2 ± 0.1
3	16.7 ± 0.8	21.6 ± 1.3	10.8 ± 0.5	14.2 ± 0.8	5.8 ± 0.4	7.6 ± 0.4
4	16.6 ± 0.6	21.8 ± 0.9	12.0 ± 0.4	15.4 ± 0.6	6.2 ± 0.5	7.2 ± 0.5
5	20.3 ± 0.7	22.3 ± 0.9	12.6 ± 0.5	14.3 ± 0.9	8.0 ± 0.5	8.2 ± 0.5
6	18.9 ± 1.4	23.4 ± 1.3	11.4 ± 0.9	14.6 ± 0.6	6.0 ± 0.6	6.6 ± 0.3
7	18.5 ± 0.9	21.1 ± 0.8	12.0 ± 0.4	14.7 ± 0.5	7.2 ± 0.4	8.0 ± 0.4
8	15.5 ± 0.7	21.7 ± 0.4	11.5 ± 0.5	16.0 ± 0.7	5.0 ± 0.4	7.2 ± 0.3
9	17.6 ± 0.9	23.8 ± 1.1	10.0 ± 0.7	13.1 ± 0.9	6.4 ± 0.4	9.0 ± 0.6
10	17.4 ± 1.1	22.3 ± 1.2	9.7 ± 0.4	11.8 ± 1.0	5.5 ± 0.5	7.6 ± 0.9
11	20.9 ± 1.1	22.4 ± 0.9	11.7 ± 0.7	12.1 ± 0.8	8.0 ± 0.4	9.2 ± 0.7
12	12.6 ± 1.0	19.3 ± 1.4	7.2 ± 0.3	10.1 ± 0.7	4.1 ± 0.3	5.4 ± 0.3
13	17.7 ± 0.8	25.4 ± 1.1	10.5 ± 0.6	16.6 ± 1.3	5.4 ± 0.3	9.3 ± 0.5
15	18.3 ± 0.8	22.4 ± 0.6	11.0 ± 0.6	15.4 ± 0.8	6.9 ± 0.3	7.2 ± 0.1
16	17.0 ± 1.1	18.9 ± 1.0	10.2 ± 0.6	12.9 ± 0.7	6.2 ± 0.4	6.7 ± 0.3
17	17.4 ± 1.1	19.3 ± 0.8	12.2 ± 0.6	14.3 ± 0.7	5.6 ± 0.3	6.8 ± 0.3
18	18.8 ± 1.2	19.6 ± 0.7	10.0 ± 0.6	11.0 ± 0.7	6.7 ± 0.5	6.8 ± 0.5
ANOVA						
Environment	***[z]		***		***	
Accession	***		***		***	
Environment x Accession	*		*		***	

[y]Values represent mean (± standard errors) of 15 observations for field environment and 9 observations for nursery environment.
[z] NS,*,**,***Non significant or significant at $P \leq 0.05$, 0.01, or 0.001, respectively.

Table 2.15. Means of leaf measures separated by origin of *Erigeron procumbens* accession along Texas coast when grown in 2.3 L containers in the nursery or planted to the field.

Location	Lamina length (mm)		Lamina width (mm)		Petiole length (mm)	
	Field	Nursery	Field	Nursery	Field	Nursery
South	18.37 ± 0.41	22.28 ± 0.38	11.23 ± 0.25	13.67 ± 0.38	6.68 ± 0.21	8.2 ± 0.25
Central	16.96 ± 0.35	21.44 ± 0.39	10.69 ± 0.22	14.34 ± 0.30	5.86 ± 0.16	7.33 ± 0.16
North	18.75 ± 1.17	19.56 ± 0.73	10.00 ± 0.58	11.00 ± 0.69	6.71 ± 0.47	6.78 ± 0.46
ANOVA						
Environment	***[z]		***		***	
Location	***		NS		***	
Environment x Location	NS		***		NS	

[y]Values represent mean (± standard errors) of 15 observations for field environment and 9 observations for nursery environment.
[z] NS,*,**,***Non significant or significant at $P \leq 0.05$, 0.01, or 0.001, respectively.

Sesuvium portulacastrum

Height, height/width ratio, flower count, flower diameter, internode, leaf length, leaf width, petiole length, and stem diameter were different among *S. portulacastrum* accessions (Tables 2.16 and 2.17). When grouped based on region of collection along the Texas coast, there were differences among the regions for height, flower diameter, leaf length, leaf width, petiole length and stem diameter. Only flower count, height/width ratio, growth index, and pedicle length had highly variable traits with CV's near 100 (Table 2.18).

Plants collected from the southern region, like southern accessions of *O. drummondii*, were on average 69 % taller than plants collected from the northern range (Table 2.19). Most of this increase in the average height could be explained by accession 1 and accession 7, with mean heights when grown in the field of 14.4 ± 0.9 cm and 24.2 ± 1.6, respectively (Table 2.16). There was not a significant environmental effect, however there was an interaction between environment and accession. All accessions of

S. portulacastrum collected from the southern coast, except one had a decrease in mean height when grown in the nursery environment. This decrease in height ranged from 13 % for accession 4 to 40 % for accession 5 (Table 2.16). In contrast all *S. portulacastrum* accessions collected from the northern region range had taller mean heights when grown in the nursery. One accession, accession 10, increased its height 60 % compared to field conditions (Table 2.16).

Internode extension was generally greater in the nursery environment, most likely from favorable cultural conditions. Not all accessions were as plastic as the others, for example accession 1 only increased internode extension by 66 % where accession 6 increased internode accession by 220 % when grown in the nursery compared to the field (Table 2.16). This difference in plasticity of internode extension would explain the interaction between accession of *S. portulacastrum* and environment. The region of collection had no effect on the internode extension in this study.

Table 2.16. Means of *Sesuvium portulacastrum* growth measures by accession when grown in 2.3 L containers in the nursery or planted to the field.

	Height (cm)		Height/width ratio ($cm \cdot cm^{-1}$)		Internode length (mm)		Flower Count (No./plant)		Flower Diameter (mm)	
Accession	Field	Nursery	Field	Nursery	Field	Nursery	Field	Nursery	Field	Nursery
1	14.4 ± 0.9[x]	11.7 ± 1.5	0.09 ± 0.01	0.26 ± 0.06	44.2 ± 3.9[y]	73.5 ± 9.6	20.8 ± 7.7	3.3 ± 0.9	14.6 ± 0.9	19.3 ± 0.3
2	10.8 ± 2.1	8.0 ± 3.0	0.08 ± 0.02	0.10 ± 0.04	40.8 ± 3.6	79.9 ± 6.1	16.8 ± 4.6	3.3 ± 0.7	15.6 ± 0.3	17.5 ± 0.5
3	14.2 ± 1.2	8.0 ± 0.6	0.10 ± 0.01	0.10 ± 0.01	25.3 ± 3.1	61.4 ± 5.1	21.8 ± 3.2	18.0 ± 7.5	13.2 ± 1.1	15.5 ± 0.6
4	8.4 ± 0.4	7.3 ± 0.7	0.04 ± 0.00	0.13 ± 0.01	26.3 ± 1.5	54.6 ± 1.7	40.2 ± 19.9	13.0 ± 1.0	16.4 ± 0.5	15.3 ± 0.4
5	12.0 ± 1.7	7.3 ± 1.2	0.06 ± 0.01	0.14 ± 0.03	20 ± 1.2	41 ± 4.2	185.0 ± 42.7	0.7 ± 0.3	15 ± 0.5	16.4 ± 0.2
6	12.7 ± 1.2	13.3 ± 2.0	0.13 ± 0.05	0.15 ± 0.02	30.2 ± 3.7	97.6 ± 5.8	14.7 ± 7.7	0.3 ± 0.3	18 ± 1.1	18.5 ± 0.6
7	24.2 ± 1.6	20.3 ± 1.9	0.18 ± 0.02	0.26 ± 0.02	45.7 ± 2.3	107.2 ± 4.7	15.4 ± 1.8	10.7 ± 0.7	20.4 ± 0.6	19.2 ± 0.5
8	10.4 ± 0.9	12.3 ± 2.3	0.07 ± 0.01	0.13 ± 0.02	27.4 ± 2	76.1 ± 2.8	55.6 ± 12.3	11.0 ± 3.5	17.7 ± 0.5	19.6 ± 0.7
9	8.0 ± 1.2	10.0 ± 2.0	0.06 ± 0.00	0.13 ± 0.04	26.9 ± 3.2	54 ± 4.1	67.7 ± 30.6	9.0 ± 2.9	16 ± 0.9	15 ± 0.4
10	5.8 ± 1.1	9.3 ± 1.3	0.03 ± 0.00	0.10 ± 0.02	37.6 ± 4	71.8 ± 4.2	70.3 ± 11.9	10.0 ± 2.1	12.8 ± 0.3	15.9 ± 0.6
11	7.8 ± 0.5	10.3 ± 2.9	0.04 ± 0.01	0.13 ± 0.03	41.6 ± 2.2	72.9 ± 3.8	79.0 ± 17.2	8.0 ± 2.7	13.5 ± 0.5	15.8 ± 0.7
12	7.0 ± 1.0	8.0 ± 2.1	0.07 ± 0.01	0.08 ± 0.02	45.7 ± 6.8	83.3 ± 2.6	46.3 ± 20.2	14.7 ± 1.9	15.9 ± 1.4	17.4 ± 0.2
13	7.5 ± 0.9	7.7 ± 0.7	0.26 ± 0.21	0.08 ± 0.01	24.3 ± 1.8	71.1 ± 4.8	25.0 ± 10.2	3.3 ± 0.3	14.7 ± 0.7	18 ± 0.4
14	16.0 ± 0.8	21.0 ± 6.0	0.09 ± 0.01	0.25 ± 0.04	29.2 ± 1.9	49.4 ± 2	36.4 ± 3.1	11.0 ± 0.6	15.3 ± 0.3	16 ± 0.4
15	12.8 ± 1.1	12.0 ± 0.6	0.07 ± 0.01	0.13 ± 0.02	37.1 ± 5.7	63.1 ± 4.1	56.5 ± 20.7	3.3 ± 0.8	16.7 ± 0.4	15.2 ± 0.3
ANOVA										
Environment	NS[z]		***		***		***		***	
Accession	***		*		***		***		***	
Environment x Accession	*		NS		***		***		***	

[w]Values represent mean (± standard errors) of 5 observations for field environment and 3 observations for nursery environment.
[y]Values represent mean (± standard errors) internode extension of 15 observations for field environment and 9 observations for nursery environment.

Table 2.17. Means of leaf measures by accession for *Sesuvium portulacastrum* when grown in 2.3 L containers in the nursery or planted to the field.

	Lamina length (mm)		Lamina width (mm)		Petiole length (mm)		Stem Diameter (mm)	
Accession	Field	Nursery	Field	Nursery	Field	Nursery	Field	Nursery
1	29.9 ± 0.8[y]	38.3 ± 1.6	8.6 ± 0.2	8.6 ± 0.3	11.7 ± 0.3	8.0 ± 0.5	4.5 ± 0.1	3.9 ± 0.4
2	20.6 ± 1.0	28.2 ± 0.8	7.5 ± 0.4	8.3 ± 0.3	9.6 ± 0.4	7.0 ± 0.5	3.5 ± 0.1	3.6 ± 0.1
3	23.7 ± 0.4	33.8 ± 1.1	8.0 ± 0.4	10.1 ± 0.3	7.6 ± 0.3	6.4 ± 0.5	3.5 ± 0.1	3.7 ± 0.1
4	24.1 ± 0.8	32.9 ± 1.3	7.1 ± 0.2	7.7 ± 0.2	7.9 ± 0.4	4.4 ± 0.2	2.8 ± 0.1	3.6 ± 0.1
5	24.7 ± 0.6	31.3 ± 1.1	6.4 ± 0.4	7.9 ± 0.3	6.2 ± 0.2	4.3 ± 0.3	3.3 ± 0.1	3.6 ± 0.2
6	28.4 ± 1.4	42.9 ± 1.6	11.9 ± 0.5	14.8 ± 0.3	10.4 ± 0.3	10.5 ± 0.4	4.2 ± 0.2	5.3 ± 0.2
7	32.8 ± 0.9	48.9 ± 1.0	15.2 ± 0.4	17.7 ± 0.6	12.8 ± 0.4	10.8 ± 0.3	5.3 ± 0.2	6.0 ± 0.2
8	25.5 ± 0.8	33.1 ± 1.3	6.1 ± 0.2	6.9 ± 0.3	6.7 ± 0.2	4.3 ± 0.2	3.2 ± 0.1	3.9 ± 0.1
9	21.5 ± 0.8	25.0 ± 0.9	6.2 ± 0.2	5.9 ± 0.3	7.9 ± 0.3	5.3 ± 0.2	2.9 ± 0.1	2.9 ± 0.1
10	26.0 ± 0.6	26.3 ± 0.3	5.6 ± 0.5	5.9 ± 0.3	6.4 ± 0.4	3.9 ± 0.2	2.5 ± 0.1	2.6 ± 0.1
11	27.2 ± 0.8	26.6 ± 0.6	6.7 ± 0.3	5.4 ± 0.2	6.1 ± 0.4	3.9 ± 0.4	2.6 ± 0.1	2.7 ± 0.1
12	29.2 ± 1.0	28.2 ± 1.0	6.9 ± 0.3	7.0 ± 0.3	8.1 ± 0.4	5.1 ± 0.4	3.2 ± 0.2	3.3 ± 0.1
13	20.7 ± 1.2	29.2 ± 0.6	5.3 ± 0.3	5.7 ± 0.2	7.3 ± 0.5	5.8 ± 0.2	2.5 ± 0.1	2.8 ± 0.1
14	26.0 ± 1.0	31.9 ± 0.9	7.4 ± 0.3	6.8 ± 0.3	8.5 ± 0.2	4.4 ± 0.3	3.7 ± 0.1	3.2 ± 0.1
15	33.7 ± 7.4	29.8 ± 0.6	6.5 ± 0.4	5.2 ± 0.2	8.8 ± 0.6	5.1 ± 0.3	3.0 ± 0.1	3.0 ± 0.1
ANOVA								
Environment	***[z]		***		***		***	
Accession	***		***		***		***	
Environment x Accession	***		***		***		***	

[y]Values represent mean (± standard errors) of 15 observations for field environment and 9 observations for nursery environment.
[z] NS,*,**,***Non significant or significant at $P \leq 0.05$, 0.01, or 0.001, respectively.

Table 2.18. Maximum, minimum, mean, standard deviation, coefficient of variation of growth measures combined for all accessions of *Sesuvium portulacastrum* across all environments.

	Max	Min	Mean	Standard Deviation	CV[x]
Plant height (cm)	29.0	3.0	11.5[y]	5.3	45.8
Flower count	303.0	0.0	33.3	47.5	142.6
Height/width ratio ($cm \cdot cm^{-1}$)	0.89	0.2	0.11	0.1	88.6
Ornamental rating	5	1	3.0	0.7	24.7
Growth index ($cm^{3)}$	785672.0	864.0	199752.0	180270.1	90.2
Internode length (mm)	126.0	8.6	48.7[z]	25.1	51.6
Flower diameter (mm)	23.0	10.9	16.4	2.4	13.7
Pedicle length (mm)	113.0	1.0	8.6	8.3	96.8
Lamina length (mm)	92.1	15.3	28.7	7.4	25.7
Lamina width (mm)	20.0	2.8	8.0	3.1	39.4
Petiole length (mm)	16.2	2.0	7.4	2.7	36.2

[x]Coefficient of variation.
[y]Means combined across all accessions and environments, N=111.
[z]Means combined across all accession and environments N=322 for internode mean and N=230 for floral data.

Table 2.19. Means of growth measures separated by origin of accession along Texas coast for *Sesuvium portulacastrum* when grown in 2.3 L containers in the nursery or planted to the field.

	Height (cm)	Flower count (No./plant)		Height/width ratio ($cm \cdot cm^{-1}$)		Internode length (mm)		Flower diameter (mm)	
Location	Combined[u]	Field	Nursery	Field	Nursery	Field	Nursery	Field	Nursery
South	13.5 ± 1.0	58.74 ± 17.21	6.6 ± 2.37	0.1 ± 0.01[v]	0.19 ± 0.02	33.57 ± 1.7[x]	74.8 ± 4.53	17.15 ± 0.46[y]	17.17 ± 0.35
Central	12.6 ± 0.8	36.63 ± 5.28	6.33 ± 1.01	0.08 ± 0.01	0.14 ± 0.02	31.5 ± 1.54	66 ± 2.46	16.19 ± 0.26	16.79 ± 0.34
North	8.0 ± 0.4	58.84 ± 8.51	10.53 ± 1.17	0.09 ± 0.04	0.1 ± 0.01	35.48 ± 1.83	70.62 ± 2.22	14.17 ± 0.36	16.42 ± 0.27
ANOVA									
Environment	NS[z]	***		***		***		***	
Location	***	NS		NS		NS		***	
Environment x Location	NS	NS		NS		NS		***	

[u]Environments combined when not significant to $P \leq 0.05$. Values represent means (± standard errors) of 38, 39, and 34 observation for south, central, and northern coast, respectively.
[v]Values represent means (± standard errors) of height/ width ratio of 23, 24, and 19 observations for south, central, and northern coast, respectively for field environment and observation of 15, 15, and 15 observations for south, central, and northern coast, respectively for nursery environment.
[x]Values represent means (± standard errors) of internode extension for 69, 69, and 51 observations for south, central, and northern coast, respectively for field environment and observation of 44, 45, and 45 observations for south, central, and northern coast, respectively for nursery environment.
[y]Values represent means (± standard errors) of flower diameter for 34, 51, and 29 observations for south, central, and northern coast, respectively for field environment and observation of 29, 42, and 45 observations for south, central, and northern coast, respectively for nursery environment.
[Z] NS,*,**,***Non significant or significant at $P \leq 0.05$, 0.01, or 0.001, respectively.

There were differences in leaf lamina length, leaf lamina width, petiole length, and stem diameter on the regional level, as well as, the accession level. Accessions from the southern region had longer leaves, wider leaves, longer petioles and thicker stems than accessions from either the central or northern collection areas (Table 2.20). Like internode extension, leaf measures generally increased when grown in the nursery environment, with some accessions like accession 6 increasing leaf length 51 % and leaf width 24 % in the nursery environment (Table 2.17). Accessions 11, 12, and 15 saw decreases in leaf measures in the nursery environment compared to the field. Leaf length was correlated with leaf width (r=0.59) and internode extension (r=0.53). Leaf width was strongly correlated with latitude of collection site (r=-0.59) and stem diameter (r=0.83). These changes in leaf size indicate that *S. portulacastrum* leaves are plastic in response to environmental conditions.

Flower count was not affected by region of collection, however there were differences among accessions, with a strong environmental effect. Accessions flowered more in the field than in the nursery environment. This is most likely because plants were larger in the field than in the nursery due to a longer growing season. Even though fewer flowers were produced in the nursery, the flowers were larger for most accessions. Flower diameter was correlated to stem diameter (r=0.52). Some accessions, such as 4 and 15, produced smaller flowers in the nursery than in the field (Table 2.16).

Table 2.20. Means of leaf measures separated by origin of *S. portulacastrum* accession along Texas coast when grown in 2.3 L containers in the nursery or planted to the field.

Location	Lamina length (mm)		Lamina width (mm)		Petiole length (mm)		Stem Diameter (mm)	
	Field	Nursery	Field	Nursery	Field	Nursery	Field	Nursery
South	27.93 ± 0.55[y]	38.86 ± 1.15	9.65 ± 0.43	11.39 ± 0.65	9.72 ± 0.34	7.53 ± 0.46	4.00 ± 0.13	4.52 ± 0.18
Central	25.23 ± 1.08	31.36 ± 0.52	7.17 ± 0.16	7.47 ± 0.27	8.19 ± 0.2	5.47 ± 0.23	3.43 ± 0.06	3.48 ± 0.06
North	25.09 ± 0.56	27.07 ± 0.39	6.14 ± 0.18	5.98 ± 0.14	6.91 ± 0.21	4.80 ± 0.16	2.70 ± 0.06	2.85 ± 0.05
ANOVA								
Environment		***[z]		*		***		***
Location		***		***		***		***
Environment x Location		***		*		NS		NS

[y]Values represent mean (± standard errors) of 15 observations for field environment and 9 observations for nursery environment.
[z] NS,*,**,***Non significant or significant at $P \leq 0.05$, 0.01, or 0.001, respectively.

Ornamental ratings were different when analyzed using Chi square analysis by collection region or accession. There was not a difference among accessions for ornamental rating. Cluster analysis formed two clusters (Fig. 2.5). One group was formed by three of the five southern accessions and all other accessions formed the remaining group. The three accessions forming their own group had larger leaves, stems, and were taller than the other accessions.

The accessions from the southern collection had larger leaves, thicker stems, and a more upright habit than collections from either the central or northern coast. Leave morphology was plastic in response to environment for most accession of *S. portulacastrum*. Flowering seemed more dependent on the environment than region of collection so a region cannot be targeted for future collection areas.

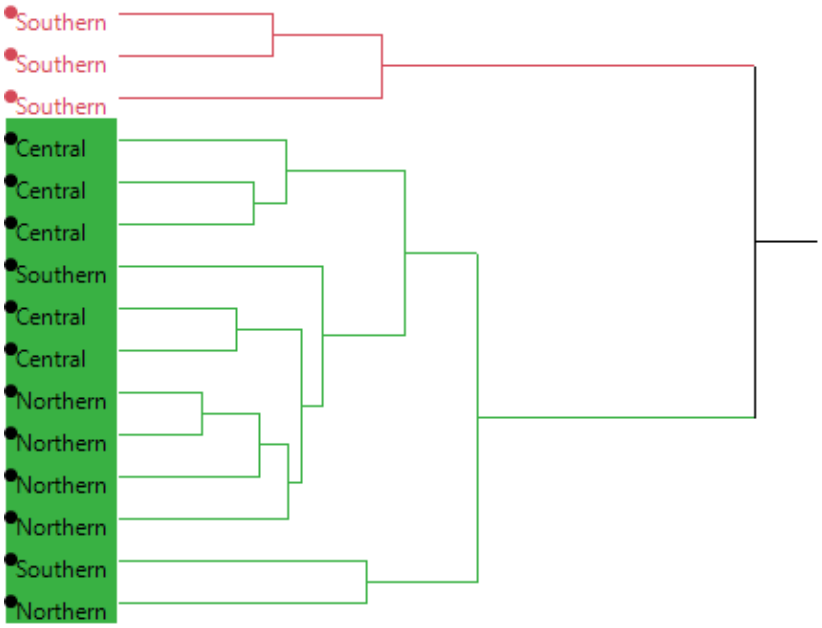

Fig. 2.5. Hierarchical Cluster analysis using Wards distance of *S. portulacastrum* accessions based on morphological traits. Different colors indicate different cluster groups and labels indicate accessions collection region.

Conclusion

We found differences among accessions for all four of the species tested and regional differences in traits of interests in *B. frutescens* and *O. drummondii*. This information can used to guide the collection of future genotypes of *B. frutescens* and *O. drummondii.* This will allow future collectors of germplasm to target their collection efforts to regions based on the characteristics of material in which they are interested. Further collection of *E. procumbens* needs to be performed to test for differences in regional populations. In the future, studies need to be performed to calculate heritability and stability of these characteristics in more environments to determine if these traits can

be used for selection to make gains in ornamental performance over a broader range of environments.

CHAPTER III

PLANT GROWTH REGULATORS*

Response of *O. drummondii* to paclobutrazol, uniconazole, and daminozide

Oenothera drummondii Hook. is a perennial shrub or subshrub native to the USA on sandy beaches from Texas to North Carolina (Correll and Johnston, 1970; USDA Plants Database, 2009). *Oenothera drummondii* has large yellow flowers approximately 4-7 cm in diameter (Correll and Johnston, 1970). These large flowers and industry feedback lead us to believe that *O. drummondii* has potential as a native plant for commercial landscapes. Plant growth retardants, such as triazole compounds and daminozide, can be used to control plant height and internode extension to produce more marketable plants (Basra 2000; Niu et al., 2002; Pallez et al., 2002; Whipker and McCall, 2000), and extend postharvest life (Keever and Kessler, 2008). To our knowledge, there are no published reports of *O. drummondii* responses to plant growth regulators (PGR).

Faust et al. (2001) found as much as a 66% reduction in height and 50% in internode length on *Euphorbia pulcherrima* Willd. *ex* Klotzsch. 'Freedom Red' (poinsettia) with drench application of paclobutrazol and rates as low as 0.118 mg active ingredient (a.i.) $\cdot pot^{-}$. Niu et al. (2002) found a 15% reduction in height of poinsettia compared to controls when paclobutrazol was applied as a drench at 2 $mg \cdot L^{-1}$ (0.24 mg

* Part of this chapter is reprinted with permission from "Growth and flowering responses of sea marigold to daminozide, paclobutrazol, or uniconazole applied as drenches or sprays" by Carver, S.T., M.A. Arnold, D.H. Byrne, A.R. Armitage, and A.R. King, 2014. Vol. 33, pp. 626-631, Copyright 2014 by Springer Science+Business Media New York.

a.i.·pot^{-1}). Arnold (1998) found paclobutrazol rates at 0-2 mg a.i.·pot^{-1} were not great enough to dramatically reduce shoot length in *Salvia greggi* Gray, *Lantana horrida* H.B.K. 'LS Red' or *Verbena canadensis* L. 'Homestead Purple', all plants with a semi-woody habits similar to *O. drummondii*.

