DROUGHT, SALINITY, AND INVASIVE PLANTS: A NEW MODEL FOR SUSTAINABLE WATER MANAGEMENT

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ABSTRACT

Long-term drought, soil salinity, and land-use intensification have increased the risk of invasive plants in the semiarid southwestern United States. However, soil-related factors that regulate plant invasions are not adequately known. We evaluated the salinity responses of three invasive plant species during a three-month seedling growth period in a greenhouse, and a two-week seed germination period in the laboratory. The species included the indigenous *Lepidium alyssoides* (mesa pepperwort), and the exotic invasive *L. draba* (whitetop) and *L. latifolium* (perennial pepperweed). Significant reductions in seedling growth and evapotranspiration (ET) of three local *L. alyssoides* populations were largely independent of various isosmotic saline irrigation solutions that included NaCl, Na_2SO_4, and $CaCl_2$, each at -0.1 MPa and -0.2 MPa (17 to 48 mM depending on salt species and osmotic potential), suggesting that ET and growth were controlled by solution osmotic potential. Based on ET and total dry matter production under similar experimental conditions, the salt tolerance of these species equaled or exceeded that of salt-tolerant cotton (*Gossypium hirsutum*), despite their combined leaf Na and Cl concentrations of 7% to 13% of dry weight and no characteristic signs of leaf injury. These species appear to exploit high leaf Na and Cl for the maintenance of turgor, and would eventually shed high-salt leaf litter to the ground at the expense of other salt-sensitive species to continue the invasive cycle. A NaCl solution at -0.2 MPa (48 mM) had no effect on germination percentages of *L. draba* and *L. latifolium*, rather, it merely delayed their mean germination time by a day or less. Under saline conditions, high germinability and vegetative propagule pressure along with high-salt litter deposition are major factors contributing to the invasiveness of these species, and this report is the first that we are aware to provide a quantitative basis for their invasions. However, the broader impact of this research is in the application to the larger diversity of invasive species to aid in the understanding of factors that govern invasions, to strengthen predictive and preventative measures, and to preserve the quality and supply of soil water in semiarid regions.

Keywords: *Lepidium alyssoides, L. draba, L. latifolium, Phaseolus vulgaris, Gossypium hirsutum,* sodium, chloride, Chihuahuan Desert, evapotranspiration, soil water, seed germination, anthropogenic disturbance, salinization, wastewater

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BACKGROUND, JUSTIFICATION, AND OBJECTIVES

The soil water supply is the hidden but indispensable component of our water budget. It represents a portion of the groundwater supply that is near the surface and interacts with plants and soils. Hence, it is vital to agricultural production, rangelands and grazing, and natural ecosystems.

In New Mexico and the Southwest, long-term drought conditions can increase the salinity of our soil water supply. In addition, brackish wastewaters have become an increasingly valuable part of our water supply as regional fresh water supplies decrease. The New Mexico Environment Department Ground Water Quality Bureau (NMED-GWQB) lists over 1200 active permits for the land application of wastewaters pertaining to a variety of industries including industrial processing plants, oil and gas drilling, dairies, and agriculture (NMED-GWQB, 2015a, b). Land application is recognized as a sustainable method for the reuse of wastewaters (Toze, 2006; Duan et al. 2010) and its use is expected to increase as our lands continue to endure drought. Nevertheless, land application of wastewater represents a novel anthropogenic disturbance to arid landscapes as the deposition process can increase salts and sodicity in the soil water supply (Ganjegunte et al. 2008; Tzanakakis et al. 2011). With our ongoing drought and changing land use patterns, the quality of the soil water supply may also change. River and rangeland drought, salt concentration effects, and reuse of saline waters for irrigation will increase soil salinity on a wide variety of managed and unmanaged landscapes.

Our recent findings (Picchioni et al. 2012a, b) support the likelihood that the quality of the soil water supply, particularly salinity, plays an important role in the spread of invasive plant species in New Mexico and the southwestern U.S., and this should be of concern to land and water managers. Invasive plant species are typically introduced into other continents where they are non-indigenous, and cause economic or environmental harm to an ecosystem (NISC, 2006). Invasive plants displace native species, reduce biodiversity and ecosystem functions, hinder crop performance, and exacerbate drought conditions by consuming the soil water supply at the expense of desired vegetation (DiTomaso, 2000). Invasive plants are estimated to infest about 100 million ha (Sheley et al. 2011) and cause $13 billion a year in economic and environmental losses in the United States (Westbrooks, 1998). Furthermore, anthropogenic disturbances change the environment and can promote the spread of invasive plants (Hobbs and Huenneke, 1992), thereby increasing the impact of globalization and land-use intensification (Belnap et al. 2012), especially in the expanding Southwest (Abella et al. 2009).