In *Oenothera fruticosa* L. 'Youngii-lapsley', a closely related species to *O. drummondii*, three foliar applications of daminozide and paclobutrazol at 5000 mg·L^{-1} and 30 mg·L^{-1}, respectively, had no effect on plant height, however foliar application of uniconazole at 15 mg·L^{-1} reduced height by 31% (Clough et al., 2001). Uniconazole reduced flower diameter of *O. fruticosa* by 36% (Clough et al., 2001). Uniconazole and daminozide treatments also reduced lateral branch length altering the plants' form (Clough et al., 2001).

The purpose of this study is to document the responses of *O. drummondii* Hook. to three commonly available PGR, daminozide, paclobutrazol, and uniconazole, all inhibitors of GA biosynthesis, (Basra, 2000; Brown et al., 1997; Rademacher, 1997) as either drench or foliar applications.

Oenothera drummondii Materials and Method

General protocols

Oenothera drummondii tip cuttings, 4-6 cm long, were taken on 21 April 2011, from containerized stock plants of accession O13 maintained in a gravel bottom nursery in College Station, TX. Basal ends of cuttings were dipped in talc based indolebutyric acid (IBA) at the concentration of 1 g·kg^{-1} (Hormodin® 1, OHP, Inc., Mainland, Pa.). Cuttings were placed in 36 cm x 51 cm x 10 cm deep flats (Kadon Corp., Dayton, Ohio)

filled with coarse perlite (Sun Gro Horticulture Canada Ltd., Seba Beach, Alta.). Intermittent mist was applied at 16 min intervals for 15 sec durations using reverse osmosis water from 1 h before sunrise to 1 h after sunset. On 2 May 2011, rooted cuttings were potted in 0.47 L black plastic pots (Dillen Products, Middlefield, Ohio) containing Metro-Mix 700 media (Sun Gro Horticulture Canada Ltd, Vancouver, B.C.). On 16 May 2011, liners of *O. drummondii*, were potted into 2.3 L black plastic containers (C300S, Nursery Supplies Inc., Kissimmee, Fla.) filled with pine bark based media (Metro Mix 700, Sun Gro Horticulture Canada Ltd, Vancouver, B.C.) amended with 6.53 $kg \cdot m^{-3}$ 15N -3.9P-9.9K controlled release fertilizer (Osmocote® Plus, Scotts Co., Marysville, Ohio) and placed on greenhouse benches in a completely randomized design. Average greenhouse temperature was 27ºC, minimum temperature was 22ºC, maximum temperature was 30ºC (HOBO Pro v2 Temp and RH Data Logger, Onset Computer Corporation, Inc., Pocasset, Mass.). The average photosynthetically active radiation (Accupar, Decagon Devices, Inc., Pullman, Wash.) as sampled on 22 July 2011 at solar noon was 1484 $\mu mol \cdot m^{-2} \cdot s^{-1}$.

Oenothera drummondii Drench study

On 23 May 2011 plants were cut back to 5 cm from the substrate surface. On 24 May 2011, plants were drenched with paclobutrazol at 0, 5, 10, 20, or 40 mg a.i.$\cdot pot^{-1}$ (Bonzi, Syngenta Crop Protection, Inc., Greensboro, N.C.) ((±)-(R*,R*)-β-[(4-Chlorophenyl)methyl]-α-(1,1-dimethylethyl)-IH-1,2,4-triazole-1-ethanol) or uniconazole at 0, 0.5, 1, 2, or 4 mg a.i.$\cdot pot^{-1}$ (Sumagic, Valent U.S.A. Corporation, Walnut Creek, Calif.) ([(E)-(p-chlorophenyl)-4,4-dimethyl-2-(1,2,4-triazol- 1-yl)- 1-penten-3-ol]).

These initial test rates were based upon work previously performed by Arnold (1998) and Arnold and McDonald (2001) on similar sized warm temperate to subtropical woody subshrubs. At the time of treatment application, five plants were destructively harvested to provide baseline data (Table 1). Plants treated with drench applied PGRs were destructively harvested 3 weeks after treatment on 15 June 2011. Growth measures collected included height, width, internode length, flower count, leaf dry mass, stem dry mass, root dry mass (10 d at 80ºC), leaf number, leaf area, growth indices (height x width at the widest point x width perpendicular to the widest point), and ornamental ratings.

In a second experiment, following the same general protocol to verify and refine the rates, plants were drenched with 0, 1.0, 1.5, 2.0, or 2.5 mg a.i.·pot^{-1} uniconazole or 0, 20, 30, 35, or 40 mg a.i.·pot^{-1} paclobutrazol on 31 Oct 2013. Plants were grown in an unshaded glass house (max. temperature 33.3ºC, min. temperature 11.6ºC average temperature 22.6ºC). Plants were destructively harvested on 8 Jan 2014 at 10 weeks after treatment. Data collected included dry shoot and root mass, height, width, internode extension, and flower count.

Both experiments were arranged as completely randomized designs with 5 replications per treatment. The ornamental rating scale was from 1 to 5, with a rating of 1) representing a dead plant or plant near death (unacceptable for sale), 2) plant with severe to mild damage and/or stunting to the canopy but surviving (unacceptable for sale), 3) plant with mild to no stunting to the canopy, with or without flowers present (marginal acceptability as a marketable plant), 4) no stunting to the plant canopy, canopy

is full and dense without stretching or holes, flowers can be present or not present (solidly marketable plant), and 5) no stunting to the plant canopy, canopy is full without stretching or holes, flowers must cover at least 15 percent of the canopy (superior market potential). Ornamental ratings were recorded by a single observer for consistency. An analysis of variance for the main effects for each plant growth regulator (PGR) within each study was conducted using JMP 2009 and SAS 9.3 (SAS Institute Inc., Cary, N.C.) for continuous variables. When significant (P<0.05) effects occurred for a PGR, regression analysis was conducted across the concentrations of that PGR. Chi squares frequency analysis was conducted on the discrete qualitative rating data within each PGR.

Oenothera drummondii Results and Discussion

Plant height was decreased by as much as 29.7% and 38.1% by paclobutrazol and uniconazole, respectively (Fig. 3.1A) in the first experiment. This reduction in height was mostly due to a decrease in the length of internodes (Fig. 3.1B) of treated plants for both PGRs and the number of internodes as indicated by a reduction in leaf number (Fig. 3.2A) for paclobutrazol treated plants (52.2% over controls at 40 mg a.i.·pot^{-1}). The reduction in internode length is consistent with paclobutrazol studies performed on poinsettias by Faust et al. (2001) and Niu et al. (2002) and other subshrubs such as *Salvia greggi* Gray (Arnold, 1998) and *Plumbago auriculata* Lam. (Arnold and McDonald, 2001). Internode length was reduced 61.2% for paclobutrazol at 10 mg·pot^{-1} and 50.4% for uniconazole at 4 mg·pot^{-1}, respectively. This reduction in internode extension resulted in reduced growth indices (height x width x width) (GI).

Paclobutrazol reduced growth index by as much as 85.9% at 40 mg a.i.·pot^{-1} (Fig. 3.3A) and uniconazole reduced growth index 73.0% at 4 mg a.i.·pot^{-1}(Fig. 3.3B). This reduction at the highest levels for both PGRs caused excessively stunted plants. This reduction was similar to the results found in the follow up experiment.

In the second experiment, internode length was reduced 92.1% by paclobutrazol (40 mg a.i.·pot^{-1}) and 80.0% by uniconazole (2.5 mg a.i.·pot^{-1}). This reduction in internode extension resulted in a reduction in growth index. Growth index was reduced 80.0 % by paclobutrazol (40 mg a.i.·pot^{-1}) and 67.2 % by uniconazole at 2.5 mg a.i.·pot^{-1}. These reductions were similar and/or within the range of results from the first experiment for both chemicals (Fig. 3.3).

A decrease in specific leaf area (SLA) was strongly associated with increasing application rates of either paclobutrazol or uniconazole (Fig. 3.2B). Drench treated plants had a reduction in SLA of 31.2% at 20 mg a.i.·pot^{-1} of paclobutrazol and 30.3% for uniconazole at 4 mg·pot^{-1}. Decreasing SLA and leaf area (Fig. 3.2C) indicate an increase in leaf thickness if tissue mass per volume remains constant. The thicker leaves in paclobutrazol treated plants were likely due to increases in the palisade and spongy mesophyll layers (Burrows et al. 1992). Leaf area, leaf counts and leaf mass were not collected in the second experiment.

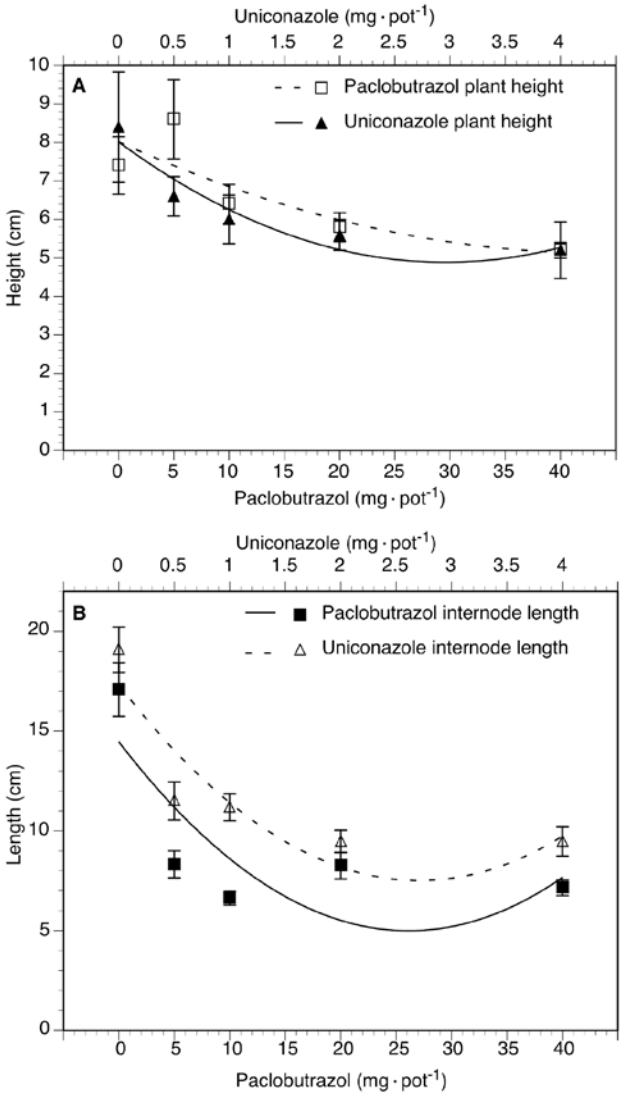

Fig. 3.1. Plant height and internode extension response to paclobutrazol and uniconazole. As rates of drench applied paclobutrazol or uniconazole increased *Oenothera drummondii* height (A), internode length (B) decreased in the first experiment. Symbols indicate means (±standard errors) of five observations. Regression equations for height are presented for paclobutrazol (R^2 = 0.23, y = 8.02-0.131*x + 0.001*x^2) and uniconazole (R^2 = 0.26, y = 8.01 - 2.11*x + 0.36*x^2 +) and for internode length for paclobutrazol(R^2 = 0.39, y = 14.46 - 0.72x + 0.01*x^2) and uniconazole (R^2 = 0.45, y = 17.33 -7.24*x + 1.33*x^2) treated plants. 17.33 -7.24*x + 1.33*x^2.

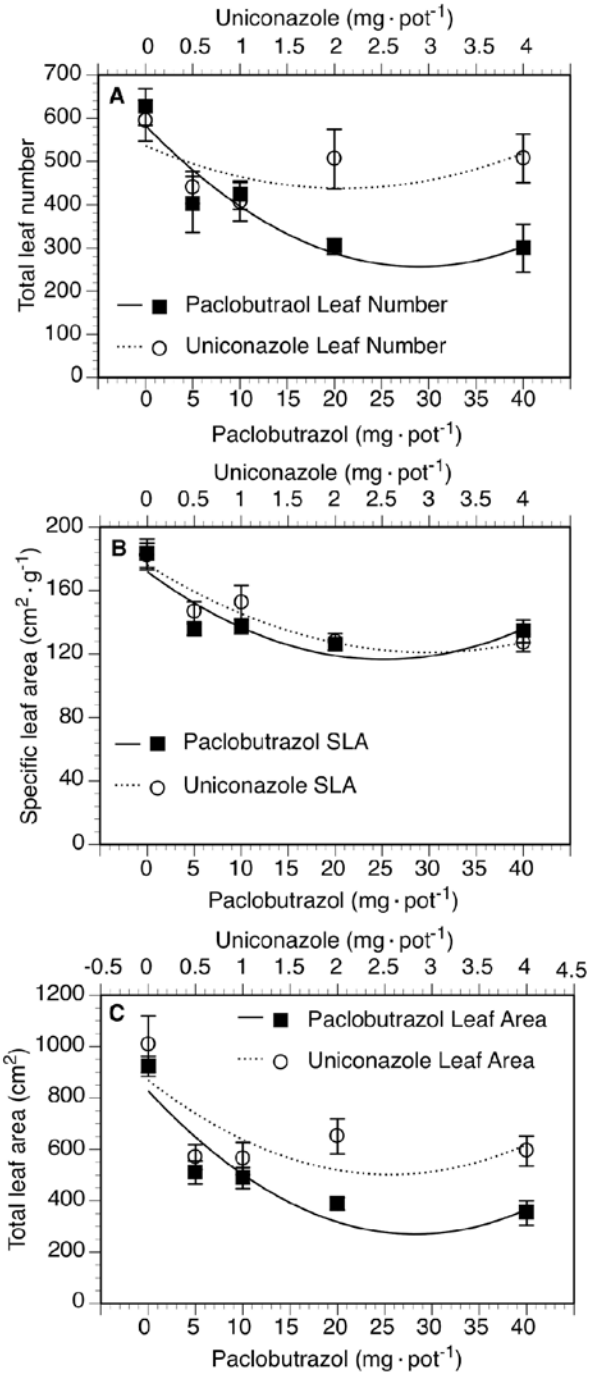

Fig. 3.2. Leaf responses to paclobutrazol and uniconazole in *Oenothera drummondii*. Effects of uniconazole and paclobutrazol drench applications on *Oenothera drummondii* mean number of leaves per plant (A), paclobutrazol leaf number = $583.26 – 22.55x + 0.39x^2$, R^2 =0.51, uniconazole leaf number = $536.32 – 94.09x + 22.48x^2$, R^2 =0.31); specific leaf area (B), paclobutrazol SLA = $171.85 - 4.39x + 0.09x^2$, R^2 =0.53; uniconazole SLA = $176.15 – 36.94x + 6.18x^2$, R^2 =0.53); and mean leaf area (C), paclobutrazol leaf area = $827.25 - 39.52x + 0.70x^2$, R^2 =0.70 , uniconazole leaf area = $868.93 - 286.88x + 56.06x^2$, R^2 =0.22. Symbols represent means (± standard errors) of five observations.

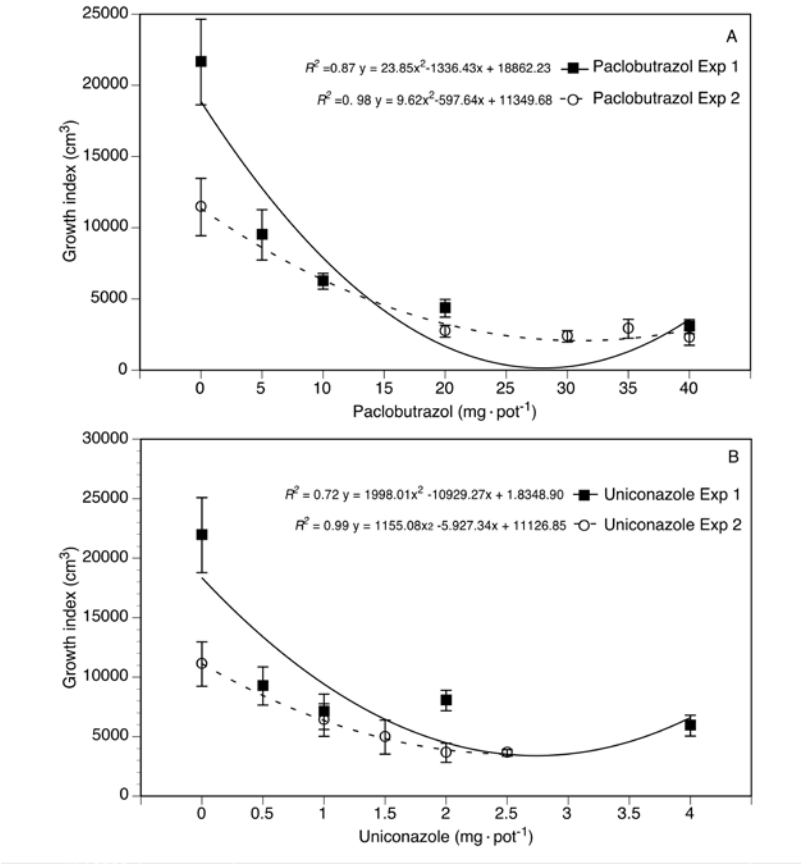

Fig. 3.3. Response of growth index of *Oenothera drummondii* to paclobutrazol and uniconazole. Means of growth index (height x width x width) for *Oenothera drummondii* treated with either drench applied paclobutrazol (A) or drench applied uniconazole (B). Solid lines represent regression equations for experiment 1 conducted in 2011 and dashed lines represent regression equations for experiment 2 conducted in 2013. Symbols indicate means (±standard errors) of five observations.

In the first experiment, paclobutrazol decreased both shoot mass (58.0%) and root mass (57.2%), but root to shoot ratio was not affected by the treatments (data not presented). Similar results were found in the second experiment in which paclobutrazol at 40 mg·pot^{-1} reduced dry shoot mass 69.3 % and reduced root mass by as much as 58.5 % at 35 mg·pot^{-1}. Uniconazole was inconsistent in its effect on shoot and root dry masses between experiments. Uniconazole drenches did not reduce shoot or root mass in the

first experiment, but in the second experiment shoot dry mass was reduced 51.6 % at 2 mg·pot^{-1} and root dry mass was reduced 40.3 % at 2 mg·pot^{-1}.

Flower number was not affected ($P \leq 0.05$) by paclobutrazol drench applications. However, flower size was very weakly negatively correlated (-0.11) ($R^2 = 0.21$) to increasing application rates of uniconazole in accordance with Clough et al. (2001), who reported a 36% reduction in flower diameter with foliar applied uniconazole on *Oenothera fruticosa* 'Youngii-lapsley'. At the rates tested, uniconazole did not affect flower size. In the second experiment all plants failed to flower prior to harvest so effects on flower size and flower count could not be determined.

Ornamental ratings were only different amongst the treatments for uniconazole in the first experiment. In the first experiment, plants treated with 2 mg a.i.·pot^{-1} of uniconazole all had ornamental ratings of 4, indicating an acceptable ornamental landscape plant. All other uniconazole treatments had ornamental ratings less than 4.

Drench application resulted in effective reductions in plant height and growth indices. Control was achieved in different environments and different production times of late spring and winter. Uniconazole applied at rates between 1.5-2.0 a.i.·pot^{-1} and paclobutrazol in the range of 30-35 mg a.i.·pot^{-1} applied as a drench should be effective in achieving approximately a one-third reduction in height and tightening up of the plant by reducing lateral internode extension and overall growth indices without overly stunting the plant during production. However, the ease of foliar application for growers could warrant testing of multiple applications or greater rates to achieve the desired canopy modifications with greater ease of application.

Response of *B. frutescens* to paclobutrazol, uniconazole, and daminozide

Borrichia frutescens (L.) DC., sea marigold or sea tansy, is a woody perennial shrub in the family Asteraceae Brecht. & J. Presl, which is native to the Gulf and Atlantic coasts of North America (U.S. Dept. Agric., 2009). *Borrichia frutescens* has the potential to serve as a groundcover or bank stabilization landscape plant in saline environments (Gilman, 1999). In some instances, when plants are grown commercially, they are given optimal cultural conditions, sometimes resulting in excess shoot extension. It then becomes desirable to control plant height and reduce internode length induced by ample fertilizer and irrigation (Basra, 2000).

Triazole plant growth regulators (PGRs) are often classified as plant growth retardants (Basra 2000), but different members of the triazole PGRs can vary in efficacy on the same species (Burnett et al. 2000; Whipker and Dasoju 1998), among species (Banko and Stefani 1988), and among cultivars of the same species (Whipker and McCall 2000). Results can also vary depending upon the substrate composition and volume of the carrier drench in which the PGR is applied (Arnold and McDonald 2002). Whipker and Dasoju (1998) reported, for the *B. frutescens* relative *Helianthus annuus* L. (sunflower), that uniconazole applied at 32 mg·L^{-1} as a foliar spray provided a 17% reduction in height over controls. Paclobutrazol applied at 40 mg·L^{-1} or 80 mg· L^{-1} as a foliar spray reduced height only 6%. Daminozide in concentrations greater than 4,000 mg· L^{-1} as a foliar spray resulted in a 17% or greater reduction in height (Whipker and Dasoju 1998). Uniconazole applied at 32 mg·L^{-1} as a foliar spray and daminozide at 4,000 to 16,000 mg·L^{-1} as a foliar spray reduced sunflower inflorescence diameter,

whereas paclobutrazol had no effect on inflorescence diameter. Daminozide, paclobutrazol, and uniconazole applications slowed flowering of sunflower by 2 to 3 d compared to non-treated controls (Whipker and Dasoju 1998). Burnett et al. (2000) reported a similar 2 to 3 d delay of flowering when paclobutrazol, uniconazole, and daminozide were applied to another sunflower relative *Coreopsis rosea* Nutt. 'American Dream'. Paclobutrazol (applied at rates of 12 to 60 mg· L^{-1} as a foliar spray) did not significantly reduce height of *C. rosea* 'American Dream', but uniconazole (applied at rates of 10 to 40 mg· L^{-1} as a foliar spray) reduced shoot height by 25-29% and daminozide (applied at rates of 2,500 to 7,500 mg·L^{-1} as a foliar spray) reduced shoot height 17-29% (Burnett et al. 2000).

The following experiments were designed to document the responses of *B. frutescens* to three commercially available plant growth regulators (PGRs), paclobutrazol, uniconazole, and daminozide, applied as either a drench or liquid spray during container production at a range of potentially effective concentrations.

Borrichia frutescens Materials and Methods

Two experiments were performed at Texas A&M Horticultural Gardens (College Station, Texas) to test the efficacy of drench and foliar applications separately. Both experiments were arranged in a completely randomized design on metal benches 0.65 m above the floor. To generate liners tip cuttings of accession B10, 4-6 cm long, were taken on 13 Feb 2011 from containerized stock plants maintained in a gravel bottom nursery in College Station, Texas (30° 37' 24.24", -97° 22' 0.17"). Basal ends of cuttings were dipped in talc based IBA at the concentration of 1 g·kg^{-1} (Hormodin® 1,

OHP, Inc., Mainland, Pa.). Cuttings were placed in 36 cm x 51 cm x 10 cm deep flats (Kadon Corp., Dayton, OH) filled with coarse perlite (Sun Gro Horticulture Canada Ltd., Seba Beach, Alta.). Intermitted mist was applied at 16 min intervals for a 15 sec duration using reverse osmosis water from 1 h before sunrise to 1 h after sunset. On 31 Mar 2011, rooted cuttings were potted in 0.47 L black plastic pots (Dillen Products, Middlefield, Ohio) containing Metro-Mix 700 media (Sun Gro Horticulture Canada Ltd., Vancouver, B.C.).

Borrichia frutescens drench application

Liners of sea marigold, *B. frutescens*, were potted into 2.3 L containers (C300S, Nursery Supplies Inc., Kissimmee, Fla.) filled with Metro-Mix 700 media (Sun Gro Horticulture Canada Ltd., Vancouver, B.C.) amended with 6.53 kg·m-3 15N -3.9P-9.9K controlled release 3 to 4 month formulation fertilizer (Osmocote® Plus, Scotts Co., Marysville, Ohio) and grown in greenhouse conditions (mean temperature was 27˚C, minimum temperature 22˚C, maximum temperature was 30˚C, average photosynthetically active radiation was 1484 µ·mol·m-2 at solar noon sampled on 22 July 2011). On 14 May 2011 plants were cut back to two nodes and were approximately 8 cm in height. Following bud break, on 25 May 2011 plants were drenched with five levels of paclobutrazol ((±)-(R*,R*)-β-[(4-Chlorophenyl)methyl]-α-(1,1-dimethylethyl)-IH-1,2,4-triazole-1-ethanol) (0, 5, 10, 20, or 40 mg a.i.·pot^{-1}) (Bonzi, Syngenta Crop Protection, Inc., Greensboro, N.C.) or uniconazole ([(E)-(p-chlorophenyl)-4,4-dimethyl-2-(1,2,4-triazol- 1-yl)- 1-penten-3-ol] (0, 0.5, 1,2, or 4 mg a.i.·pot^{-1}) (Sumagic, Valent

U.S.A. Corporation, Walnut Creek, Calif.). Daminozide was not included in drench applications because it is not labeled for drench application.

Borrichia frutescens foliar application.

In a separate experiment, plants grown in 2.3 L containers (same cultural conditions as above except mean temperature was 28˚C, minimum temperature 25˚C, maximum temperature was 33˚C) were cut back to two nodes and approximately 8 cm in height on 24 June 2011. Following budbreak plants were treated with paclobutrazol (0, 50, 100, 200, or 400 mg a.i.·L^{-1}), uniconazole (0, 25, 50, 100, or 200 mg a.i.·L^{-1}) or daminozide [butanedioic acid mono (2,2-dimethylhydrazide)] (0, 2500, 5000, 10000, or 20000 mg a.i.·L^{-1}) (B-Nine, Chemtura USA Corporation, Middlebury, Conn.) as spray applications on 7 July 2011. Plants were sprayed to run-off using approximately 30 mL per container. This provided approximately 0.0, 1.5, 3.0, 6.0, and 12.0 mg a.i.·pot^{-1} of paclobutrazol, 0.0, 0.75, 1.5, 3.0, and 6.0 mg a.i.·pot^{-1} of uniconazole, or 0.0, 75, 150, 300, 600 mg a.i.·pot^{-1} of daminozide.

Plants treated with drench applied PGRs were destructively harvested on 21 July 2011 at 8 weeks after treatment and plants treated with spray application of PGRs were destructively harvested on 18 Aug 2011 at 6 weeks after treatment. Growth measures included height, width, internode length, flower count, leaf dry mass, stem dry mass, root dry mass (10 d at 80˚C), leaf number, leaf area and ornamental ratings for both experiments. The ornamental rating scale was from 1 to 5, with 1) representing a dead plant or plant near death (unacceptable for ornamental performance), 2) plant with severe to mild damage and/or stunting to the canopy but surviving (unacceptable for

ornamental performance), 3) plant with mild to no stunting to the canopy, with or without flowers present (marginal acceptability as a marketable plant), 4) no stunting to the plant canopy, canopy is full and dense without stretching or holes, flowers can be present or not present (acceptable marketable plant), and 5) no stunting to the plant canopy, canopy is full without stretching or holes, flowers must cover at least 15 % of the canopy (superior market potential). For consistency, ornamental ratings were determined by a single observer. Qualitative data were analyzed using JMP 2009 and SAS (SAS Institute Inc., Cary, N.C.) for analysis of variance (ANOVA) using a completely random model for each experiment (drench and spray studies) and regression analysis was conducted for quantitative variables where significant ($P \leq 0.05$) main effects were found for PGR types. Discrete qualitative ornamental ratings were analyzed by Chi square frequency analysis and deemed significant at $P \leq 0.05$.

***Borrichia frutescens* Results and Discussion**

Drench Applications. Drench applications of PGRs significantly affected shoot mass and root mass ($P \leq 0.05$). The most reduction in both shoot mass and root mass (52.9 % and 48.5 %, respectively) was induced by paclobutrazol at 40 mg a.i.·pot^{-1}(Fig. 3.4A and 3.4C). Uniconazole was inconsistent in its effects on root mass. At the highest level (4 mg a.i.·pot^{-1}) uniconazole did not reduce root mass significantly from controls, but at the lowest level (0.5 mg a.i.·pot^{-1}) it reduced root mass 32.4 % (Fig. 3.4B). Shoot mass for uniconazole at 0.5 mg a.i.·pot^{-1} was reduced from the control and uniconazole at 4.0 mg a.i.·pot^{-1}, 21.2% and 5.3%, respectively (Fig. 3.4A). While shoot mass and root mass were significant among the treatment groups, predictive equations for root and

shoot masses with uniconazole were not. Paclobutrazol had a more consistent linear relationship with increasing levels resulting in linear reductions in both root and shoot masses (Fig. 3.4A and 3.4B).

Increasing levels of paclobutrazol and uniconazole reduced leaf number by 56.7 % and 23.8 %, respectively (Fig. 3.4C) in a linear fashion (r^2=0.48 and r^2= 0.20, for paclobutrazol and uniconazole, respectively). Since leaves of this species are consistently borne two to a node (Correll and Johnston, 1979), this also indicates a reduction in the number of nodes on the plant with increasing PGR levels. Uniconazole showed differences ($P \leq 0.03$) amongst the treatment groups for leaf area (Fig. 3.4D) but there was not a significant regression model, indicating an inconsistent pattern of impact on leaf area. Height reduction of 54.9 % was achieved on *B. frutescens* with paclobutrazol at 40 mg a.i.·pot^{-1} and 34.9% with uniconazole at 2 mg a.i.·pot^{-1}(Figs. 3.4E and 3.5). This reduction in height could be explained by a concurrent reduction in internode extension (Fig. 3.4F) and internode number as indicated by reduced leaf numbers (Fig. 3.4C) for both drench applied PGRs. The highest levels of paclobutrazol and uniconazole decreased growth index, a pseudo-volumetric estimate of canopy size for *B. frutescens*, by 72.3 % and 50.1 %, respectively (Fig. 3.4G). Growers will attempt to produce a finished plant that is within the golden mean of height and diameter relative to container height (Sachs et al., 1976). The golden mean or Phi, considered aesthetically pleasing by many, is achieved when the length of the longer segment divided by the shorter equals 1.62 (Navon, 2011). To achieve the golden mean between plant height

and container height in this study plants would have to be reduced approximately 30-33 % from controls.

Height reduction and growth index was similar to that found by Whipker and McCall (2000) who found a 33 % reduction with paclobutrazol at 4 mg a.i.·pot^{-1} in sunflower. A reduction of 33 % was attained in the current research with 5 mg a.i.·pot^{-1} in *B. frutescens*. Uniconazole drenches however were not as effective in controlling height in *B. frutescens* as it was in *Osteospermum ecklonis* (DC.) Norl for Gibson and Whipker (2003) in a similar volume of substrate (2.53 L compared to 2.3 L). Gibson and Whipker (2003) observed severe stunting in *O. ecklonis* with a drench rate of 1 mg a.i.·pot^{-1} and in this study *B. frutescens* was only reduced in height by 24.2 % at 4 mg a.i.·pot^{-1} with no stunting. Woody subshrubs from several families of plants have been reported to require greater rates of PGRs than those typically used on more herbaceous species to achieve similar reductions in canopy proportion (Arnold, 1998; Arnold and McDonald, 2001), and such appears to be the case with *B. frutescens*.

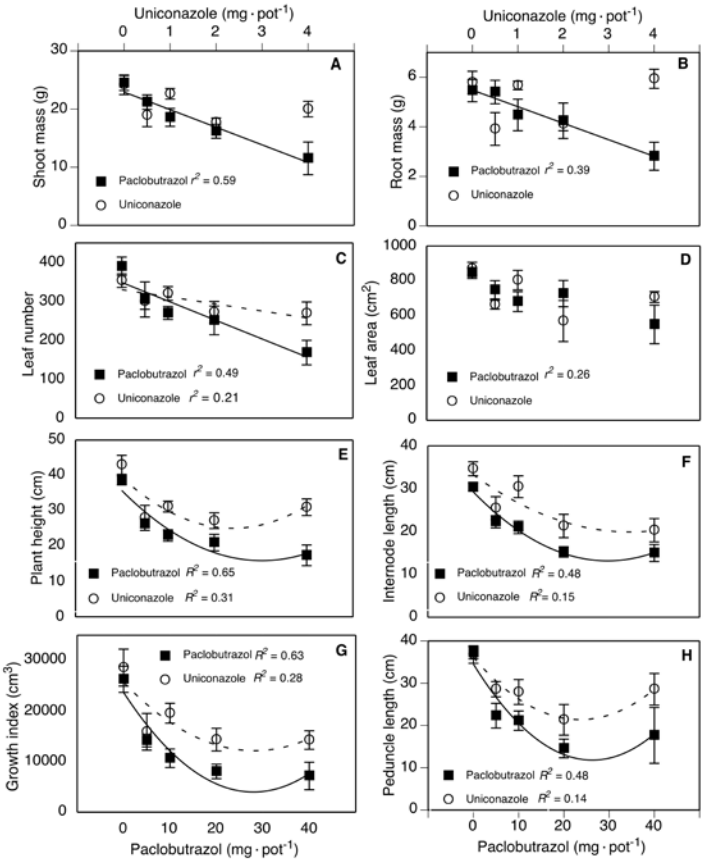

Fig. 3.4. Drench application. Main effects of increasing concentrations of drench applied paclobutrazol or uniconazole on *Borrichia frutescens* grown in 2.3 L containers. Solid lines indicate regression equations for paclobutrazol, while dashed lines indicate regression equations associated with uniconazole means. Regression equation for shoot mass presented is for paclobutrazol (A) (r^2 =0.59, y =22.92 – 0.30x). Regression equation root mass is for paclobutrazol treated plants (r^2 = 0.39, y = 5.48 – 0.07x), regression equations for root mass of uniconazole treated plants were not significant (B). Regression equations for leaf number are presented for paclobutrazol (C) (r^2 = 0.49, y = 348.58 – 4.78x) and uniconazole (r^2 = 0.21, y = 329.96 – 18.08x). Regression equations for leaf area are presented for paclobutrazol (D) (r^2 = 0.26, y = 803.45 – 6.19x). Regression equations for plant height are presented for paclobutrazol (E) (R^2 = 0.65, y = 35.74 – 1.31x + 0.02x^2) and uniconazole (R^2 = 0.31, y = 39.46 – 11.99x + 2.50x^2). Regression equations for internode length are presented for paclobutrazol (F) (R^2 = 0.48, y = 29.92 – 1.10x + 0.02x^2) and uniconazole (R^2 = 0..15, y = 30.99 – 3.46x + 0.14x^2). Regression equations for growth index are presented for paclobutrazol (G) (R^2 = 0.63, y = 23686 – 1408.79x + 25.19x^2) and uniconazole (R^2 = 0.28, y = 25697 – 9614.11x +1706.86x^2). Regression equations for peduncle length are presented for paclobutrazol (H) (R^2 = 0.48, y = 34.71 – 1.80x + 0.04x^2) and uniconazole (R^2 = 0.14, y = 35.83 – 12.16x +2.58x^2). All graph symbols indicate means (±standard errors) of either five observations (A-E, G) or fifteen observations (F and H).

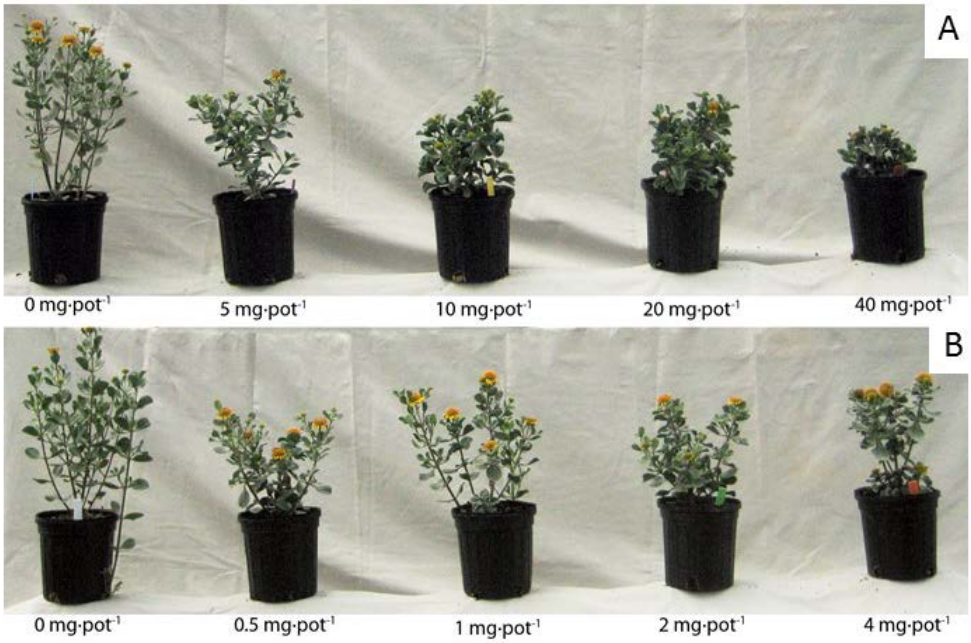

Fig. 3.5. *Borrichia frutescens* response to drench applied paclobutrazol and uniconazole. Drench application. Images showing increasing treatment levels of PGRs from left to right, (A) paclobutrazol drench treated plants (B) uniconazole drench treated *Borrichia frutescens.*

In general, neither drench applied paclobutrazol nor uniconazole substantially affected flowering morphology. Flower number and diameter were not significantly different (data not presented). Peduncle length was decreased by the application of either paclobutrazol or uniconazole with reductions as high as 60.8 % for paclobutrazol at 20 mg a.i.·pot^{-1} and 42.0 % for uniconazole at 2 mg a.i.·pot^{-1} (Fig.3.4H). This shortening of the peduncle length would be consistent with reductions observed for internode extension on the shoots. Since inflorescences were typically in a terminal position on the branches, even with a reduction in peduncle length (Fig. 3.4H), inflorescences were still readily visible above the foliage for all but the highest application rates (Fig. 3.5).

Ornamental ratings aimed at estimating market appeal on the day of harvest were deemed significant ($P \leq 0.002$), through Chi square frequency analysis, for paclobutrazol treated plants. All plants treated with 10 or 20 mg a.i.·pot^{-1} paclobutrazol received ornamental ratings of 4, whereas all other treatments were spread equally between ornamental ratings of 2-4. No plants were rated with either a 1 or 5. Ornamental ratings were not different at $P \leq 0.05$ for uniconazole treated plants.

Borrichia frutescens Spray Applications.

No growth measures recorded for *B. frutescens* were found to be different at $P \leq 0.05$ with spray applied paclobutrazol at the levels tested. Spray application of daminozide was found to be significant only for growth indices by ANOVA and regression analysis (Fig. 3.6A), while effects of spray applied uniconazole were found to be significant for growth index (Fig. 3.6A), height (not presented), and internode length (Fig 3.6B). Uniconazole applied at intermediate rates, peaking at around 100 mg a.i.·L^{-1}, appears to have slightly stimulated growth indices (Fig. 3.6A). In this study spray applied PGRs did not affect flower number, flower diameter, or peduncle length amongst the treatment groups (data not presented). Ornamental ratings were not different among treatment groups (data not presented).

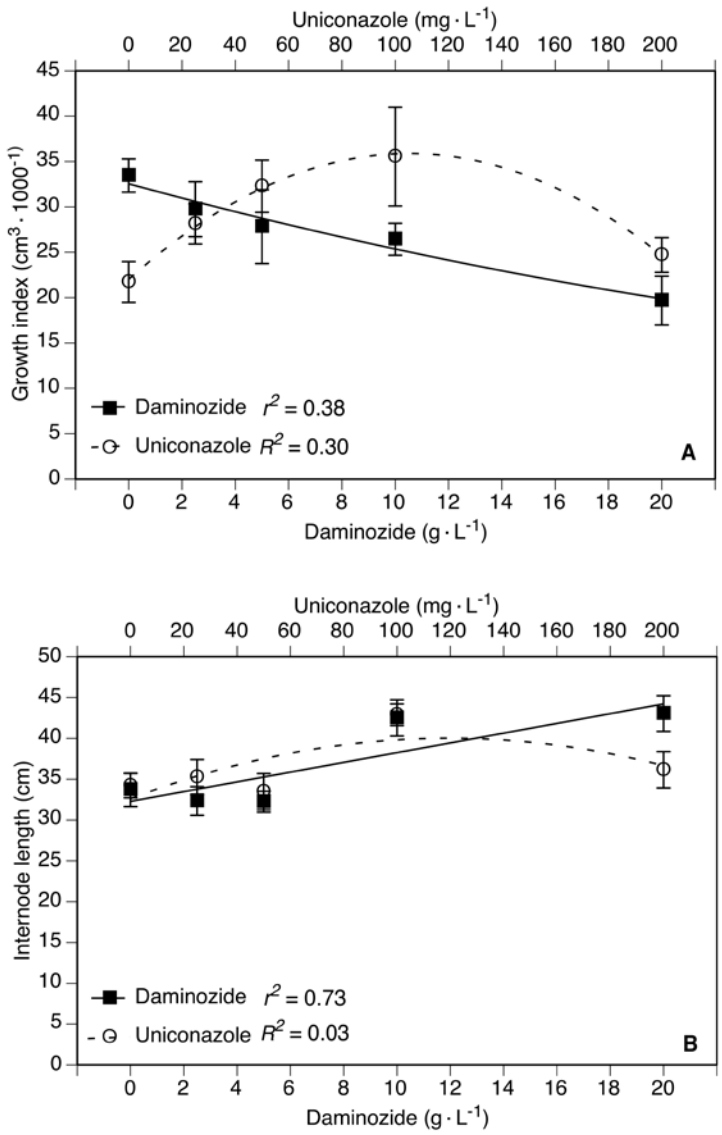

Fig. 3.6. Growth index and internode response to spray applications of daminozide and uniconazole. Spray Application. Main effects of spray applied daminozide (r^2 = 0.38, y = 32124 – 0.63x) and uniconazole on growth indices (height x width x width) (A) (R^2 = 0.30, $y = 21945 – 258.92x + 1.23x^2$) of *Borrichia frutescens*. Internode extension with daminozide regression equations were significant (r^2 = 0.73, y = 32.3 – 0.60x), but explained little of the variation in the data for uniconazole responses (B) (R^2 = 0.03, $y = 32.6 – 0.12x – 0.0005x^2$). Internode length was not significant for paclobutrazol treated plants. Regression equations are for means. Equations with solid lines represent those for daminozide and dashed lines those for uniconazole. Symbols indicate means (±standard errors) of five observations for growth indices (A) and fifteen observations for internode length (B).

***Borrichia frutescens* Conclusion**

In accordance with studies performed on other members of the *Asteraceae* by Whipker and McCall (2000) and Gibson and Whipker (2003), we observed greater reductions in height, plant indices, leaf number and internode extension with less active ingredient in drenches than with spray applications. All levels of the spray application treatments were generally ineffective in this experiment at controlling height. Peduncle length was reduced in drench application treatments of both paclobutrazol and uniconazole. This may not be desirable as *B. frutescens* does not have an excessively long peduncle and reducing its length could result in flowers being obscured by foliage at the greatest application rates.

Based on the regression equations for height, (Fig. 3.4E) the best rate of drench applied paclobutrazol and uniconazole would be 7 mg a.i.·pot^{-1} of paclobutrazol or 2 mg a.i.·pot^{-1} of uniconazole to achieve an approximate 33% reduction in height without overly stunting the plant.

The spray application of paclobutrazol, uniconazole, and daminozide was generally ineffective at controlling growth of *B. frutescens* even at four times the spray concentration of the label rate with a single application. Arnold (1998) and Arnold and McDonald (2001) reported similar challenges with spray applications on woody perennials *Salvia greggii* Gray and *Plumbago auriculata* Lam. in which drench applications resulted in more consistent reductions in shoot extension. Additional studies need to be performed to determine if multiple spray applications during production of any of the three PGRs tested could be effective at height reduction.

Response of *E. procumbens* to paclobutrazol, uniconazole, and daminozide

Erigeron procumbens (Houst. *ex* Mill.) G.L. Nesom is a stoloniferous herbaceous coastal native to Texas with potential as a ground cover plant, spiller in containers, or hanging basket plant. Plants grown commercially are usually given optimal cultural conditions, this sometimes results in excess shoot extension. If optimal cultural conditions result in excessive shoot extension it is desirable to control plant height and reduce internode extension (Basra, 2000).

Triazole plant growth regulators (PGRs) are often classified as plant growth retardants (Basra, 2000), but different members of the triazole PGRs can vary in efficacy on the same species (Burnett et al., 2000; Whipker and Dasoju, 1998), among species (Banko and Stefani, 1988), and among cultivars of the same species (Whipker and McCall, 2000). Results can also vary depending upon the substrate composition and volume of the carrier drench in which the PGR is applied (Arnold and McDonald, 2002). Whipker and Dasoju (1998) reported, for the *E. procumbens* relative *Helianthus annuus* L. (sunflower), that uniconazole applied at 32 $mg \cdot L^{-1}$ as a foliar spray provided a 17% reduction in height over controls. Paclobutrazol applied at 40 $mg \cdot L^{-1}$ or 80 $mg \cdot L^{-1}$ as a foliar spray reduced height only 6%. Daminozide in concentrations greater than 4,000 $mg \cdot L^{-1}$ as a foliar spray resulted in a 17% or greater reduction in height (Whipker and Dasoju, 1998). Uniconazole applied at 32 $mg \cdot L^{-1}$ as a foliar spray and daminozide at 4,000 to 16,000 $mg \cdot L^{-1}$ as a foliar spray reduced sunflower inflorescence diameter, whereas paclobutrazol had no effect on inflorescence diameter. Daminozide, paclobutrazol, and uniconazole applications slowed flowering of sunflower by 2 to 3 d

compared to non-treated controls (Whipker and Dasoju, 1998). Burnett et al. (2000) reported a similar 2 to 3 d delay of flowering when paclobutrazol, uniconazole, and daminozide were applied to another sunflower relative *Coreopsis rosea* Nutt. 'American Dream'. Paclobutrazol (applied at rates 12 to 60 mg· L^{-1} as a foliar spray) did not significantly reduce height of *C. rosea* 'American Dream', but uniconazole (applied at rates of 10 to 40 mg· L^{-1} as a foliar spray) reduced shoot height by 25-29% and daminozide (applied at rates of 2,500 to 7,500 mg·L^{-1} as a foliar spray) reduced shoot height 17-29% (Burnett et al., 2000).

This research was designed to document the responses of *E. procumbens*, to three commercially available plant growth regulators (PGRs), paclobutrazol, uniconazole, and daminozide, applied as either a drench or liquid spray during container production at a range of potentially effective concentrations.

Erigeron procumbens Materials and Methods

General protocols

Erigeron procumbens tip cuttings, 4-6 cm long, were taken on 25 April 2011, from containerized stock plants of accession E18 maintained in a gravel bottom nursery in College Station, Texas. Basal ends of cuttings were dipped in talc based indole butyric acid at the concentration of 1 g·kg^{-1} (Hormodin® 1, OHP, Inc., Mainland, Pa.). Cuttings were placed in 36cm x 51cm x 10cm deep flats (Kadon Corp., Dayton, Ohio) filled with coarse perlite (Sun Gro Horticulture Canada Ltd., Seba Beach, Alta.). Intermittent mist was applied at 16 min intervals for 15 sec duration using reverse osmosis water from 1 h before sunrise and 1 h after sunset. On 2 May 2011, rooted cuttings were potted in 0.47

L black plastic pots (Dillen Products, Middlefield, Ohio) containing Metro-Mix 700 media (Sun Gro Horticulture Canada Ltd., Vancouver, B.C.). On 16 May 2011, liners of *O. drummondii*, were potted into 2.3 L black plastic containers (C300S, Nursery Supplies Inc., Kissimmee, Fla.) filled with pine bark based media (Metro Mix 700, Sun Gro Horticulture Canada Ltd, Vancouver, B.C.) amended with 6.53 kg·m-3 15N -3.9P-9.9K controlled release fertilizer (Osmocote® Plus, Scotts Co., Marysville, Ohio) and placed on greenhouse benches in a completely randomized design. Average greenhouse temperature was 27ºC, minimum temperature 22ºC, and maximum temperature was 30ºC (HOBO Pro v2 Temp and RH Data Logger, Onset Computer Corporation, Inc., Pocasset, Mass.). The average photosynthetically active radiation (Accupar, Decagon Devices, Inc., Pullman, Wash.) as sampled on 22 July 2011 at solar noon was 1484 $\mu \cdot mol \cdot m^{-2} \cdot s^{-1}$.

Drench study

On 23 May 2011 plants were cut back to 6 cm from the substrate surface. On 24 May 2011, plants were drenched with paclobutrazol at 0, 5, 10, or 20 mg a.i.$\cdot pot^{-1}$ (Bonzi, Syngenta Crop Protection, Inc., Greensboro, N.C.) ((±)-(R*,R*)-β-[(4-Chlorophenyl)methyl]-α-(1,1-dimethylethyl)-IH-1,2,4-triazole-1-ethanol) or uniconazole at 0, 0.5, 1, or 2 mg a.i.$\cdot pot^{-1}$ (Sumagic, Valent U.S.A. Corporation, Walnut Creek, Calif.) ([(E)-(p-chlorophenyl)-4,4-dimethyl-2-(1,2,4-triazol- 1-yl)- 1-penten-3-ol]). These rates were based upon work previously performed by Arnold (1998) and Arnold and McDonald (2001) on similar sized warm temperate to subtropical woody subshrubs. At the time of treatment application, five plants were destructively harvested to provide

baseline data. Liners had a mean height of 5.6 cm ± 0.74, mean leaf number of 17.0 ± 2.9 and mean leaf area of 34.0 cm^2 ± 4.9.

Foliar study

In a separate experiment, plants grown in 2.3 L containers were cut back to 6 cm from substrate on 5 July 2011 and were treated with paclobutrazol (0, 25, 50, 100, or 200 mg a.i.·L^{-1}), uniconazole (0, 12.5, 25, 50, or 100 mg a.i.·L^{-1}) or daminozide (0, 1250, 2500, 5000, or 10000 mg a.i.·L^{-1}) (B-Nine, Chemtur\a USA Corporation, Middlebury, Conn.) ([butanedioic acid mono (2,2-dimethylhydrazide)]) as foliar applications on 12 July 2011 after bud break began. Liners were sprayed to runoff using approximately 30 mL per container. This provided approximately 0.0, 0.75, 1.5, 3.0, and 6.0 mg a.i.·pot^{-1} of paclobutrazol, 0.0, 0.375, 0.75, 1.5, and 3.0 mg a.i.·pot^{-1} of uniconazole, or 0.0, 37.5, 75.0, 150.0, 300.0 mg a.i.·pot^{-1} of daminozide. Liners were produced in the same manner as the first experiment. Cuttings were taken on 30 May 2011, potted 0.47 L black plastic on 5 June 2011 and potted into 2.3L containers on 24 June 2011. Average greenhouse temperature was 28ºC, minimum temperature 25ºC, and maximum temperature was 33ºC.

Both experiments were arranged as completely randomized designs with 5 replications per treatment. Plants treated with drench applied PGRs were destructively harvested 6 weeks after treatment on 29 June 2011 and plants treated with foliar application of PGR's were destructively harvested 1 month after treatment on 3 August 2011. Growth measures included height, width, internode length, flower count, leaf dry mass, stem dry mass, root dry mass (10 d at 80ºC), leaf number, leaf area, growth indices

(height x width at the widest point x width perpendicular to the widest point), and ornamental ratings for both experiments. Ornamental rating scale was from 1 to 5, with a rating of 1) representing a dead plant or plant near death (unacceptable for sale), 2) plant with severe to mild damage and/or stunting to the canopy but surviving (unacceptable for sale), 3) plant with mild to no stunting to the canopy, with or without flowers present (marginal acceptability as a marketable plant), 4) no stunting to the plant canopy, canopy is full and dense without stretching or holes, flowers can be present or not present (solidly marketable plant), and 5) no stunting to the plant canopy, canopy is full without stretching or holes, flowers must cover at least 15 percent of the canopy (superior market potential). Ornamental ratings were recorded by a single observer to ensure consistency. An analysis of variance for the main effects for each plant growth regulator (PGR) within each study was conducted using JMP 2009 and SAS 9.3 (SAS Institute Inc., Cary, N.C.) for continuous variables. When significant (P<0.05) effects occurred for the main effect of a PGR, regression analysis was conducted across the concentrations of that PGR. All non-normal data was analyzed using permutations in the lmPerm package (Wheeler, 2010) in R (R Core Team, 2013), set to defaults.

Erigeron procumbens Results and Discussion

Erigeron procumbens Drench applications.

Both drench applied paclobutrazol and uniconazole were effective at controlling growth of *E. procumbens*. Shoot mass was decreased 40 % by paclobutrazol 20 mg a.i.·pot^{-1} and decreased 27 % by uniconazole at 1 mg a.i.·pot^{-1}(Fig. 3.7).

Growth index (GI) (height x width at the widest x width perpendicular to first width) was decreased by as much as 62 % for paclobutrazol and 29 % for uniconazole (Fig. 3.8A). Height was not significantly different amongst treatment groups this can be explained by the growth habit of *E. procumbens*. *Erigeron procumbens* is a prostrate stoloniferous plant (Correll and Johnston, 1970). If the dense rosettes and stolons are against the substrate and there is no change in leaf morphology there will little to no change in plant height.

While total mean leaf area and total mean leaf mass were reduced, individual leaf mean area and leaf mass were not reduced (Fig.3.9A and B). This is mostly likely a function of the concurrent reduction in leaf number (Fig. 3.9C). Specific leaf area (SLA) (leaf area / leaf mass) was not significantly different amongst treatment groups for both drench applied paclobutrazol and uniconazole (data not reported). A lack of decrease in specific leaf area is in contrast to Matsoukis et al. (2007) who found a decrease in SLA in *Lantana camara* L. with increasing rates of paclobutrazol at similar rates as those tested in this study and Burrows et al. (1992) who found paclobutrazol treated plants had

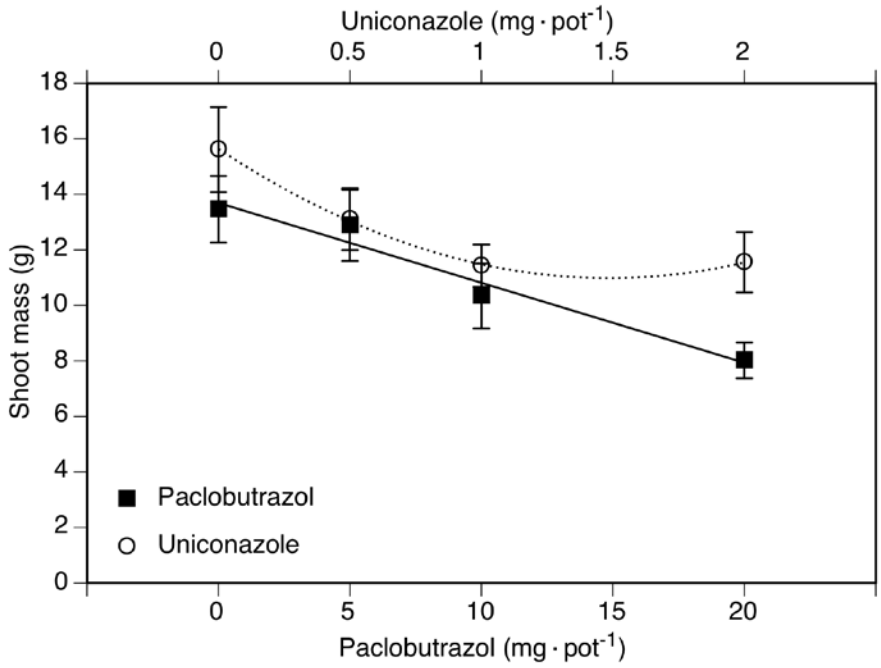

Fig. 3.7. Effect of drench applied paclobutrazol or uniconazole on shoot mass of *E. procumbens*. Effect of drench applied paclobutrazol (r^2=0.47, y = 13.69- 0.29x) or uniconazole (R^2= 0.27, y = 15.64 - 6.27x + 2.11x^2) on shoot mass of *Erigeron procumbens*. Solid lines indicate regression lines for paclobutrazol and dashed lines indicate regression lines for uniconazole. All symbols represent means (n=5) with solid symbols indicating paclobutrazol treated plants and open symbols indicating means for uniconazole treated plants.

increased leaf thickness. No change in SLA, no difference in individual leaf mean area, and individual leaf mass indicated drench applied paclobutrazol and uniconazole had little effect on leaf size or leaf thickness, and therefore little effect on plant height.

Most of the reduction in growth was due to reduced width at the widest point (Fig. 3.8B). Paclobutrazol reduced width 39 % at 20 mg a.i.·pot^{-1} and uniconazole reduced width 27 % at 1 mg a.i.·pot^{-1}. The reduction in width can be attributed to two growth parameters, internode extension and total number of nodes. Mean internode extension was reduced by as much as 45 % for paclobutrazol at 10 mg a.i.·pot^{-1} and 35 % by uniconazole at 2 mg a.i.·pot^{-1} (Fig. 3.8C). and a reduction in the total number of

number of nodes as evidenced by a reduction in leaf number (Fig. 3.9C) This reduction in growth caused by reduced internode length is consistent with paclobutrazol's and uniconazole's mode of action of blocking GA synthesis and therefore reducing internode extension (Basra, 2000; Fletcher and Hofstra, 1985).

Flower diameter was slightly increased by both PGRs (Fig. 3.10). Paclobutrazol increased flower diameter by as much as 9 % (10 mg a.i.·pot^{-1}) and uniconazole increased flower diameter by as much as 8 % (1 mg a.i.·pot^{-1}). In sunflower Dasjou et al.(1998) found a reduction of inflorescence diameter by as much as 30 % with drench applied paclobutrazol at 32 mg a.i.·pot^{-1} compared to untreated controls. Neither PGR tested as drench application affected peduncle length, which is in contrast to reductions seen in *B. frutescens* (L.) DC. (Carver et al., 2014) and in *Achillea* spp. (Burnett et al., 2000).

Paclobutrazol did not affect ornamental ratings ($P \leq 0.05$). Uniconazole affected ($P \leq 0.05$) ornamental ratings with plants receiving 2 mg a.i.·pot^{-1} being the only treatments to be scored a 5 on the ornamental rating scale.

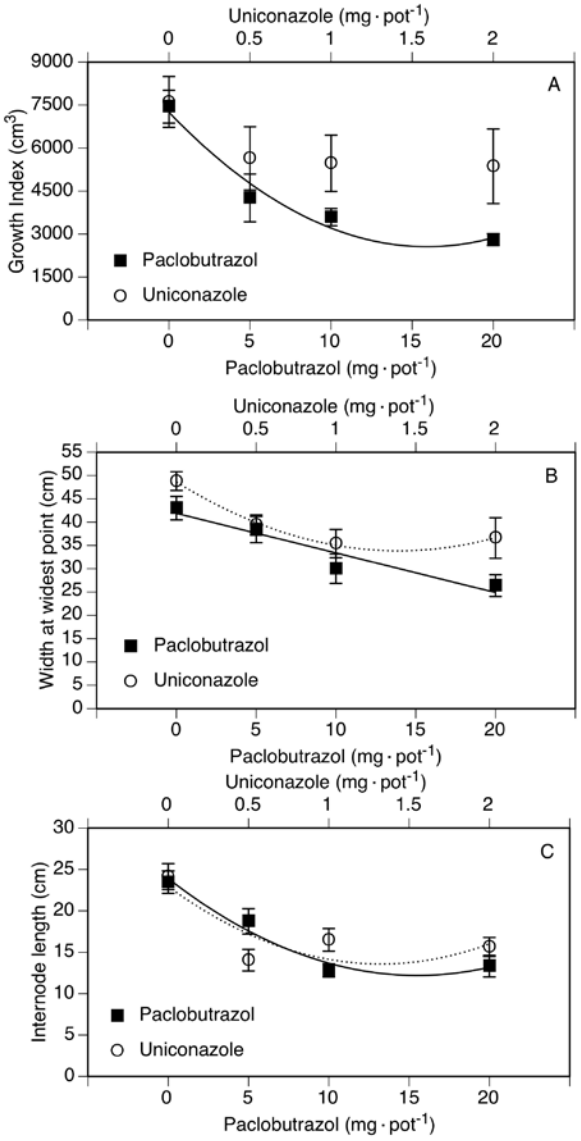

Fig. 3.8. Main effects of drench applied PGR on general growth parameters of *Erigeron procumbens*. Solid lines indicate regression lines for paclobutrazol and dashed lines indicate regression lines for uniconazole. Mean growth index (A) for paclobutrazol (R^2=0.67, y = 7253.05 - 589.87x +18.54x^2) (n=5). Mean width at the widest point (B) for paclobutrazol (r^2=0.54, y = 41.88 - 0.85x) and uniconazole (R^2=.36, y = 48.61 - 21.21x + 7.62x^2) (n=5). Mean internode extension (C) for paclobutrazol (R^2=0.42, y = 23.90 - 1.51x + 0.05x^2) and uniconazole (R^2=0.24, y = 22.94 - 14.14x + 5.34x^2) (n=15). Solid symbols indicate paclobutrazol treated plants and open symbols indicate means for uniconazole treated plants.

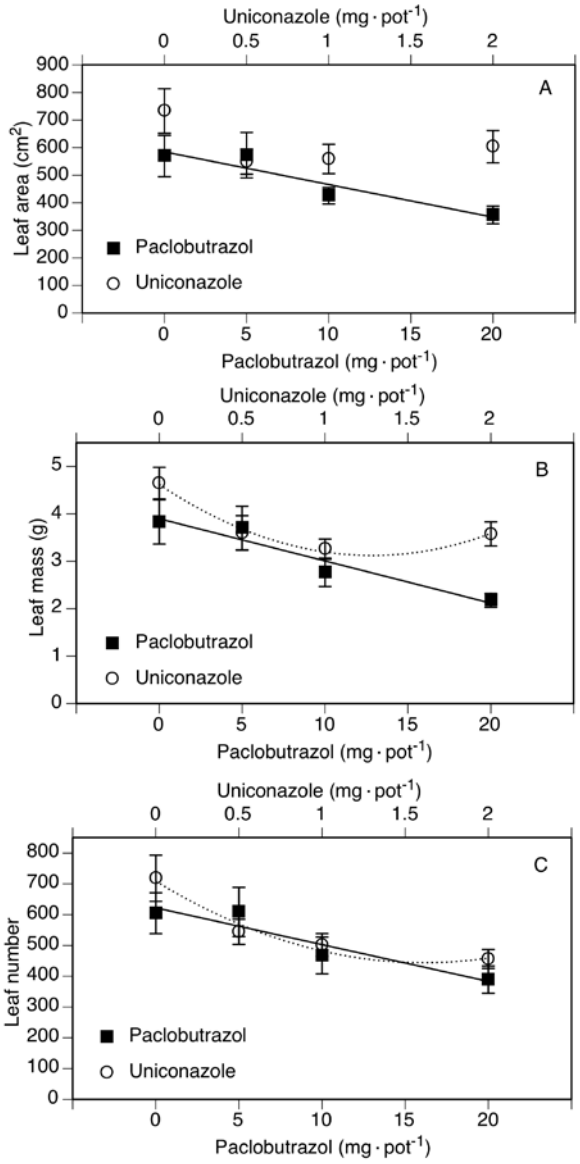

Fig. 3.9. Main effects of increasing drench applied PGR on leaf characteristics of *Erigeron procumbens*. Solid lines indicate regression lines for paclobutrazol and dashed lines indicate regression lines for uniconazole. Mean leaf area (A) for paclobutrazol (r^2=0.33, y = 584.64 - 11.85x). Mean total leaf mass (B) for paclobutrazol (r^2=0.43, y = 3.90 - 0.09x) and uniconazole (R^2= 0.13, y = 4.62 - 2.33x + 0.91x^2). Mean leaf number (C) for paclobutrazol (r^2=0.31, y = 622.12 - 11.95x) and uniconazole (R^2= 0.43, y = 708.49 - 328.01x - 101.69x^2). All symbols represent means (n=5) with solid symbols indicating paclobutrazol treated plants and open symbols indicating means for uniconazole treated plants.

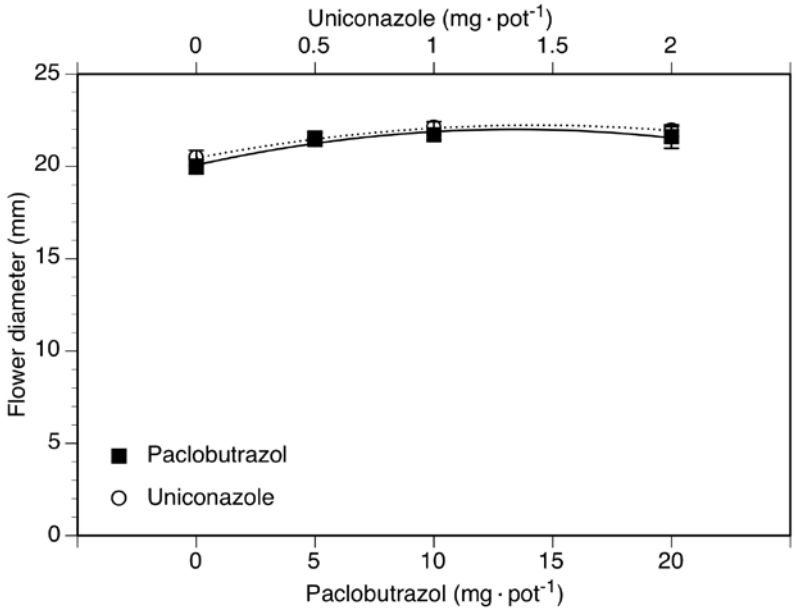

Fig. 3.10. Response of flower diameter of *E. procumbens* to paclobutrazol or uniconazole. Effect of drench applied paclobutrazol (r^2=0.47, y = 13.69- 0.29x) or uniconazole (R^2= 0.27, y = 15.64 -6.27x + 2.11x^2) on inflorescence diameter of *Erigeron procumbens.* Solid lines indicate regression lines for paclobutrazol and dashed lines indicate regression lines for uniconazole. All symbols represent means (n=15) with solid symbols indicating paclobutrazol treated plants and open symbols indicating means for uniconazole treated plants.

Erigeron procumbens Spray applications

At the levels tested, spray application of paclobutrazol did not affect ($P \leq 0.05$) any growth measurements recorded. Spray applied uniconazole minimally affected plant height reducing it 0.08% at 100 mg·L^{-1}(data not presented), but did not affect leaf area. This may indicate a change in the width to length ratio of the leaf blade, since *E. procumbens* is a prostrate plant without erect stems. Uniconazole also affected internode and peduncle length reducing each 66.3 % and 44.2 %, respectively at the highest level

(100 mg·L^{-1}) (Fig. 3.11). This is in contrast to the drench studies where peduncle length was not affected. Spray applied daminozide reduced flower number by 68.0 % at the highest level tested (10000 mg·L^{-1}), increased leaf area by 41.4 %, and reduced internode length 21.7 % at the highest level (10000 mg·L^{-1}) (Fig. 3.12). None of the spray applied PGR's affected ornamental rating when analyzed using Chi square analysis.

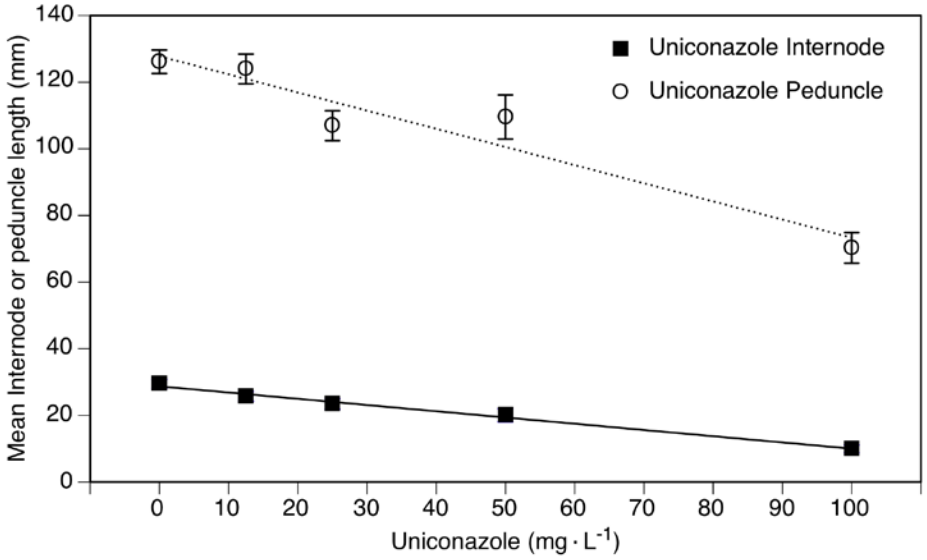

Fig. 3.11. Main effect of spray applied uniconazole on internode and peduncle extension of *Erigeron procumbens*. Internode extension (r^2= 0.57 , y = 28.80 - 0.19x) represented by solid regression line and symbols. Peduncle length (r^2= 0.51, y = 127.80 - 0.54x) represented by dashed regression line and open symbols.

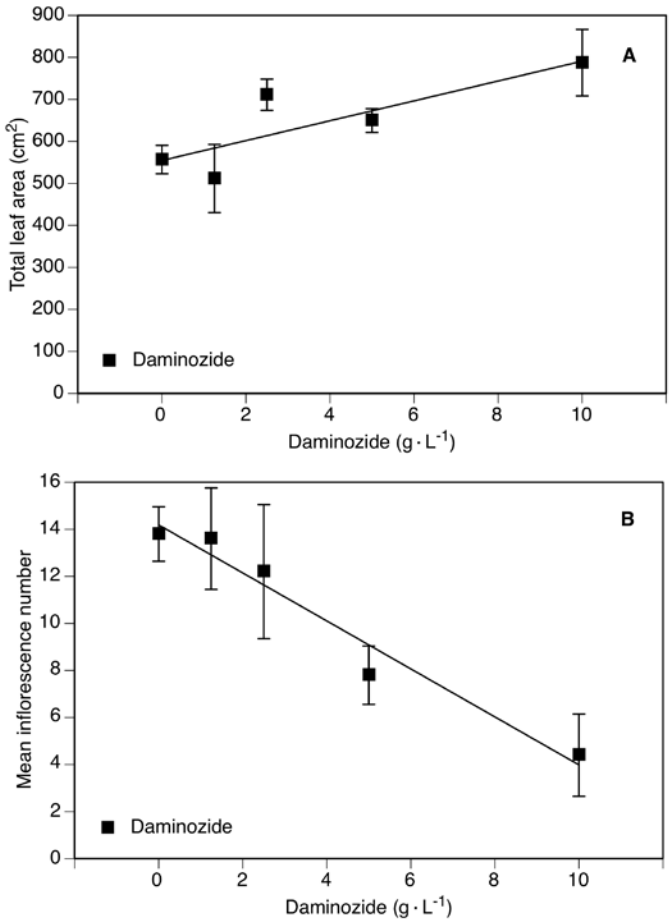

Fig. 3.12. Leaf area and inflorescence diameter response to foliar applied daminozide for *E procumbens*. Total mean leaf area (A) (r^2= 0.36, y = 552.04 + 24.41x) (n=5). Mean inflorescence number (B) (r^2= 0.43, y = 13.82 - 0.95x) (n=5).

Erigeron procumbens Conclusion

Both drench applied paclobutrazol and uniconazole were effective in controlling growth of *E. procumbens*. Growth indices were reduced by 62 % for paclobutrazol (20 mg a.i.·pot^{-1}) and 29 % for uniconazole (2 mg a.i.·pot^{-1}). Spray applied uniconazole was

effective at reducing internode extension but did not reduce GI and should be tested at higher rates or as multiple applications. Daminozide reduced total inflorescence number per plant. Daminozide like foliar applied uniconazole should be tested with multiple applications.

Since *E. procumbens* is a prostrate ground cover type plant, controlling height should not be a major concern. Based on the authors personal observations drench paclobutrazol should be applied at 4 mg a.i.·pot^{-1} and drench applied uniconazole should be applied at 2 mg a.i.·pot^{-1} in order to produce compact plants with an aesthetically pleasing growth habit.

Response of *S. portulacastrum* to paclobutrazol, uniconazole, and daminozide

Sesuvium portulacastrum is a fleshy native perennial herb with trialing stems which root at the nodes (Correll and Johnston, 1970). It is commonly found growing in sand dunes and also along edges of bays with clay soils and appears to flower year round in warm environments (USDA Plants Database, 2009). The long season of flower and growth habit of the plant suggest it may have potential as a ground cover plant, spiller for mixed species patio containers, or hanging basket plant (Arnold, 2011). Plants grown commercially are usually given optimal cultural conditions, this sometimes results in excess shoot extension. If optimal cultural conditions result in excessive shoot extension it is desirable to control plant height and reduce internode extension using plant growth regulators (PGRs) (Basra, 2000).

Triazole PGRs are often classified as plant growth retardants (Basra, 2000), but different members of the triazole PGRs can vary in efficacy on the same species (Burnett

et al., 2000; Whipker and Dasoju, 1998), among species (Banko and Stefani, 1988), and among cultivars of the same species (Whipker and McCall, 2000). Results can also vary depending upon the substrate composition and volume of the carrier drench in which the PGR is applied (Arnold and McDonald, 2002). Currently there is a lack of literature regarding the effects of paclobutrazol, uniconazole or daminozide on the containerized plant production of members of the Aizoaceae Martinov and even the closely related *Portulaca grandiflora* Hook. So suggested rates will be drawn from other herbaceous species.

Whipker and Dasoju (1998) reported, for *Helianthus annuus* L. (sunflower), that uniconazole applied at 32 mg·L^{-1} as a foliar spray provided a 17% reduction in height over controls. Paclobutrazol applied at 40 mg· L^{-1} or 80 mg·L^{-1} as a foliar spray reduced height only 6%. Daminozide in concentrations greater than 4,000 mg·L^{-1} as a foliar spray resulted in a 17% or greater reduction in height (Whipker and Dasoju, 1998). Uniconazole applied at 32 mg·L^{-1} as a foliar spray and daminozide at 4,000 to 16,000 mg·L^{-1} as a foliar spray reduced sunflower inflorescence diameter, whereas paclobutrazol had no effect on inflorescence diameter. Daminozide, paclobutrazol, and uniconazole applications slowed flowering of sunflower by 2 to 3 d compared to non-treated controls (Whipker and Dasoju, 1998). Burnett et al. (2000) reported a similar 2 to 3 d delay of flowering when paclobutrazol, uniconazole, and daminozide were applied to another sunflower relative *Coreopsis rosea* Nutt. 'American Dream'. Paclobutrazol (applied at rates of 12 to 60 mg·L^{-1} as a foliar spray) did not significantly reduce height of *C. rosea* 'American Dream', but uniconazole (applied at rates of 10 to 40 mg·L^{-1} as a

foliar spray) reduced shoot height by 25-29% and daminozide (applied at rates of 2,500 to 7,500 mg·L^{-1} a foliar spray) reduced shoot height 17-29% (Burnett et al., 2000).

Whipker and McCall (2000) reported that paclobutrazol drenches at 2 mg a.i.·pot^{-1} and 4 mg a.i.·pot^{-1} controlled height of sunflower by 24% and 33%, respectively. Gibson and Whipker (2003) found that paclobutrazol sprays ≤ 80 mg·L^{-1} and uniconazole sprays ≤ 24 mg·L^{-1} were ineffective at controlling height in *Osteospermum ecklonis* (DC.) Norl., however paclobutrazol drenches of 16 mg a.i.·pot^{-1} reduced height 16%, and uniconazole drenches of 0.5 mg a.i.·pot^{-1} reduced height 35% and 1.0 mg a.i.·pot^{-1} caused severe plant stunting. Paclobutrazol was ineffective at controlling plant height when applied as a foliar spray to *Oenothera fruticosa* L. 'Youngii-lapsley', *Coreopsis rosea* Nutt. 'American Dream' and *Helianthus annuus* L. (Burnett et al., 2000; Clough et al., 2001; Whipker and Dasoju, 1998), but was effective when applied as a drench on *Helianthus annuus* L. (Pallez et al., 2002; Whipker and McCall 2000) and *O. ecklonis* (Gibson and Whipker, 2003). The difference in efficacy between foliar applications and drench applications suggest in new species both modes of application need to be tested. These rates reported for other plants provide suggestions on rates to test with *S. portulacastrum*.

The research herein was designed to document the responses of *S. portulacastrum*, to three commercially available plant growth regulators (PGRs), paclobutrazol, uniconazole, and daminozide, applied as either a drench or liquid spray during container production at a range of potentially effective concentrations.

Sesuvium portulacastrum Materials and Methods

Sesuvium portulacastrum General protocols

Sesuvium portulacastrum tip cuttings, 4-6 cm long, were taken on 25 April 2011, from containerized stock plants of accession S007 maintained in a gravel bottom nursery in College Station, Texas (lat. 30˚37'45"N, long. 96˚20'34" W). Basal ends of cuttings were dipped in talc based indole butyric acid at the concentration of 1 $g \cdot kg^{-1}$ (Hormodin® 1, OHP, Inc., Mainland, Pa.). Cuttings were placed in 36cm x 51cm x 10cm deep flats (Kadon Corp., Dayton, Ohio) filled with coarse perlite (Sun Gro Horticulture Canada Ltd, Seba Beach, Alta.). Intermitted mist was applied at 16 min intervals for 15 sec duration using reverse osmosis water from 1 h before sunrise and 1 h after sunset. On 2 May 2011, rooted cuttings were potted in 0.47 L black plastic pots (Dillen Products, Middlefield, Ohio) containing Metro-Mix 700 media (Sun Gro Horticulture Canada Ltd, Vancouver, B.C.). On 16 May 2011, liners of *S. portulacastrum*, were potted into 2.3 L black plastic containers (C300S, Nursery Supplies Inc., Kissimmee, Fla.) filled with pine bark based media (Metro Mix 700, Sun Gro Horticulture Canada Ltd, Vancouver, B.C.) amended with 6.53 $kg \cdot m^{-3}$ 15N -3.9P-9.9K controlled release fertilizer (Osmocote® Plus, Scotts Co., Marysville, Ohio) and placed on greenhouse benches in a completely randomized design for each experiment. Average greenhouse temperature was 27ºC with a minimum temperature of 22ºC and maximum temperature of 30ºC (HOBO Pro v2 Temp and RH Data Logger, Onset Computer Corporation, Inc., Pocasset, Mass.). The average photosynthetically active radiation (Accupar, Decagon Devices, Inc., Pullman, Wash.) as sampled on 22 July 2011 at solar noon was 1484 $\mu mol \cdot m^{-2} \cdot s^{-1}$.

Drench study

On 23 Sept 2011 plants were cut back to 6 cm from the substrate surface. On 02 Oct 2011, plants were drenched with paclobutrazol at 0, 5, 10, or 20 mg a.i.·pot^{-1} (Bonzi, Syngenta Crop Protection, Inc., Greensboro, N.C.) ((±)-(R*,R*)-β-[(4-Chlorophenyl)methyl]-α-(1,1-dimethylethyl)-IH-1,2,4-triazole-1-ethanol) or uniconazole at 0, 0.5, 1, or 2 mg a.i.·pot^{-1} (Sumagic, Valent U.S.A. Corporation, Walnut Creek, Calif.) ([(E)-(p-chlorophenyl)-4,4-dimethyl-2-(1,2,4-triazol- 1-yl)- 1-penten-3-ol]). These rates were based upon work previously performed by Arnold (1998) and Arnold and McDonald (2001) on similar sized warm temperate to subtropical woody subshrubs and prior research with composite plants. At the time of treatment application, five plants were destructively harvested to provide baseline data. The liners had mean height of 10.2 cm ± 0.67, mean of 19.0 ± 2.9 leaves, and a mean leaf area of 47.13 cm^2 ± 7.9.

Foliar study

In a second experiment, cuttings were taken on 30 May 2011, then rooted cuttings were potted in 0.47 L black plastic containers on 5 June 2011 and transplanted into 2.3L containers on 24 June 2011. Plants grown in 2.3 L containers were cut back to 10 cm from substrate on 18 Aug 2011 and were treated with paclobutrazol (0, 25, 50, 100, or 200 mg a.i.·L^{-1}), uniconazole (0, 12.5, 25, 50, or 100 mg a.i.·L^{-1}) or daminozide (0, 1250, 2500, 5000, or 10000 mg a.i.·L^{-1}) (B-Nine, Chemtura USA Corporation, Middlebury, Conn.) ([butanedioic acid mono (2,2-dimethylhydrazide)]) as foliar applications on 28 Aug 2011 after bud break began. Liners were sprayed to runoff using approximately 30 mL of solution per container. This provided approximately 0.0, 0.75, 1.5, 3.0, and 6.0 mg a.i.·pot^{-1} of paclobutrazol, 0.0, 0.375, 0.75, 1.5, and 3.0 mg a.i.·pot^{-1}

of uniconazole, or 0.0, 37.5, 75.0, 150.0, 300.0 mg a.i.·pot^{-1} of daminozide. Liners were produced in the same manner as the first experiment. Average greenhouse temperature during the experiment was 28ºC, with a minimum temperature of 25ºC and maximum temperature of 33ºC.

Both experiments were arranged as completely randomized designs with 5 replications per treatment. Plants treated with drench applied PGRs were destructively harvested 6 weeks after treatment on 29 June 2011 and plants treated with foliar application of PGR's were destructively harvested 1 month after treatment on 3 August 2011. Growth measures included height, width, internode length, flower count, leaf dry mass, stem dry mass, root dry mass (10 d at 80ºC), leaf number, leaf area, growth indices (height x width at the widest point x width perpendicular to the widest point), and ornamental ratings for both experiments. Ornamental rating scale was from 1 to 5, with a rating of 1) representing a dead plant or plant near death (unacceptable for sale), 2) plant with severe to mild damage and/or stunting to the canopy but surviving (unacceptable for sale), 3) plant with mild to no stunting to the canopy, with or without flowers present (marginal acceptability as a marketable plant), 4) no stunting to the plant canopy, canopy is full and dense without stretching or holes, flowers can be present or not present (solidly marketable plant), and 5) no stunting to the plant canopy, canopy is full without stretching or holes, flowers must cover at least 15 percent of the canopy (superior market potential). Ornamental ratings were recorded by a single observer for consistency. An analysis of variance for the main effects for each plant growth regulator (PGR) within each study was conducted using JMP 2009 and SAS 9.3 (SAS Institute Inc., Cary, N.C.)

for continuous variables. When significant (P<0.05) effects occurred for a PGR, regression analysis was conducted across the concentrations of that PGR. All non-normal data was analyzed using permutations in the lmPerm package (Wheeler, 2010) in R (R Core Team, 2013), set to defaults.

***Sesuvium portulacastrum* Results and Discussion**

Both drench applied paclobutrazol and uniconazole affected height of *S. portulacastrum* similarly at the time of harvest. Paclobutrazol reduced height 45 % compared to controls at 20 mg a.i.·pot^{-1} and uniconazole reduced height 43 % compared to controls at 2 mg a.i.·pot^{-1} (Fig. 3.13.A). These reductions in height were more severe than the height reductions found in *O. ecklonis* which was reduced 16 % by 16 mg a.i.·pot^{-1} of paclobutrazol and reduced 35 % by 0.5 mg a.i.·pot^{-1} of uniconazole (Gibson and Whipker, 2003), but not as severe as reductions reported in *H. annuum* which saw a 33 % reduction in height from 4 mg a.i.·pot^{-1} paclobutrazol drench. Producers will usually try to produce plants that fit in the golden mean ratio of the height and diameter of the container (Sachs et al., 1976). The largest application of a.i. for both PGR's in this study resulted in height reductions of more than the 33 %, which is well with the golden mean.

The reduction in height was coupled with a reduction in growth indices (GI) for both PGRs tested. Growth indices were reduced 72 % when 20 mg a.i.·pot^{-1} of paclobutrazol is applied and 67 % when 2 mg a.i.·pot^{-1} of uniconazole is applied (Fig. 3.13.b).

Dry root and dry shoot masses were only affected ($P \leq 0.05$) by application of paclobutrazol drench. Drench applied paclobutrazol at 20 mg a.i.·pot^{-1} reduced dry root mass 35 % (Fig. 3.14.A) and reduced shoot mass 37 % at 20 mg a.i.·pot^{-1} (Fig. 3.14.B).

The reduction in height is associated with a reduction in internode length for both PGR's. Paclobutrazol reduced internode elongation 61 % at 20 mg a.i.·pot^{-1} and uniconazole reduced 71 % 2 mg a.i.·pot^{-1} compared to controls (Fig 3.15). This reduction in internode extension is similar but more pronounced than 25 % found by Arnold (1998) in *Verbena canadensis* (L.) Britton and *S. greggii* A. Gray with a paclobutrazol drench of 1.0 mg a.i.·pot^{-1}. This reduction of internode length is consistent with other research reports and the mode of action for both paclobutrazol and uniconazole (Arnold, 1998; Basra, 2000; Fletcher and Hofstra, 1985; Tsegaw et al., 2005; Wang, 1991).

Even though height, internode, and shoot dry mass were affected ($P \leq 0.05$), leaf morphology and leaf number were not for both paclobutrazol and uniconazole. This is in contrast to several reports in other species where total leaf number was decreased, leaves were thickened, individual leaf area was increased, and total leaf area was decreased (Burrows et al., 1992; Matsoukis et al., 2002). Perhaps the succulent nature of the leaves affected their responses to the applied PGRs. Therefore the reduction in dry shoot mass, height and GI for paclobutrazol treated plants is most likely a result of the reduction in internode length and the associated loss of mass from reduced amounts of succulent tissues in the shortened internodes.

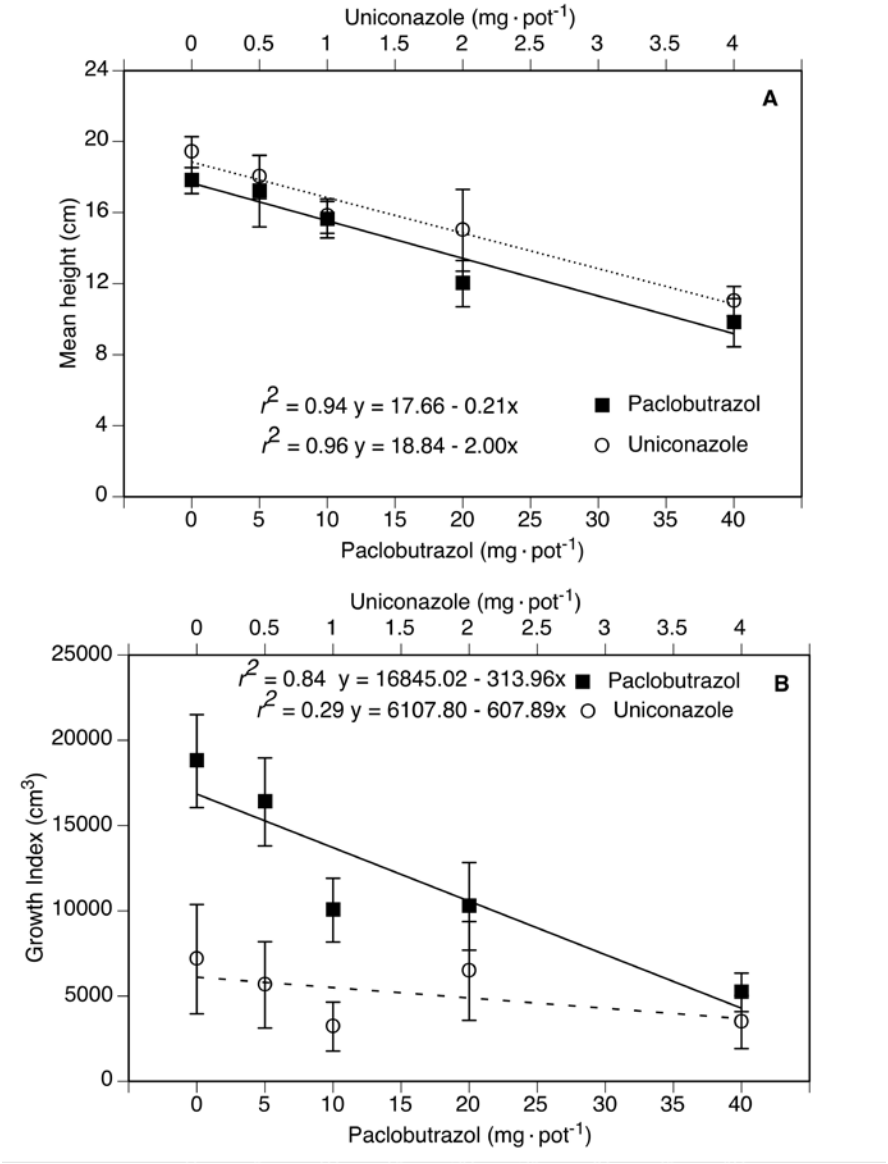

Fig. 3.13. Growth index and plant height response of *S. portulacastrum*. Main effects of drench applied paclobutrazol or drench applied uniconazole on mean height (A) (n = 5) and growth index (height x width x width) (B) (n = 5) of *Sesuvium portulacastrum*. All regression equations were based on means. Solid lines indicate paclobutrazol treated plants and dashed lines indicate uniconazole treated plants.

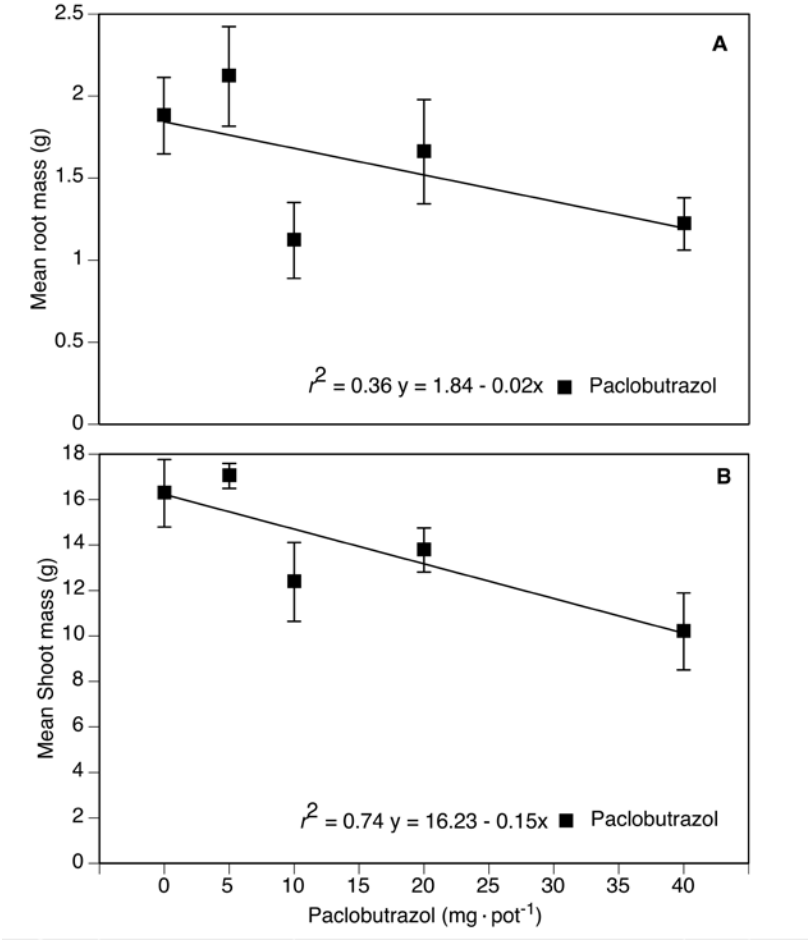

Fig. 3.14. Response of shoot mass and root mass of *S. portulacastrum* Main effects of drench applied paclobutrazol or drench applied uniconazole on mean dry root mass (A) (n = 5) and mean dry shoot mass (B) (n = 5) of *Sesuvium portulacastrum.* All regression equations were based on means.

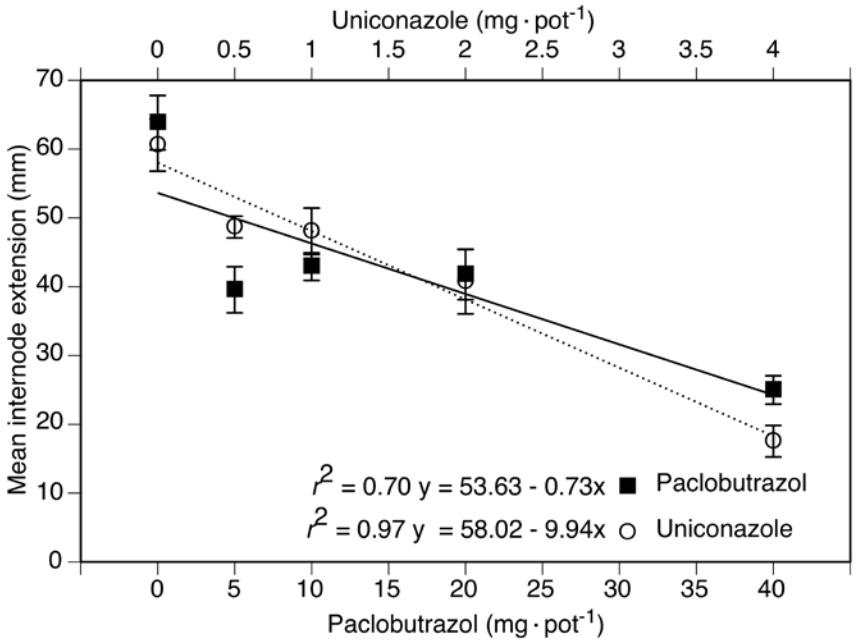

Fig. 3.15. *Sesuvium portulacastrum* internode extension in response to drench applied paclobutrazol or uniconazole. Main effects of drench applied paclobutrazol or drench applied uniconazole on mean internode extension (n = 5) of *Sesuvium portulacastrum.* All regression equations were based on means. Solid lines indicate paclobutrazol treated plants and dashed lines indicate uniconazole treated plants.

Only paclobutrazol affected ($P \leq 0.05$) ornamental ratings. All plants in the control treatment and treated with 10 mg a.i.·pot^{-1} were scored with an ornamental rating of 3. Forty percent of plants treated with 20 mg a.i.·pot^{-1}drench applied paclobutrazol and 60 % of plants treated with 5 mg a.i.·pot^{-1} were scored an ornamental rating of 4. Plants treated with 40 mg a.i.·pot^{-1} of drench applied paclobutrazol fared worse than the controls with 40 % of treated plants receiving an ornamental rating of 2 and the remain receiving an ornamental rating of 3.

Foliar Spray Applications

Internode elongation was reduced 29 % at 200 mg a.i.·L^{-1} for paclobutrazol and 30 % 50 mg a.i.·L^{-1} for uniconazole (Fig. 3.16). However no other growth measures were significantly impacted by the foliar application of either paclobutrazol or uniconazole. This is in alignment with other researchers who have reported the decreased efficacy of both uniconazole and paclobutrazol when applied as a foliar spray (Burnett et al., 2000; Clough et al., 2001; Gibson and Whipker, 2003; Whipker and Dasoju, 1998). Only daminozide affected other growth of *S. portulacastrum*, even though daminozide did not affect internode elongation. Daminozide reduced specific leaf area (SLA) 25 % at 10000 mg a.i.·L^{-1} (Fig. 3.17.A), reduced leaf number 27 % at 10000 mg a.i.·L^{-1} (Fig. 3.17.B), and reduced dry shoot mass 20 % at 5000 mg a.i.·L^{-1} (Fig. 3.17.C). None of the tested PGRs in this study affected ($P \leq 0.05$) ornamental ratings when applied foliarly. The ineffectiveness of both uniconazole and paclobutrazol as a foliar application is consistent with other research reports were the sprays were completely ineffective or required applications higher than suggested label rates.

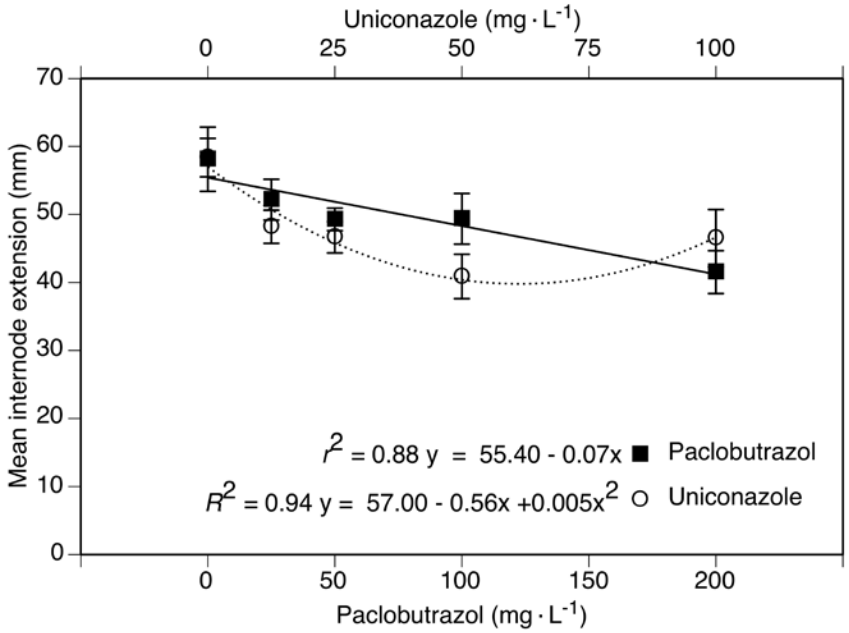

Fig. 3.16. Main effects of spray applied paclobutrazol or spray applied uniconazole on mean internode extension of *Sesuvium portulacastrum*. All regression equations were based on means (n = 5) . Solid lines indicate paclobutrazol treated plants and dashed lines indicate uniconazole treated plants.

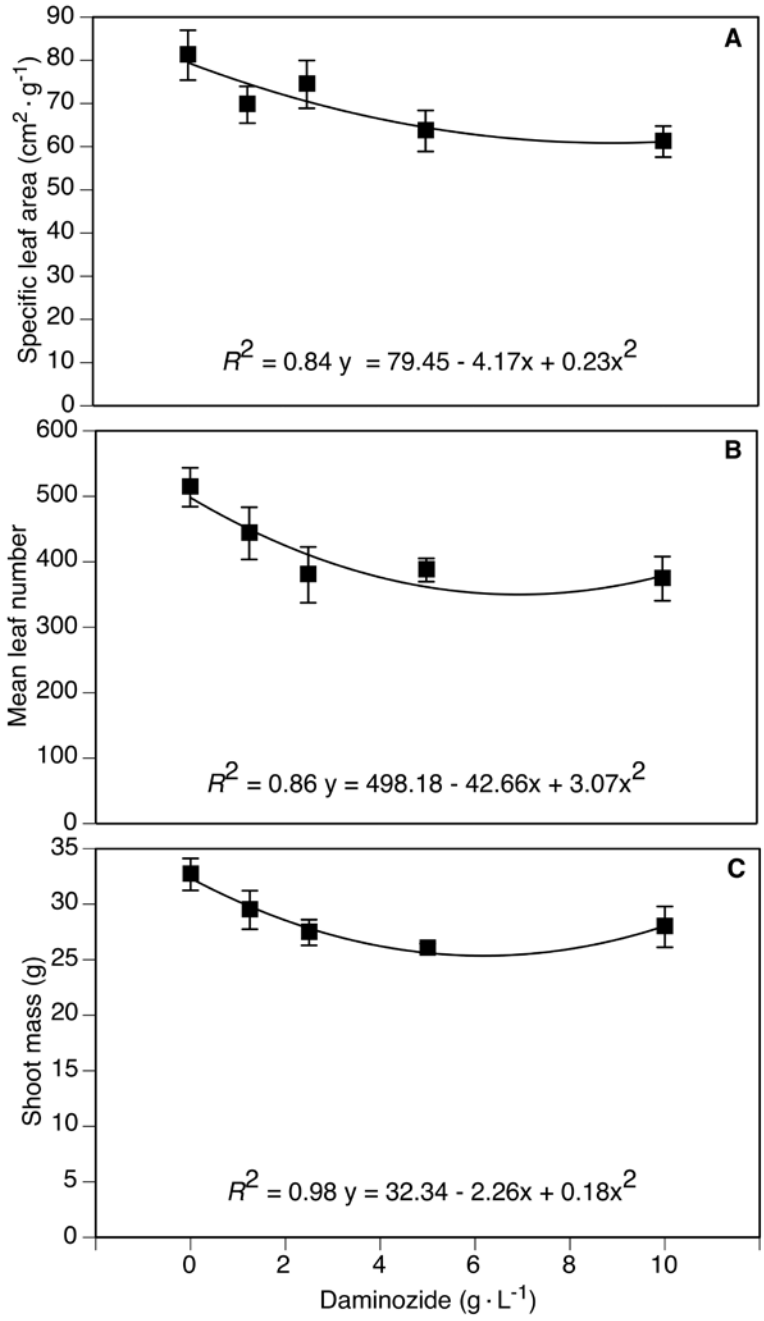

Fig. 3.17. Leaf and shoot response of *S. portulacastrum* to daminozide. Main effects of the spray applied daminozide on specific leaf area (n = 5) (A), mean leaf number (n = 5) (B), and dry shoot mass (n = 5) (C) of *Sesuvium portulacastrum.* All regression equations were based on the means.

Sesuvium portulacastrum Conclusion

Both uniconazole and paclobutrazol reduced the height of *S. portulacastrum*. Uniconazole reduced height by as much as 43 % at 2 mg a.i.·pot^{-1} and paclobutrazol reduced height by as much as 45 % at 20 mg a.i.·pot^{-1}. Growth indices were reduced 72 % when 20 mg a.i.·pot^{-1} of paclobutrazol is applied and 67 % when 2 mg a.i.·pot^{-1} of uniconazole is applied. Producers will usually try to produce plants that fit in the golden mean ratio of the height and diameter of the container (Sachs et al., 1976). The largest application of a.i. for both PGR's in this study resulted in height reductions of more than the 33 %, which is well within the golden mean. Daminozide reduced SLA 25 % at 10000 mg a.i.·L^{-1}, reduced leaf number 27 % at 10000 mg a.i.·L^{-1}, and reduced dry shoot mass 20 % at 5000 mg a.i.·L^{-1}. The ineffectiveness of both uniconazole and paclobutrazol as foliar applications on *S. portulacastrum* is consistent with other research reports were the sprays were completely ineffective or required applications higher than suggested label rates.

CHAPTER IV
SALINITY TOLERANCES*

With the decreasing availability of high quality irrigation water in urban areas in arid environments new ornamental plants need to be developed for landscapes that will thrive with use of lower quality irrigation water. A strategy often employed in built environments in arid regions is to stretch water supplies via utilization of recycled or poorer quality non-potable water sources, which are often higher in salinity, for landscape irrigation (Miyamoto et al., 2001, 2002). An important ingredient in successfully implementing such a strategy will be the identification and development of suitable landscape taxa capable of thriving with saline irrigation water. Texas and the Southwestern USA have regions that are classic arid environments and even the more mesic portions are prone to extended droughts and limited water resources (Arnold, 2008). Demand for high quality water for human consumption may make the use of recycled water to irrigate urban landscapes inevitable (Niu and Rodriguez, 2006). The composition of treated waste water varies among communities and depends on the composition of the original source of water, and type and number of industrial, commercial, and residential users that are contributing to the source of treated waste water (Harivandi, 2000). With recycled water, such as treated effluent, the major concern is elevated salinity which can be as much as two to three times the level of

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potable water (Khurram and Miyamoto, 2005). After most recycled water treatment processes, sodium chloride is the most deleterious chemical remaining (Wu et al., 2001). Foliar necrosis and plant death are major concerns of using irrigation water with high concentrations of salts (Fox et al., 2005; Miyamoto et al., 2001, 2002).

Salts can induce a number of stress responses in plants: salts can affect general water balance, ion toxicity especially Na^+, Cl^-, or SO_4^{2-}, and inhibit nutrient uptake (Taiz and Zeiger, 2006). Water balance in the plant is affected by the dissolved solutes in the root zone causing a low osmotic potential which reduces soil water potential (Taiz and Zeiger, 2006). This results in an analogous situation to water deficit even when an otherwise sufficient amount of water is available (Taiz and Zeiger, 2006). Plants can adjust, to some extent, to prevent a loss of turgor pressure through a reduced osmotic potential, however growth may be slowed and often plants exhibit stress responses similar to adjustment to water deficit (Taiz and Zeiger, 2006). Ions of Na^+, Cl^-, or SO_4^{2-} can accumulate to toxic levels in plants in saline environments (Marschner, 1995). The accumulation of ions especially a high ratio of Na^+ to K^+ can inhibit protein synthesis and inactivate enzymes and may eventually lead to cell death (Taiz and Zeiger, 2006).

All of these responses to salt stress can lead to a loss of ornamental appeal. A buildup of ions from the transpirational stream can lead to leaves with necrotic margins, chlorosis, or leaf abscission giving the plant a general unhealthy appearance that is unacceptable for ornamental use (Maas, 1993; Marschner, 1995; Sykes, 1993). Salt ions interfering or interacting with nutrient absorption can also lead to chlorotic

plants that are unacceptable in ornamental applications (Arnold, 2008; Maas, 1993; Marschner, 1995; Sykes, 1993).

The appearance of plants in the landscape is extremely important to landscape managers, designers, and people using the space (Fox et al., 2005). The use of salt tolerant or halophytic species could potentially reduce the ornamental liabilities of salt stress, allowing lower quality irrigation water to be used in production of plant materials and landscape irrigation without a loss of ornamental function.

Sesuvium portulacastrum (L.) L., *Borrichia frutescens* (L.) DC., *Oenothera drummondii* Hook., and *Erigeron procumbens* (Houst. *ex* Mill.) G.L. Nesom. are species native to Texas coastal regions (Richardson, 2002) selected for these studies because of their potential natural tolerance to salinity, especially in the form of sodium and chlorine ions, associated with seawater exposure in their coastal ranges. All of these species offer interesting foliage, form and/or flowering attributes which would make them potentially desirable landscape plants. Native plants were also selected because they pose a low potential for invasiveness compared to exotics. The purpose of this study is to quantify the salinity tolerances of these four potential landscape species in support of cultivar selection research.

Materials and Methods

Four separate experiments were conducted with the same general protocols but on different dates (Table 4.1). Tip cuttings, 4-6 cm long, were taken from containerized stock plants of single accessions maintained in a gravel nursery in College Station, Texas. Basal ends of cuttings were dipped in talc based IBA at the concentration of 1

$g \cdot kg^{-1}$ (Hormodin® 1, OHP, Inc., Mainland, Pa.). Cuttings were placed in 36 cm x 51 cm x 10 cm deep flats (Kadon Corp., Dayton, Ohio) filled with coarse perlite (Sun Gro Horticulture Canada Ltd., Seba Beach, Alta.). Intermittent mist was applied at 16 min intervals for 15 sec duration using reverse osmosis water from 1 h before sunrise to 1 h after sunset. Rooted cuttings were potted in 0.47 L black plastic pots (Dillen Products, Middlefield, Ohio) containing calcined clay amended with 6.53 $kg \cdot m^{-3}$ 15N-3.9P-9.9K controlled release fertilizer (Osmocote® Plus, Scotts Co., Marysville, Ohio), 0.89 $kg \cdot m^{-3}$ micronutrient fertilizer (Micromax®, Scotts Co., Marysville, Ohio), 1.78 $kg \cdot m^{-3}$ $CaSO_4$ (United States Gypsum Co., Chicago, Ill.), and 4.15 $kg \cdot m^{-3}$ $CaMg(CO_3)_2$ as per the methods of Denny (2007). Liners were later potted into 2.3 L containers filled with the media described above and placed on greenhouse benches in a completely randomized design with five replicates of each treatment. Mean temperature, minimum, and maximum temperature was recorded for each experiment (Table 4.1).

Table 4. 1. Transplant dates and mean greenhouse temperatures for each separate salinity experiment with the four species tested.

Species	Cuttings Rooted	Planted in 0.47 L Pots	Planted in 2.3 L pots	Treatment Started	Harvest	Max. Temp (ºC)	Min. Temp (ºC)	Mean Temp (ºC)
B. frutescens	13 Feb 2011	2 Mar 2011	1 Apr 2011	9 Apr 2011	5 Mar 2011	31.6	17.2	24.7
E. procumbens	25 Apr 2011	2 Mar 2011	16 Mar 2011	25 Mar 2011	23 Jun 2011	31.9	20.5	27.1
O. drummondii	9 Mar 2011	16 Mar 2011	31 Mar 2011	9 Apr 2011	29 Apr 2011	31.6	18.0	24.6
S. portulacastrum	18 Mar 2011	23 Mar 2011	7 Jun 2011	15 Jun 2011	14 Jul 2011	32.7	22.3	28.0

Treatments were 2NaCl:1$CaCl_2$ (Denny, 2007; Solis-Perez, 2009) added at the rates of 0.00, 8.75, 17.50, 35.00, or 70.00 $g \cdot L^{-1}$ to reverse osmosis water, representing electrical conductivities (EC) of 0.8, 15.1, 23.8, 51.3, and 92.5 $mS \cdot cm^{-1}$, applied either

over the top of the canopy contacting the foliage or sub-canopy not contacting the foliage. Irrigation water with a salt concentration of 35 $g \cdot L^{-1}$ roughly represents the salinity of seawater (Denny, 2007; Miyamoto et al., 2004; Southorn, 1995). All treatments were watered simultaneously as needed with 800 mL (>25 % leaching factor).

At the time of harvest, plant heights, widths, leaf count, leaf area, and internode lengths were recorded. Chlorophyll content was sampled spectrophotometrically by removing five leaf discs from each plant and extracting in 80 % acetone (Bryan, 2008; Harborne, 1984). A foliar damage rating of 1-5 was taken by the same observer at harvest, with 1) representing a dead plant or plant near death (unacceptable for ornamental use), 2) plant with severe damage to the canopy but surviving (unacceptable for ornamental use with 50-90% of the foliage exhibiting necrotic regions), 3) plant with severe to mild damage to the canopy, (20-40 % of the foliage exhibiting necrotic regions), 4) very mild damage to the plant canopy, canopy is full with <10% of the foliage having necrotic regions (acceptable ornamental landscape plant), and 5) no damage to the plant canopy, (acceptable ornamental landscape plant). Fresh and dry shoot and root weights were recorded. Shoots from three plants in each treatment were rinsed in distilled water to remove external salts and analyzed for N, P, K, Ca, Mg, S, Na, Fe, Mn, Zn, Cu, and B content (Texas A&M AgriLife Ext. Ser. Soil, Water and Forage Testing Laboratory, College Station, Texas). Electrical conductivity of the media was determined using the 1:5 method described by Lang (1996).

An analysis of variance for the interactions among application methods and salinity level treatments within each study was conducted using JMP 2009 and SAS 9.3

(SAS Institute Inc., Cary, N.C.) for continuous variables. If interactions were not significant ($P \leq 0.05$), then data were pooled into the main effects and they were analyzed for significance. All non-normal data was analyzed using permutations in the lmPerm package (Wheeler, 2010) in R (R Core Team, 2013), set to defaults.

Results and Discussion

Growth Responses

Interactions among application modes and salinity levels were not significant ($P \leq 0.05$) for any of the growth measures, thus only main effects are presented. The main effects of salinity level were significant ($P \leq 0.05$) for all growth measures and media EC (Table 4.2). Increasing salinity levels generally resulted in progressive decreases in all growth measures recorded except for those of *S. portulacastrum* and height of *O. drummondii* (Table 4.2). *Sesuvium portulacastrum* leaf area, leaf number, and shoot mass were stimulated by the lowest salinity exposure (38 %, 15 %, and 25 %, respectively, compared to the controls), consistent with reports that it is a halophyte (Lonard and Judd, 1997). *Sesuvium portulacastrum* was able to survive regular irrigation with salinity at 70 $g \cdot L^{-1}$, equivalent to nearly twice the salinity of seawater, which resulted in an elevated mean substrate EC of 27.8 $mS \cdot cm^{-1}$. This is consistent with the natural occurrence of *S. portulacastrum* immediately adjacent to the ocean on dunes (Richardson, 2002). The mean height of *O. drummondii* was increased 41% by low levels of salt in the irrigation solution, but other measurements decreased with increasing salinity exposure and *O. drummondii* typically succumbed to salinity of 35 $g \cdot L^{-1}$ or greater (Table 4.2). Treating *B. frutescens* and *E. procumbens* with salt

concentrations of 70 $g \cdot L^{-1}$ killed them, but they survived regular irrigation with salinity equivalent to that of seawater. However, *E. procumbens* plants treated with 35 $g \cdot L^{-1}$ foliar applied died while plants treated with the same concentration applied sub-canopy survived.

Internode length was reduced 51 % at 70 $g \cdot L^{-1}$ and only 7 % at 35 $g \cdot L^{-1}$ for *S. portulacastrum* (Table 4.2). Salt treatments also reduced internode length for *E. procumbens* (61 % at 35 $g \cdot L^{-1}$), *O. drummondii* (46 % at 17.5 $g \cdot L^{-1}$), and *B. frutescens* (57 % at 35 $g \cdot L^{-1}$). The reduction in internode extension could be beneficial during nursery or greenhouse production by potentially reducing the need for plant growth retardants.

The interaction among application modes and salinity levels for *O. drummondii* was significant ($P \leq 0.001$) for chlorophyll concentrations, but only the main effects of salinity were significant ($P \leq 0.05$) for *B. frutescens*, *E. procumbens*, and *S. portulacastrum*. For all four species tested, chlorophyll content increased with increasing salinity then declined (Fig. 4.1). At the highest salinity level tested chlorophyll concentration for *S. portulacastrum* was reduced 36 % compared to controls at 70 $g \cdot L^{-1}$. In *O. drummondii* there was an interaction among the concentrations of total salts and modes of application in which chlorophyll concentration was reduced 4 % at 17.5 $g \cdot L^{-1}$ when applied foliarly but increased 67 % when applied sub-canopy at the

Table 4.2. Main effects of salinity on growth measures for Borrichia *frutescens, Erigeron procumbens, Oenothera drummondii,* and *Sesuvium portulacastrum* grown in 2.3 L containers in four separate experiments.

		Leaf Area (cm^2)	Leaf Count	Height (cm)	Internode (cm)	Shoot Mass (g)	Foliar Damage Rating (1-5)	E. C. of Media ($mS \cdot cm^{-1}$)
B. frutescens	Control (0 $g \cdot L^{-1}$)	521.3±44.3[x]	125.3±8.9	36.0±1.2	65.7±1.6	7.9±0.8	5.0±0.0[y]	0.5±0.2
	Low (8.75 $g \cdot L^{-1}$)	498.0±46.0	132.4±12.1	33.4±1.1	54.8±1.5	7.9±0.8	5.0±0.0	4.3±0.9
	Medium (17.5 $g \cdot L^{-1}$)	307.4±29.0	111.3±10.6	29.2±1.0	42.7±1.6	5.4±0.5	5.0±0.0	7.7±0.4
	Med-High (35 $g \cdot L^{-1}$)	123.5±18.8	42.7±6.1	18.6±1.4	28.0±1.5	2.4±0.3	5.0±0.0	12.2±1.5
	High (70 $g \cdot L^{-1}$)	-	-	-	-	-	-	16.1±1.8
	Linear	***[z]	***			***		
	Quadratic							
E. procumbens	Control (0 $g \cdot L^{-1}$)	684.8±59.5	614.5±52.0	6.1±0.3	22.8±1.0	9.1±0.6	4.9±0.1	2.2±0.2
	Low (8.75 $g \cdot L^{-1}$)	232.5±25.6	238.5±28.3	5.6±0.4	19.9±1.0	3.3±0.5	4.5±0.2	7.7±1.1
	Medium (17.5 $g \cdot L^{-1}$)	121.9±20.7	131.9±21.4	5.1±0.3	12.5±0.6	2.4±0.4	3.7±0.2	10.5±1.1
	Med-High (35 $g \cdot L^{-1}$)	26.2±8.5	28.5±9.2	5.2±1.24	8.9±1.0	0.7±0.2	2.8±0.6	15.7±2.1
	High (70 $g \cdot L^{-1}$)	-	-	-	-	-	-	32.3±1.8
	Linear	***	***			***		
	Quadratic							
O. drummondii	Control (0 $g \cdot L^{-1}$)	1033.2±41.1	565.0±26.7	12.6±1.0	12.2±0.6	7.9±0.8	5.0±0.0	0.9±0.1
	Low (8.75 $g \cdot L^{-1}$)	491.5±39.0	275.1±18.7	17.8±1.0	9.0±0.4	7.9±0.8	4.5±0.5	6.6±0.5
	Medium (17.5 $g \cdot L^{-1}$)	192.6±28.5	99.2±12.1	11.7±0.9	6.6±0.6	5.4±0.5	3.6±0.3	8.9±0.6
	Med-High (35 $g \cdot L^{-1}$)	-	-	-	-	2.4±0.3	-	14.2±1.0
	High (70 $g \cdot L^{-1}$)	-	-	-	-	-	-	25.6±1.3
	Linear	***	***			***		
	Quadratic							
S. portulacastrum	Control (0 $g \cdot L^{-1}$)	607.7±26.3	288.1±20.2	23.5±2.5	68.2±2.6	26.4±1.1	5.0±0	1.0±0.2
	Low (8.75 $g \cdot L^{-1}$)	837.7±32.1	331±18.2	15.6±0.8	69.7±1.8	33.1±1.1	5.0±0	8.1±0.3
	Medium (17.5 $g \cdot L^{-1}$)	617.2±21.1	278.6±12.7	18.5±0.8	63.5±1.9	29.5±0.8	4.9±0.1	15.0±1.2
	Med-High (35 $g \cdot L^{-1}$)	357.5±9.2	153.6±8.3	19.8±1.3	63.5±1.7	19.3±0.4	4.9±0.1	18.4±1.4
	High (70 $g \cdot L^{-1}$)	75.0±8.9	54.8±5.5	9.6±0.8	33.7±1.8	8.0±0.4	1.8±0.2	27.8±1.7
	Linear	***	***			***		
	Quadratic							

[x]Values represent means (± standard errors) of 10 observations. Lack of observations indicates mortality.
[y]Foliar damage ratings range from a 1 (most severe damage) to a 5 (no observable damage).
[z]*,**,***, or n.s. indicates significance of the linear or quadradic regression component at $P \leq 0.05$, 0.01, 0.001, or not significant, respectively.

same concentration. In all cases initial treatment with saline water was accompanied by an increase in the concentration of chlorophyll per unit of leaf area (Fig. 4.1). This increase might be due to the decrease in leaf area and a possible increase in leaf thickness (Poljakoff-Mayber, 1975; Longstreth and Strain, 1977).

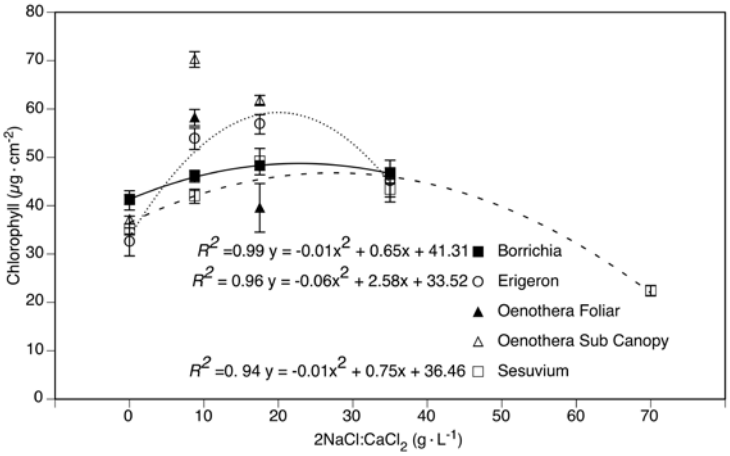

Fig. 4.1. Chlorophyll Concentration. Interactions among salt (0, 8.75, and 17.5 g·L^{-1}) levels and application methods for *O. drummondii* and main effects of salt levels for *B. frutescens*, *E. procumbens*, and *S. portulacastrum*. Symbols for *O. drummondii* represent means (± s.e.) of n=5. Symbols for *B. frutescens*, *E. procumbens*, and *S. portulacastrum* represent means (± s.e.) of n=10. Regressions equations were based on means and are presented when significant at $P \leq 0.05$. Equations were not included for *O. drummondii* due to mortality of the two most saline treatments.

Sesuvium portulacastrum Mineral Content

Phosphorus was not affected ($P \leq 0.05$) by the application of salt solution in *S. portulacastrum* (Table 4.3). For all other mineral concentrations tested there was not an interaction among the modes of application and amounts of salt solutions (Table 4.3). In *S. portulacastrum* the application of salt solutions resulted in an increase in the concentrations of N, K, Ca, Cu, and Na at all levels tested (Table 4.3). This is in contrast to results of Teixeira and Carvalho (2009) in the salt tolerant *Portulaca oleracea* L., Kachout et al. (2011) in the halophyte *Atriplex* L., and Carter and Grieve (2010) in *Zinnia elegans* Jacq. where they found decreases in K and Ca concentrations when plants were treated with saline irrigation water. Tissue levels of Na were elevated in all salinity treatments compared to the controls for *S. portulacastrum* (Table 4.3) as was the

case for *O. drummondii* (Table 4.4), *E. procumbens* (Table 4.5), and *B. frutescens* (Table 4.6). However, salinity levels in tissues of *S. portulacastrum* were greatest at the lower salinity levels (Table 4.3), rather than at the greater salinity levels as was seen with the other three species (Tables 4.4, 4.5, 4.6). This suggests some Na accumulation may have taken place at lower salinity levels. The K/Na ratios for *S. portulacastrum* were also different from that of the other three species (Fig. 4.2 A, B, and C versus D). Foliar applied salinity resulted in a more rapid and severe drop in the K/Na ratio for the other three species (Fig. 4.2.A-C) than for *S. portulacastrum* in which the interaction was not significant ($P \leq 0.05$) indicating similar effects for either foliar or substrate exposure to elevated salinity (Fig. 4.2.D). Competition between Na and K uptake have been reported for a number of plants (Marschner, 1995).

Nitrogen concentration was increased 38 % by the application of a salt solution of 70 $g \cdot L^{-1}$ to *S. portulacastrum*. Concentrations of Fe, Mg, and S were decreased at all levels tested (Table 4.3). Boron, Mn, and Zn shoot contents decreased with low levels of salt then increased with the application of more concentrated salt solutions, as compared to controls (Table 4.3). In *S. portulacastrum*, Zn showed a decrease in shoot content at all levels except 70 $g \cdot L^{-1}$ where Zn concentration was increased 62 % (Table 4.3). This was similar to Teixeira and Carvalho (2009) who also found a decrease in Zn, however they did not treat plants with irrigation water having a total salt concentration near the greatest concentration in this study.

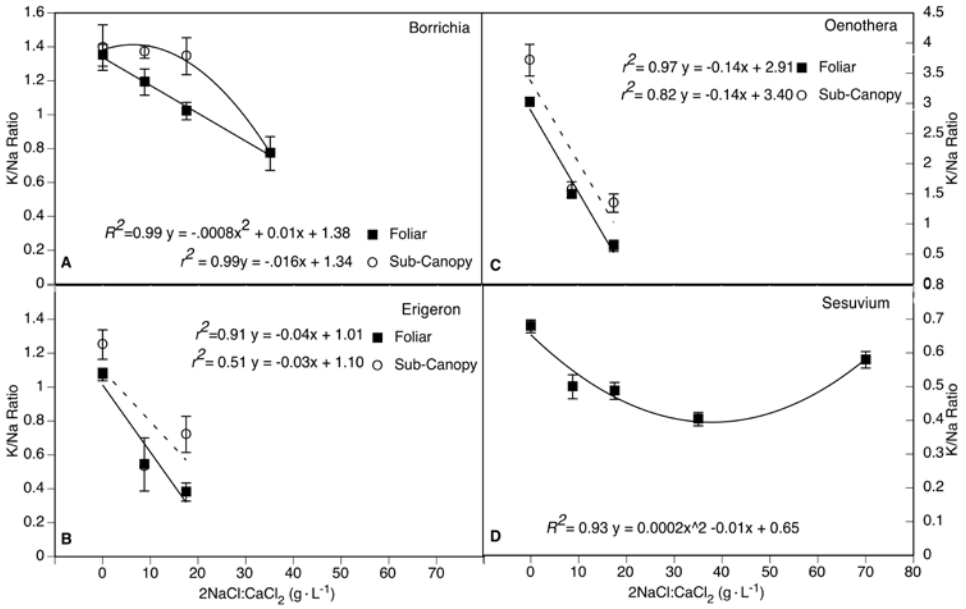

Fig. 4.2. Ratios of K/Na. Ratios of K/Na with significant ($P \leq 0.05$) interactions among application modes and salt levels for *B. frutescens* (A), *O. drummondii* (B), and *E. procumbens* (C). There was not a significant interaction or main effect of modes of application for *S. portulacastrum* (D). Symbols for *B. frutescens*, *O. drummondii*, and *E. procumbens* represent means (± standard errors) for n=3; those for *S. portulacastrum* represent means (± s.e.) for n=6. Absence of symbols indicates mortality. All regression equations were generated from the means.

Table 4.3. Mean shoot concentrations for minerals with significant interactions among modes of application and salt concentrations for *S. portulacastrum* grown in 2.3 L containers irrigated with varied concentrations of 2NaCl:$CaCl_2$ solutions.

				Macronutrients			
		N (%)	**P (ppm)**	**K (ppm)**	**Ca (ppm)**	**Mg (ppm)**	**S (ppm)**
Sub-Canopy	0 g·L^{-1}	2.6±0.1[y]	4285.7±334.8	30680.7±557.6	7731.3±870.5	4023±405.8	6019±495.5
	8.75 g·L^{-1}	2.4±0.1	4748.7±334.1	40327±702.9	9954±1192.4	2603±159.2	5125.7±627.4
	17.5 g·L^{-1}	2.5±0.0	4676.3±420.2	39953.3±1342.9	10678.3±1011.7	2459.3±177	4408.3±219.7
	35 g·L^{-1}	2.8±0.1	4637±391.1	32051±406.5	11234±377	2153±24.8	4125.3±245.4
	70 g·L^{-1}	3.9±0.3	5415.3±203	42433.3±1280.1	17855±1770.8	3981.3±68.7	5617.7±50.2
Foliar	0 g·L^{-1}	2.7±0.1	4910±167.1	33271.3±932.2	8499.3±1147.3	3876.3±168.8	6291.7±455.1
	8.75 g·L^{-1}	2.4±0.1	3910.7±259.8	39273.3±2750.8	7892±135.2	2275.7±274.2	4327.7±402.7
	17.5 g·L^{-1}	2.4±0.1	5006±157.9	37207±1812	11473.7±1154.5	2361.7±131.7	4181.7±403.1
	35 g·L^{-1}	2.8±0.1	5340.7±609.1	31920±798.8	11229.3±276.6	2182.7±40.6	3835.7±139.6
	70 g·L^{-1}	3.6±0.1	5266.7±222.7	37707.3±1234.4	21959±586.4	3792.3±104	4987±371.9
ANOVA Effects	Irrigation mode	0.583[z]	0.401	0.172	0.322	0.165	0.192
	Salt concentration	<0.0001***	0.057	<0.0001***	<0.0001***	<0.0001***	<0.0001***
	Mode x salt concentration	0.786	0.182	0.132	0.065	0.937	0.669
				Micronutrients			
		Zn (ppm)	**Fe (ppm)**	**Cu (ppm)**	**Mn (ppm)**	**Na (ppm)**	**B (ppm)**
Sub-Canopy	0 g·L^{-1}	15.3±0.9	43.3±8.8	5.7±0.3	153.3±11.6	46860.7±476.6	85±2.5
	8.75 g·L^{-1}	14.3±2.4	33.7±5.5	7±0.6	133±10.5	82387.3±10881.1	68.7±6.2
	17.5 g·L^{-1}	12.3±2.4	25±3	7.7±0.9	106.3±15.4	81218.7±7270.3	58.3±4.1
	35 g·L^{-1}	11.7±1.5	21.3±0.9	6.3±0.7	101±15	79868.3±4983.7	58.3±4.7
	70 g·L^{-1}	24±0.6	26.7±2.7	9.7±0.7	184±9.7	69476.7±1665.7	96.3±6
Foliar	0 g·L^{-1}	16±1.5	39±4.6	5.7±0.3	137.7±19.8	47433.7±1106.4	88±3.6
	8.75 g·L^{-1}	15.3±2	22±3.6	6.7±0.3	114.3±10.4	80804±8616.8	61±3.6
	17.5 g·L^{-1}	13.3±1.5	30±1.5	7±0.6	93.3±8.4	78715±4696.5	61±2
	35 g·L^{-1}	14.3±0.9	27.3±3	7±0	94.3±8.6	80388±6802.2	58.7±0.3
	70 g·L^{-1}	26.7±1.5	24.3±3	9±0.6	187.3±20.7	69478.7±4904	100.3±5.8
ANOVA Effects	Irrigation mode	0.177	0.824	0.686	0.310	0.804	0.980
	Salt concentration	<0.0001***	0.006	<0.0001***	<0.0001***	<0.0001***	<0.0001***
	Mode x salt concentration	1.000	0.239	0.913	1.00	1.000	0.665

[y]Values represent means (± standard errors) of 3 observations.
[z] NS,*,**,***Non significant or significant at $P \leq 0.05$, 0.01, or 0.001, respectively. Mode of application either foliar or sub-canopy. Level is 0, 8.75, 17.5, 35, and 70 g·L^{-1} of 2NaCl:$CaCl_2$. *P* values are permutation test p-values.

Oenothera drummondii Mineral Content

For *O. drummondii* all mineral concentrations had significant effects except for B (Table 4.4). There were interactions among the modes of application and salt concentrations for N, Ca, Mg, Na, Zn, Fe, and Cu in *O. drummondii* (Table 4.4). Concentrations of Ca, Cu, Na, and Zn increased as compared to controls for both modes of application and all salt concentrations tested. Potassium, Mg, and P had decreased

mineral concentrations compared to controls for all salt concentrations in *O. drummondii*. This suggests Ca, Cu, and Na were preferentially taken up by the plants as salt concentrations increased (Table 4.4). This is similar to studies with *Anitrrhinum majus* L., considered tolerant of saline irrigation, which also showed increasing concentrations of Ca and Na with decreasing concentrations of K and P (Carter and Grieve, 2008) and *Z. elegans* which also showed decreasing K with increasing salt concentrations (Carter and Grieve, 2010). Nitrogen was decreased 17 % by the foliar application of irrigation water with a total salt concentration of 8.75 $g \cdot L^{-1}$, but was increased 63 % by sub-canopy application of irrigation water at the same concentration with *O. drummondii* (Table 4.4).

***Erigeron procumbens* Mineral content**

Treatments did not have effect ($P \leq 0.05$) on N, Mg, Cu, S or B in *E. procumbens*. There were interactions ($P \leq 0.05$) among the modes of application and salt concentrations for Na, Ca, and P (Table 4.5). Mode of application was only significant for P and Zn. Zinc and P were increased by as much as 86 % and 27 %, respectively, in sub-canopy irrigation treatments of *E. procumbens* at 8.75 $g \cdot L^{-1}$ total salts. With foliar applications at the same concentrations, Zn was only increased 46 % and P was unaffected (Table 4.5).

Table 4.4. Mean shoot concentrations for minerals with significant interaction between mode of application and salt concentration for *O. drummondii* grown in 2.3 L containers irrigated with varied concentrations of 2NaCl:$CaCl_2$ solutions.

		Macronutrients					
		N (%)	P (ppm)	K (ppm)	Ca (ppm)	Mg (ppm)	S (ppm)
Sub-Canopy	0 g·L^{-1}	2.6±0.3[y]	5402.7±501.5	29439±1234.7	17240.3±1229.9	3597±92.5	6568.7±261.2
	8.75 g·L^{-1}	4.2±0.2	5097.3±181.8	24035±260.7	22062.3±668.8	2518.3±128.7	7005.3±305.2
	17.5 g·L^{-1}	4.0±0.2	5047.3±153.7	23667.3±837.6	26425.3±897.8	2661±53.2	5366±420.1
Foliar	0 g·L^{-1}	3.4±0.4	5019.3±12.7	30272.3±483.5	15585.7±652.4	2984±162.3	7258.7±549.9
	8.75 g·L^{-1}	2.8±0.2	4573.3±277.1	24509.7±583.6	24090±761.9	2160.3±150	5159.3±529.2
	17.5 g·L^{-1}	3.0±0.2	3966.3±298.8	22500.7±980.3	31809±1930.5	2892.3±76.2	5070±644
ANOVA Effects	Irrigation mode	0.028*[z]	0.003**	0.922	0.055	0.018*	0.198
	Salt concentration	0.097	0.058	<0.0001***	<0.0001***	<0.0001***	0.007**
	Mode x salt concentration	0.002**	0.770	0.500	0.026	0.009	0.073
		Micronutrients					
		Zn (ppm)	Fe (ppm)	Cu (ppm)	Mn (ppm)	Na (ppm)	B (ppm)
Sub-Canopy	0 g·L^{-1}	47.3±1.8	112±11.2	13.7±1.2	312.7±53.5	8045±868.3	71±0.6
	8.75 g·L^{-1}	108±8	80.3±15.7	32±3.6	306±32.8	15523.3±1259.1	69.3±3.3
	17.5 g·L^{-1}	91.7±12.2	125.7±25.7	22±2.5	226.3±6.3	17995.7±1801.1	67.3±3.8
Foliar	0 g·L^{-1}	55.7±6.7	80.7±1.9	15.3±2.3	258.7±20.7	10033.3±339.1	68±4.2
	8.75 g·L^{-1}	68.7±1.2	97±33.5	19±1	220.7±23.7	16485.7±304.2	58.3±1.9
	17.5 g·L^{-1}	85.7±10.2	25.7±1.2	21±2.3	260.7±34	36851.7±5320.5	72±9
ANOVA Effects	Irrigation mode	0.075	0.028	0.047	0.143	0.002**	0.370
	Salt concentration	0.001**	0.550	0.001**	0.449	<0.0001***	0.329
	Mode x salt concentration	0.039*	0.038*	0.020*	0.153	0.003**	0.287

[y]Values represent means (± standard errors) of 3 observations.
[z] NS,*,**,***Non significant or significant at $P \leq 0.05$, 0.01, or 0.001, respectively. Mode of application either foliar or sub-canopy. Level is 0, 8.75, 17.5, 35, and 70 g·L^{-1} of 2NaCl:$CaCl_2$. *P* values are permutation test p-values.

Table 4.5. Mean shoot concentrations for minerals with significant interaction between mode of application and salt concentration for *E. procumbens* grown in 2.3 L containers irrigated with varied concentrations of 2NaCl:$CaCl_2$ solutions.

		Macronutrients					
		N (%)	P (ppm)	K (ppm)	Ca (ppm)	Mg (ppm)	S (ppm)
Sub-Canopy	0 g·L^{-1}	3.2±0.1[x]	3907±272	20921.3±502.6	7973.7±540.8	2455.7±222.6	4838±221
	8.75 g·L^{-1}	3.4±0.2	4958.7±130.2	15611.7±311.3	13652±521.4	2819.3±65	5445.3±174
	17.5 g·L^{-1}	3.3±0.3	4318.7±199.4	19063.3±2066	14393.7±362.7	2634.7±217	4829.7±268.4
	35 g·L^{-1}	3.3±0.3[y]	3978.3±80.4	16050.0±281.6	25918.3±1253.0	3382.0±73.1	5723.0±223.3
Foliar	0 g·L^{-1}	3.4±0.1	4438±151.5	20806±128	8174.3±258.4	2849±123.8	5615.7±120.7
	8.75 g·L^{-1}	3.2±0.1	4067.3±196.5	15202.7±1811.3	16233±3302.6	2667±165.7	5743.3±586
	17.5 g·L^{-1}	3.3±0.1	4609.7±250.7	16396.3±1953.1	24873.3±1526	2558.7±87.2	4643±165.5
ANOVA Effects	Irrig. mode	0.784[z]	0.014*	0.667	0.302	0.563	0.534
	Salt concentration	0.697	0.013*	0.002**	<0.0001***	0.172	0.076
	Mode x salt concentration	0.578	0.015*	0.635	0.015	0.210	0.295

		Micronutrients					
		Zn (ppm)	Fe (ppm)	Cu (ppm)	Mn (ppm)	Na (ppm)	B (ppm)
Sub-Canopy	0 g·L^{-1}	41.3±1.5	74±16.1	19.7±0.7	302.7±15.6	16917.3±1392.5	80.7±3.2
	8.75 g·L^{-1}	77±7.1	115.7±33	21.7±1.7	359.3±26.6	29346.3±466.7	75±2.3
	17.5 g·L^{-1}	53.3±2.9	58.3±4.4	19.7±1.2	344.3±38.1	26757±1325.4	80.3±7.3
	35 g·L^{-1}	61.0±7.1	252.7±38.6	26.3±2.0	475.3±56.0	41780.0±1167.5	84.7±2.2
Foliar	0 g·L^{-1}	38.3±0.9	79.3±17.3	20.3±0.3	293±10.6	19410±549.9	84±2.6
	8.75 g·L^{-1}	56.3±9.8	138.3±10.7	20.3±0.9	349.7±44.8	30932.7±5329.2	83±9
	17.5 g·L^{-1}	55.3±3.5	91±25.4	21±1.5	334.3±14.2	43436±2140.1	75.3±2
ANOVA Effects	Irrig. mode	0.021	0.332	0.583	0.643	0.473	0.312
	Salt concentration	0.005	0.045*	0.544	0.010*	<0.0001***	0.593
	Mode x salt concentration	0.127	0.778	0.468	1.00	0.018*	0.579

[x]Values represent means (± standard errors) of 3 observations.
[y]*E. procumbens* treated with 35 g·L^{-1} foliar did not survive.
[z] NS,*,**,***Non significant or significant at $P \leq 0.05$, 0.01, or 0.001, respectively. Mode of application either foliar or sub-canopy. Level is 0, 8.75, 17.5, 35, and 70 g·L^{-1} of 2NaCl:$CaCl_2$. P values are permutation test p-values.

Borrichia frutescens Mineral Content

Shoot concentrations of Fe and S of *B. frutescens* were not affected ($P \leq 0.05$) by any of the treatments. There were interactions ($P \leq 0.05$) among modes of application and salt concentrations for Ca, Zn, and Cu in *B. frutescens*, with Zn concentrations

increasing by as much as 230 % at 35 $g \cdot L^{-1}$ of salinity as compared to controls (Table 4.6). The increase in Zn could be the result of a defense mechanism to elevated levels of Na in the irrigation water and substrate (Tavallali et al., 2009). Zinc has been shown to mitigate some effects of Na and Zn concentrations in tissues have been shown to increase in other plants such as *Capsicum annuum* L. (Cornillon and Palloix, 1997) and *Pistacia vera* L. (Tavallali et al., 2009) in response to Na application. Mode of application did not affect any other minerals tested in *B. frutescens*.

Foliar Damage Ratings

Sesuvium portulacastrum only had foliar damage ratings at the highest level (70 $g \cdot L^{-1}$), but exhibited essentially no signs of damage at levels equivalent to the salinity of seawater (Table 4.2). *Oenothera drummondii* had increasing amounts of damaged foliage with increasing salt concentrations with mean foliar damage ratings decreased from 5 for controls to 3.5 at 17.5 $g \cdot L^{-1}$. Treatments with salt concentrations greater than 17.5 $g \cdot L^{-1}$ resulted in mortality of *O. drummondii*. The lesser salinity tolerance of *O. drummondii* compared to *S. portulacastrum* is consistent with its occurrence typically associated with the landward side of the dunes, whereas *S. portulacastrum* is often found on the seaward side of the dunes. Surviving *B. frutescens* while stunted did not exhibit any foliar damage up to 35 $g \cdot L^{-1}$ salinity exposure, but were killed by treatments with salt concentrations of 70 $g \cdot L^{-1}$. Mode of application (either foliar application or sub canopy) only had effect ($P \leq 0.05$) on *E. procumbens*. Increasing levels of salt in the irrigation water decreased the mean damage rating from 4.8 to 3.25 at 17.5 $g \cdot L^{-1}$ for sub-

Table 4.6. Mean shoot concentrations for minerals with significant interaction between mode of application and salt concentration for *B. frutescens* grown in 2.3 L containers irrigated with varied concentrations of 2NaCl:$CaCl_2$ solutions.

		Macronutrients					
		N (%)	P (ppm)	K (ppm)	Ca (ppm)	Mg (ppm)	S (ppm)
Sub-Canopy	0 g·L^{-1}	2.7±0.2[y]	2292.3±176	33226±1924	8721.7±599.2	3773±213	18024.3±717.4
	8.75 g·L^{-1}	2.7±0.1	3326.7±320.2	38315.7±420.3	10765±461	3209.3±93.6	16770±557
	17.5 g·L^{-1}	2.8±0.1	3579±222.7	38057±859.2	10899±421.3	2536.7±170.1	17052.7±1366.8
	35 g·L^{-1}	3±0.3	2810.7±305.6	25893.7±1637.1	11212.7±372.7	2628.7±144.7	14537±1317.7
Foliar	0 g·L^{-1}	3.0±0.4	2543.3±203.4	32554.7±2596.3	9883.7±1492.4	4025.3±332.6	16885.3±449.1
	8.75 g·L^{-1}	2.2±0.1	2602.3±108.7	35874.3±1817	10115.7±916.5	2860±102.1	13112.3±746.6
	17.5 g·L^{-1}	2.5±0.1	3115.7±209.1	33544.3±1176.5	11547.3±215.2	2798.3±34.4	15702.7±266.6
	35 g·L^{-1}	3.2±0.1	3056.7±164.2	29400.3±2370.2	18296±1977.7	3629±603.6	17444.3±2183.9
ANOVA Effects	Irrig. mode	0.583[z]	0.564	0.470	0.006	0.115	0.205
	Salt concetration	0.029*	0.002**	<0.0001***	<0.0001***	0.005**	0.179
	Mode x salt concentration	0.153	0.128	0.184	0.010	0.159	0.052

		Micronutrients					
		Zn (ppm)	Fe (ppm)	Cu (ppm)	Mn (ppm)	Na (ppm)	B (ppm)
Sub-Canopy	0 g·L^{-1}	13.3±0.3	26.3±1.2	11.3±0.7	194.3±13.9	24046±1414.1	120±11.0
	8.75 g·L^{-1}	24.3±0.3	43.3±23.8	15.3±0.7	201±8.1	28007.7±828.8	86.7±0.7
	17.5 g·L^{-1}	31±4.7	28.3±4.1	15.7±0.7	212.7±39.9	28653.7±2323.2	83.7±9.8
	35 g·L^{-1}	21.7±1.9	34±9.6	13±0.6	202.7±27.3	34355.7±3063.4	108.7±5.2
Foliar	0 g·L^{-1}	13.7±0.7	24.3±1.2	11.7±0.3	179.0±8.7	24222.7±2317.4	111.3±5.2
	8.75 g·L^{-1}	16.7±0.9	21.7±0.9	12.7±0.3	162.7±20.3	30419.7±2950.7	82±6.5
	17.5 g·L^{-1}	18.7±2	22.3±1.8	13±0.6	172.3±6.3	32994.7±1726.1	93.7±3.8
	35 g·L^{-1}	45.3±5.8	30.7±1.5	17±1.5	388.7±45	38137.3±3541.1	137±19.1
ANOVA Effects	Irrig. mode	0.706	0.451	0.522	0.002**	0.706	0.451
	Salt concentration	<0.0001***	0.842	0.001	<0.0001***	<0.0001***	0.842
	Mode x salt concentration	<0.0001***	0.875	0.002	0.003**	<0.0001***	0.875

[x]Values represent means (± standard errors) of 3 observations.
[y]*E. procumbens* treated with 35 g·L^{-1}foliar did not survive.
[z] NS,*,**,***Non significant or significant at $P \leq 0.05$, 0.01, or 0.001, respectively. Mode of application either foliar or sub-canopy. Level is 0, 8.75, 17.5, 35, and 70 g·L^{-1} of 2NaCl:$CaCl_2$. P values are permutation test p-values.

canopy applications and from 5 to 2.8 at 35 $g \cdot L^{-1}$. *Erigeron procumbens* was killed at concentrations greater than 35 $g \cdot L^{-1}$.

Conclusions

Sesuvium portulacastrum had increasing levels of K while other species treated with similar levels of saline irrigation water either had decreasing concentrations of K or unchanged concentrations of K while tissue concentrations of Na increased. Salt tolerance seems to be linked with the ability of the plant to maintain a high K/Na ratio (Navarro et al., 2008; Taiz and Zeiger, 2006). As shown in Fig. 4.2, *S. portulacastrum* has a relatively steady K/Na ratio while all other species show a decrease in the ratio with treatment of salty irrigation water and death occurring with K/Na ratios between 0.2-0.8 (Fig. 4.2). Continued growth and survival of *S. portulacastrum* when irrigated with water containing salinity approximately twice seawater, lack of adverse responses to foliar application of salinity, and its enhanced growth at milder elevations in substrate salinity are consistent with its designation as a halophyte. Although less tolerant to irrigation with saline water than *S. portulacastrum*, the other three species included in these studies tolerated chronic exposure to irrigation water with salinity half as salty as seawater and in some cases survived even greater concentrations. *Borrichia frutescens*, *E. procumbens* and *S. portulacastrum* may use the accumulation of Zn as a method to mitigate increasing amounts of Na in shoot tissues.

A slight decrease in growth from the shortening of internodes could be a beneficial aspect of using saline irrigation water on these species. The reduction in growth could eliminate or reduce the amount of plant growth regulators needed during a

commercial production cycle where plant retardants are regularly used to increase compactness of plants.

All four species in this study can be irrigated with water resulting in a substrate EC of 15 $mS \cdot cm^{-1}$ without affecting their ability to perform as ornamentals in container production, permitting the use of low quality irrigation water. Tolerance of these salinity levels may prove useful in landscape settings with recycled irrigation water, in coastal restoration or landscape development, and in areas where highway runoff or splash of deicing salts would be encountered.

CHAPTER V

MATING SYSTEM

Oenothera drummondii Hook. is a perennial shrub or subshrub native to the USA on sandy beaches from Texas to North Carolina (Correll and Johnston, 1970; USDA Plants Database, 2009) with large yellow flowers approximately 4-7 cm in diameter (Correll and Johnston, 1970). These large flowers and industry interest lead us to believe that *O. drummondii* has potential as a native plant for commercial landscapes. However currently there is little literature regarding *O. drummondii* especially referencing its culture, breeding system, or phenotypic variability in the wild.

The breeding system of *Oenothera drummondii* needs to be documented to ease the process of controlled pollination under greenhouse conditions for introgressing traits, e.g., blue foliage and upright habits found in non-floriferous accessions from southern Texas into floriferous accessions with green foliage. If herkogamy (separation of the stigma above the anthers) in combination with heavy viscin threads of pollen can be demonstrated, the need to emasculate flowers where pollinators are excluded could be avoided (Wagner et al., 2007; Theiss et al., 2010).

Theiss et al. (2010) used pollination tests to determine if 10 species of *Oenothera* L. were self-compatible or self-incompatible in relation to life history and flower morphology. The species were a mix of annuals and perennials in the sections *Anogra* (Spach) W.L. Wagner & Hoch and *Kleinia* Munz. Most of the species in the study exhibited herkogamy. The authors grew plants under greenhouse conditions and

allocated equal number of flowers to each pollination treatment. Pollination treatments were pollination from pollen of the same plant as the flower (selfing) or pollen from another plant of the same species (outcrossing). Flowers were not emasculated, but stigmas were visually checked for contamination by unwanted pollen. Swollen fruit with seed were considered successful and recorded. One aspect they may have overlooked is the number of seed set to determine the preference to one type of pollen treatment. Also, with addition of an un-emasculated un-pollinated flower with an emasculated un-pollinated flower the percent of self-fertilization and agamospermy could have been determined. They found of the ten species six to be self-incompatible, two of variable compatibility, and two that are self-compatible (Theiss et al., 2010). There also was not a relationship between breeding system and life history.

Wolin et al. (1984) performed a similar experiment on *Oenothera speciosa* Nutt. Plants were enclosed in wire mesh to exclude pollinators. Flowers were either manually self-pollinated, crossed with pollen from a different genotype (outcrossed), flowers were enclosed for self-pollination, or emasculated or left open for insect pollination. Then fruit set and seed per fruit were recorded. The emasculated and enclosed flowers did not set fruit. Self-pollination, intra-clonal pollination, emasculation and bagging, and bagging the flowers resulted in lower fruit set than open pollinated controls. Outcrossed flowers set fruit at 100 % and set more seed than the other treatments. From the pollination study Wolin et al. (1984) concluded that *Oenothera speciosa* is partially self-incompatible and is not apomictic.

From this study we hope to determine the breeding system of a promising accession of *O. drummondii* for ornamental horticulture, whether emasculation of flowers is necessary for controlled crosses, and if this accession is apomictic. Determining this information will aid future breeding projects, as well as, facilitate possible commercial seed production if this species is adopted by commercial producers.

Materials and Methods

Oenothera drummondii tip cuttings, 4-6 cm long, were taken on 1 Feb 2013, from containerized stock plants of accession O13 maintained in a gravel bottom nursery in College Station, Texas. Basal ends of cuttings were dipped in talc based indole butyric acid at the concentration of 1 $g \cdot kg^{-1}$ (Hormodin® 1, OHP, Inc., Mainland, Pa.). Cuttings were placed in 36 cm x 51 cm x 10 cm deep flats (Kadon Corp., Dayton, Ohio) filled with coarse perlite (Sun Gro Horticulture Canada Ltd., Seba Beach, Alta.). Intermittent mist was applied at 16 min intervals for 15 sec duration using reverse osmosis water from 1 h before sunrise and 1 h after sunset. On 13 Feb 2013, rooted cuttings were potted in 0.47 L black plastic pots (Dillen Products, Middlefield, Ohio) containing Metro-Mix 700 media (Sun Gro Horticulture Canada Ltd., Vancouver, B.C.). On 7 Mar 2013, 75 liners of *O. drummondii*, were potted into 2.3 L black plastic containers (C300S, Nursery Supplies Inc., Kissimmee, Fla.) filled with pine bark based media (Metro Mix 700, Sun Gro Horticulture Canada Ltd., Vancouver, B.C.) amended with 6.53 $kg \cdot m^{-3}$ 15N -3.9P-9.9K controlled release fertilizer (Osmocote® Plus, Scotts Co., Marysville, Ohio) and placed on benches at 0.66 m spacings in an unshaded glasshouse at the Horticulture and Forestry Science Building at Texas A&M University (30° 36'

34.419", -96° 21' 1.047"). Glasshouse set point temperature was 29ºC for day and 21ºC for night. Plants were watered by hand as necessary and insect pests controlled with imidacloprid (Marathon 1% granular, OHP Inc., Mainland, Pa.) applied at 5 $g \cdot pot^{-1}$.

Three plants of accession 10-GL-11-9-12-5 were produced in a similar manner and maintained on an adjacent bench in the same greenhouse to provide outcross pollen for pollination treatments. Accession 10-GL-11-9-12-5, hereafter referred to as (O12-5), is a low growing blue foliaged accession with redlines down the sutures of the floral buds. Accession O13 is a low growing green form, that also lacks redlines on the sutures of the floral buds. Accession O13 was the maternal parent for all treatments.

Before treatments began several flowers were observed to determine timing and emasculation procedures. Ventilation was only allowed through the cool pads to limit pollinating insects. Treatments were in a full factorial and included intact or emasculated flowers cross pollinated using accession O12-5, self-pollinated, or left un-pollinated. The experiment was repeated on three dates (8 May 2013, N=3; 12 May 2013, N=3; 19 May 2013, N=6). All pollinations were performed before 11am per the methods of Theiss et al. (2010). Seed pods were harvested after turning approximately 70 % brown and before opening. All seed pods were checked for seed and seed was counted and then stored at room temperature (????) and humidity (????) for approximately 1 year in paper envelopes.

Seed germination percent was determined on filter paper in petri dishes in the dark. Seeds were checked for germination after 10 d.

Results and Discussion

Emasculation

During this process we observed the stigma is superior to the anthers early on in the development stages (Fig. 5.1.C) and remains above the anthers even after pollen dehiscence (Fig 5.1.A, B, and D). From these observations the following emasculation procedure was proposed and used. Emasculations were performed in the morning two days prior to pollination. First the sepals were split at the distal end of the floral bud and peeled back to reveal the four unexpanded petals (Fig. 5.2.A). The four unexpanded petals are then removed in twisting motion while grasping the petals. This exposes the un-dehisced anthers and unexpanded stigma (Fig. 5.2.B). Then the anthers are removed (Fig. 5.2.C) and the sepals are folded back to cover the stigma. When the sepals reopen exposing the receptive stigma (Fig. 5.2.D), usually the following evening, the flowers are ready to be pollinated. Pollen then can be applied liberally to the receptive stigma (Fig. 5.2.E). Pollen either from deliberate application or from contamination is visible to the unaided eye because of its sticky nature and large grain size (Fig 5.2.E and F) (Theiss et al., 2010).

Fig. 5.1. Floral Structure of *Oenothera drummondii*. Three petals removed exposing anthers and stigma (A), All petals removed showing position of stigma in relation to anthers (B), immature floral bud with sepal removed showing position of stigma above anthers (C), flower with petals removed one day before anthesis and after pollen dehiscence (D).

Fig. 5. 2 Stages of the emasculation of *Oenothera drummondii* flowers. Sepals split open exposing petals and stigma (A), petals removed exposing anthers (B), anthers removed (C), receptive stigma (D), pollinated stigma (E) and viscin pollen.

Pollination

Date and pollen source were the only significant ($P \leq 0.05$) main effects. There was not a difference in seed set between emasculated flowers or intact flowers (Table 5.1). There were no significant interactions among the main effects (Table 5.1). A lack of adverse effect on seed set is somewhat surprising as most genera surveyed by Lloyd and Schoen (1992) had reduced seed or fruit set when flowers were selfed. Selfed flowers across all dates and treatments had a mean seed count of 285.0 (± 14.1) seeds·fruit^{-1} and outcrossed flowers had a mean seed count of 240.5 (± 17.5) seeds·fruit^{-1} across all treatments and dates.

In general the un-pollinated treatments had low seed set with intact un-pollinated flowers producing the least amount of seed (Fig. 5.3). This low seed set in un-pollinated flowers could be attributed to pest infestations, e.g. thrips, aphids, or spider mites, (Lloyd and Schoen, 1992), or apomictic seed set. Thrips and other non-flying insects have been shown to transfer small amounts of pollen. *Oeonthera speciosa* is not apomictic (Wolin et al., 1984), and this could extend to *O. drummondii*. However, it is not possible to discern between gietonogamy and apogamy in this study due to the presence of thrips.

Another indicator of plants with self-compatible breeding systems is a reduction and/or absence of inbreeding depression (Charlesworth and Charlesworth, 1987). In related work, there was not an appearance of inbreeding depression. Plants generated from the selfing of O13 and another accession O6 did not result in inferior plants in height or flowering capability in *O. drummondii* and there was no heterosis in height or

flowering in the F_1 generation (unpublished data). The lack of inbreeding depression, no decrease in seed set from self-pollination, and herkogamy are indicative of a plant with an intermediate to self-compatible breeding system (Charlesworth and Charlesworth, 1987; Lloyd, 1992).

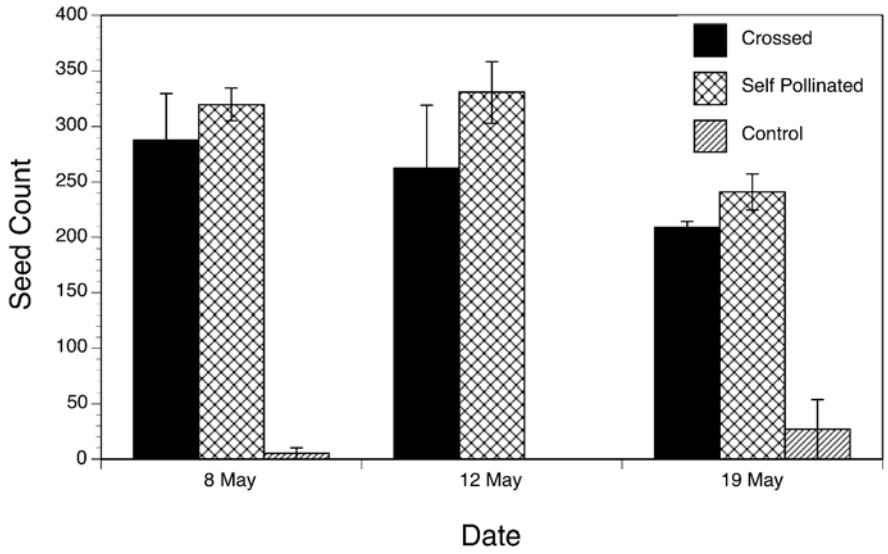

Fig. 5.3. Mean seed count (± standard error) per fruit for *Oenothera drummondii*. Mean totals with a common letter are not different ($P \leq 0.05$) based on Tukey's mean comparisons.

Table 5.1 Partial ANOVA for main effects of date, emasculation, and pollen source on breeding system of *Oenothera drummondii*.

Source	DF	SS	F Ratio	Prob > F
Date	2	44325.8	6.0631	*[z]
Emasculation	1	9580.1	2.6208	NS
Date*Emasculation	2	7135.6	0.976	NS
Pollen	2	1009730	138.1146	***
Date*Pollen	4	22351.4	1.5287	NS
Emasculation*Pollen	2	7806.8	1.0678	NS
Date*Emasculation*Pollen	4	3743	0.256	NS

Seed Germination

There were no differences in percent germination among the treatments that produced mature seed ($P = 0.59$). All treatments had near 100 % germination 10 d after imbibing on filter paper in darkness. This implies there is not a preference for pollen source for the viability of seed produced between these two accessions and seeds can successfully store for 1 yr at room temperature in paper envelopes. In addition to storage capability of one year, light is most likely not necessary for the germination of *O. drummondii* seed in laboratory conditions.

Conclusion

From these experiments we determined emasculation in an environment with pollinators excluded is not necessary to perform controlled crosses. Seeds can be stored at room temperature and humidity for at least 1 yr without a detrimental effect on germination, light is not required for germination, and *O. drummondii* is most likely a facultative outcrossing species. This is evidenced by the presence of herkogamy in flowers, and similar seed set in self-pollinated flowers compared to out crossed flowers.

CHAPTER VI

CONCLUSION

The work described herein was to determine if four Texas coastal natives could provide an alternative to potentially invasive exotic species. They were screened for phenotypic variation for use in improvement and breeding programs, and assessed for responses to PGR's which is often necessary in commercial nursery production. They were also tested for tolerances to saline irrigation representing non-traditional sources of irrigation and harsh saline environments. Based on interest from commercial entities, methods to more easily facilitate the controlled crossing of *O. drummondii* were investigated through pollen storage and mating system studies.

All four species exhibited differences among accessions for a variety of morphological and physiological traits. Regional differences in traits of interest were found in *B. frutescens* and *O. drummondii*. This information can used to guide the collection of future genotypes of *B. frutescens* and *O. drummondii*. This will allow germplasm collectors to target their collection efforts to regions based on the characteristics of material in which they are interested. Further collection of *E. procumbens* needs to be performed to test for differences in regional populations, as few were found in our accessions. In the future, studies need to be performed to calculate heritability and stability of these characteristics in diverse environments to determine if these traits can be used for selection to make gains in ornamental performance over a broader range of environments.

Drench application of PGR's resulted in effective reductions in plant height and growth indices for *O. drummondii*. Control was achieved in different environments and different production times of late spring and winter. Uniconazole applied at rates between 1.5-2.0 a.i.·pot^{-1} and paclobutrazol in the range of 30-35 mg a.i.·pot^{-1} applied as a drench should be effective in achieving approximately a one-third reduction in height and tightening up of the plant by reducing lateral internode extension and overall growth indices without overly stunting the plant during production. However, the ease of foliar application for growers could warrant testing of multiple applications or greater rates to achieve the desired canopy modifications with greater ease of application for *O. drummondii*.

Drench application of 7 mg a.i.·pot^{-1} of paclobutrazol or 2 mg a.i.·pot^{-1} of uniconazole should achieve an approximate 33% reduction in height without overly stunting the plant. However, the spray application of paclobutrazol, uniconazole, and daminozide was generally ineffective at controlling growth of *B. frutescens* even at four times the spray concentration of the label rate with a single application. Arnold (1998) and Arnold and McDonald (2001) reported similar challenges with spray applications on woody perennials *Salvia greggii* Gray and *Plumbago auriculata* Lam. in which drench applications resulted in more consistent reductions in shoot extension. Additional studies need to be performed to determine if multiple spray applications during production of any of the three PGRs tested could be effective at height reduction in *B. frutescens*.

Both drench applied paclobutrazol and uniconazole were effective in controlling growth of *E. procumbens*. Growth indices were reduced by 62 % for paclobutrazol (20

mg a.i.·pot-1) and 29 % for uniconazole (2 mg a.i.·pot^{-1}). Spray applied uniconazole was effective at reducing internode extension but did not reduce GI and should be tested at higher rates or as multiple applications. Daminozide like foliar applied uniconazole should be tested with multiple applications. Since *E. procumbens* is a prostrate ground cover type plant, controlling height should not be a major concern. Based on the author's personal observations, drenches of paclobutrazol should be applied at 4 mg a.i.·pot^{-1} and drenches of uniconazole should be applied at 2 mg a.i.·pot^{-1} in order to produce compact plants with aesthetically pleasing growth habits.

Both PGR's reduced the height of *S. portulacastrum*. Uniconazole reduced height by as much as 43 % at 2 mg a.i.·pot^{-1} and paclobutrazol reduced height by as much as 45 % at 20 mg a.i.·pot^{-1}. Growth indices were reduced 72 % when 20 mg a.i.·pot^{-1} of paclobutrazol is applied and 67 % when 2 mg a.i.·pot^{-1} of uniconazole is applied. Daminozide reduced SLA 25 % at 10000 mg a.i.·L^{-1}, reduced leaf number 27 % at 10000 mg a.i.·L^{-1}, and reduced dry shoot mass 20 % at 5000 mg a.i.·L^{-1}. The ineffectiveness of both uniconazole and paclobutrazol as a foliar application on *S. portulacastrum* is consistent with other research reports were the sprays were completely ineffective or required applications higher than suggested label rates.

Sesuvium portulacastrum had increasing levels of K while other species treated with similar levels of saline irrigation water either had decreasing concentrations of K or unchanged concentrations of K while tissue concentrations of Na increased. Salt tolerance seems to be linked with the ability of the plant to maintain a high K/Na ratio (Navarro et al., 2008; Taiz and Zeiger, 2006). Continued growth and survival of *S.*

portulacastrum when irrigated with water containing salinity approximately twice seawater, lack of adverse responses to foliar application of salinity, and its enhanced growth at milder elevations in substrate salinity are consistent with its designation as a halophyte. Although less tolerant to irrigation with saline water than *S. portulacastrum*, the other three species included in these studies tolerated chronic exposure to irrigation water with salinity half as salty as seawater and in some cases survived even greater concentrations. *Borrichia frutescens, E. procumbens* and *S. portulacastrum* may use the accumulation of Zn as a method to mitigate increasing amounts of Na in shoot tissues. All four species in this study can be irrigated with water resulting in a substrate EC of 15 $mS \cdot cm^{-1}$ without affecting their ability to perform as ornamentals in container production, permitting the use of low quality irrigation water. Tolerance of these salinity levels may prove useful in landscape settings with recycled irrigation water, in coastal restoration or landscape development, and in areas where highway runoff or splash of deicing salts would be encountered.

From the mating system experiments with *O. drummondii* we determined emasculation in an environment with pollinators excluded is not necessary to perform controlled crosses. Seeds can be stored at room temperature and humidity for at least 1 yr without a detrimental effect on germination, light is not required for germination, and *O. drummondii* is most likely a facultative outcrossing species. Pollen storage procedures we tested were not effective at preserving viable pollen. From all of the studies performed, *O. drummondii* is the most likely candidate for introduction into ornamental trade. *Oenothera drummondii* had variation in growth forms and flowering

present in wild populations, is responsive to applications of commercial PGR's, tolerant to saline irrigation water, and is easily emasculated to perform controlled crosses.

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APPENDIX

Table A. 1. GPS coordinates and location description of collection site for each accession of *Oenothera drummondii*.

Accession	*Latitude*	*Longitude*	*Description of location*	*Regional location along Texas coast*
1	26° 06.879	97° 09.965	Gulf and Gardenia on Gulf Beach South Padre Island, TX	South
2	27° 38.623	97° 11.288	Hwy 361 and Gulf Access Rd. 3 Top of Dune	Central
3	26° 06.070	97° 09.864	Gulf and E. Martin Gulf Beach South Padre Island, TX	South
4	26° 07.472	97° 10.039	Gulf and Georgia Ruth Gulf Beach South Padre Island, TX	South
5	26° 14.445	97° 11.120	Where Park Rd 100 ends north of South Padre Island, TX	South
6	26° 11.814	97° 10.643	Park Rd 100 North of South Padre Island, Tx	South
7	28° 35.886	95° 58.718	Beach in Matagorda Beach TX	Central
8	28° 36.291	95° 57.588	Beach in Matagorda Beach TX	Central
9	28° 57.043	95° 17.588	Surfside Beach	North
10	29° 06.698	95° 04.956	Beach Access 2 Jamaica Beach	North
11	29° 40.203	94° 03.950	Side of Rd Near end of Hwy 87 Mcfaddin NWR	North
12	29° 12.519	94° 55.596	Galveston 3005 Rd Beach Access 14 in Dunes	North
13	29° 33.076	94° 23.333	Hwy 87 and 124	North
14	29° 26.297	94° 39.666	Off of HWY 87 on Gulf View on Crystal Beach	North
15	27° 51.816	97° 20.057	Sunset Park Portland Texas growing in oyster shell	Central
16	28° 5.1027	97° 20.057	Fulton Beach Rd in front of Royal Oaks Subdivision	?Central

[z]Latitude and Longitude presented in degrees and decimal minutes format

Table A. 2. GPS coordinates and location description of collection site for each accession of *Borrichia frutescens.*

Accession	*Latitude*	*Longitude*	*Description of location*	*Regional location along Texas coast*
1	27° 42.341	97° 09.224	Hwy 361 and Gulf Access Rd. 2	central
2	27° 38.867	97° 11.587	Hwy 361 and Gulf Access Rd. 3	central
3	26° 06.742	97° 10.212	Laguna St. and Campeche	southern
4	27° 17.363	97° 39.710	End of Rd. 771 in Rivera Beach	southern
5	26° 06.068	97° 09.864	Gulf and E. Martin South Padre Island, TX	southern
6	26° 08.435	97° 10.492	Convention Center in South Padre Island, TX	southern
7	26° 04.353	97° 22.510	Port Isabel Texas next to Whataburger	southern
8	26° 04.715	97° 12.712	Shore Dr. Port Isabel, TX	southern
9	26° 33.535	97° 25.568	Mansfield and North Shore Port Mansfield, TX	southern
10	26° 07.175	97° 09.945	Gulf and E. Mars South Padre Island, TX	southern
11	27° 38.647	97° 17.057	Laguna Shores Rd. Flour Bluff	central
12	26° 34.163	97° 25.774	Fred Stone Park Port Mansfield, TX	southern
13	28° 41.805	95° 57.570	Matagorda Beach along main road	central
14	28° 39.614	96° 24.754	End of 172 Rd in Port Alto, TX	central
15	28° 23.470	96° 50.245	Town Park in Austwell, TX	central
16	28° 33.601	96° 32.247	Public Beach in Magnolia Beach, TX	central
17	28° 27.159	96° 24.326	Park in Port O'Connor, TX	central
18	28° 24.581	96° 43.542	Park living in effluent stream, Sea Drift, TX	central
19	28° 02.160	97° 02.520	Beginning of Fulton Beach Rd. Rockport TX	central
20	28° 57.017	95° 17.142	End of RD332 Surfside	northern
21	29° 22.040	94° 45.607	Hwy 87 Side of RD Bolivar	northern
22	29° 40.091	94° 04.279	McFaddin NWR on Beach	northern
23	29° 42.612	93° 51.539	1st St. in Sabine TX	northern
24	29° 22.042	94° 45.606	Hwy 87 Side on side of RD Bolivar	northern
26	28° 56.415	95° 17.888	On Bay Beach Park View and Port Velasco	northern
27	29° 33.079	94° 22.336	124 @ Hwy 87 High Island in Ditch	northern
28	29° 12.522	95° 55.598	3005 Rd Beach Access 14 Beach in Galveston	northern

[z]Latitude and Longitude presented in degrees and decimal minutes format

Table A. 3. GPS coordinates and location description of collection site for each accession of *Erigeron procumbens*.

Accession	*Latitude*	*Longitude*	*Description of location*	*Regional location along Texas coast*
1	27° 48.886[z]	97° 04.355	2016 11TH St. Port Aransas, TX	Central
2	27° 42.343	97° 09.240	Hwy 361 and Gulf Access Rd. 2	Central
3	27° 53.658	97° 18.440	Walmart Parking lot Portland, TX	Central
4	27° 40.141	97° 17.239	Wells Fargo Parking lot Flour Bluff, TX	Central
5	26° 07.093	97° 10.165	Park Rd 100 and Mars St. South Padre Island, TX	Southern
6	27° 54.524	97° 08.947	Central Park Aransas Pass, TX	Central
7	27° 08.072	97° 47.561	Hwy 77 Kennedy County Rest Stop	Southern
8	26° 07.185	97° 10.256	Laguna and Constellation South Padre Island, TX	Southern
9	26° 07.598	97° 10.069	Gulf and Cora Lee South Padre Island, TX	Southern
10	26° 30.713	97° 27.841	Hwy 186 in Ditch with Sand	Southern
11	26° 06.738	97° 10.210	Laguna and Mars South Padre Island, Texas	Southern
12	27° 37.411	97° 13.468	14175 Jack Fish Ave. The Island Corpus Christi, TX	Central
13	27° 48.341	97° 04.823	11th St. and Gulf Access Rd 1A Port A, TX	Central
14	27° 38.808	97° 16.958	Laguna Shores Rd. Flour Bluff, TX	Central
15	27° 38.877	97° 11.582	Hwy 361 and Gulf Access Rd. 3	Central
16	28° 27.154	96° 24.327	Water front park in Port O'Connor, TX	Central
17	28° 08.317	96° 58.153	4th St growing in ditch, Lamar, TX	Central
18	29° 05.631	95° 06.601	Just north of Toll Bridge on County RD 3005	Northern

[z]Latitude and Longitude presented in degrees and decimal minutes format

Table A. 4. GPS coordinates and location description of collection site for each accession of *Sesuvium portulacastrum*.

Accession	*Latitude*	*Longitude*	*Description of location*	*Regional location along Texas coast*
1	26° 34.164[z]	97° 25.745	Fred Stone Park Port Mansfield	Southern
2	27° 48.201	97° 4.654	Hwy 361 and Gulf Access Rd 1A	Central
3	27° 17.363	97° 39.71	End of Rd 771 in Rivera Beach	Central
4	26° 4.715	97° 12.714	Shore Dr. Port Isabel, TX	Southern
5	26° 4.354	97° 12.718	Port Isabel, TX	Southern
6	26° 4.716	97° 12.715	Port Isabel, TX	Southern
7	26° 7.47	97° 10.042	Gulf Beach South Padre Island, TX	Southern
8	28° 27.163	96° 24.325	Park in Port O'Connor, TX	Central
9	29° 6.698	95° 4.956	Beach Access Rd 2 Jamaica Beach	Northern
10	29° 40.09	94° 4.279	McFaddin NWR	Northern
11	29° 33.076	94° 23.338	124 @ HWY87 High Island	Northern
12	28° 57.02	95° 17.148	End of Rd 332 Surfside Beach	Northern
13	29° 12.523	95° 55.598	Beach Access 14 in dunes Galveston	Northern
14	27° 51.719	97° 20.446	Sunset Park Portland Texas	Central
15	28° 8.671	97° 3.506	Fulton Beach Rd	Central

[z]Latitude and Longitude presented in degrees and decimal minutes format