In the Rio Grande watershed of New Mexico, soil salinity, increased use of marginal water for irrigation, human impacts of water use, and elevated ecosystem pressure of invasive plants are major environmental problems (Creel, 2010; Lacewell et al. 2010). Concerns about invasive plants in New Mexico are not confined to the Rio Grande watershed, but are statewide. Between 1915 and 2000, the

number of alien plant species in New Mexico increased by about three-fold (from 136 to 390), with Brassicaceae (which includes *Lepidium*) increasing at the highest rate of all represented families (Cox, 2001). The increase has been exponential and attributed in large part to agriculture and urban development, both of which may create favorable environments for invasive plants (Cox, 2001). The latter inventory predicted high potential for future invasions that was later confirmed by Allred (2008), who reported 455 exotic plant species in New Mexico (Brassicaceae third highest in abundance), a 17% increase in seven years.

Preventing the spread of invasive plants is critically important to the conservation and management of natural ecosystems. Management strategies focused on preventing invasive plants from invading a new area are considered to be the most economical and ecologically viable methods for limiting the impacts of invasive species (Abella et al. 2009). However, prevention management is difficult due to the limited capability of predicting and identifying the early stages of an invasion (Hohmann et al. 2013). For successful long-term intervention and prevention, an understanding of the biology and ecology of an invasive species and the factors that influence its ability to invade are required (DiTomaso, 2000; Byers et al. 2002; Abella et al. 2012). "Non-resource" edaphic factors may regulate plant species populations on semiarid lands (Cox et al. 2006; Miller et al. 2006). It has been suggested that salinity is an important "non-resource" factor in this context, but additional data are needed to reinforce this hypothesis (Grace, 2001).

Plant invasions may be stimulated by soil salinity (Cox et al. 2006). The plant salinity database is largely restricted to agricultural crops, thus water managers of salinizing natural terrestrial systems lack knowledge of salt responses of relevant species (Blacklow, 2003). Soil salinity and sodicity are neglected factors in the vegetation science literature (Bui, 2013), which has been strongly voicing the need for predictive tools to assess the risk of plant invasions. Voluminous documentation, including that on *Lepidium* spp., is adamant about a need for better understanding of site-specific factors, especially edaphic ones like salinity, that lead to the proliferation of weedy, invasive plants (Byers et al. 2002; Brooks, 2003; Abella et al. 2009; Grace, 2001; Hobbs et al. 2003; Nielsen et al. 2003; Hart et al. 2003; D'Antonio and Myerson, 2002; Andrew and Ustin, 2009; Larson and Kiemnec, 2005; Reynolds and Boyer, 2010). Conclusions and recommendations from the aforementioned literature are highly applicable to New Mexico and the semiarid Southwest.

To address these issues, we propose that the quality of the soil water supply, specifically salinity, can be a useful metric for assessing and predicting the risk of our lands to invasive species. Our research is broad in scope and will benefit New Mexico and the southwestern U.S. by providing new information to reveal the importance of soil water salinity as a predictive tool. This concept is strongly aligned with the need for preventative management (Davies and Johnson, 2011), particularly to fill significant "hard

data" gaps in the literature pertaining to the biology of invasive plant species and the potential influence of soil water salinity. A critical outcome of reducing the impact of invasive species is conservation of the soil water supply for desired native vegetation, and to preserve natural ecosystems. By better understanding the relationship between salinity and the spread of invasive plant species, we can positively influence land and water management decisions for rangelands, riparian areas, grazing, crops, dairies, and the oil and gas industry.

In order to develop this new research model, we focus on salinity responses of three potentially invasive plant species: *Lepidium alyssoides* A. Gray var. *alyssoides* (mesa pepperwort), *L. draba* L. [= *Cardaria draba* (L.) Desv.] (whitetop), and *L. latifolium* L. (perennial pepperweed). *Lepidium alyssoides* is indigenous to Arizona, New Mexico, Utah, Colorado, Wyoming, Texas, and Michigan (NRCS, 2015), whereas *L. draba* and *L. latifolium* are Eurasian introductions to much of the U.S., including New Mexico, and are considered as noxious, invasive species (Francis and Warwick, 2007; Renz et al. 2012; Andrew and Ustin, 2009; Cripps et al. 2009; NRCS, 2015). In New Mexico, *L. draba* and *L. latifolium* were recently designated, respectively, as Class A and Class B noxious weeds (Cattaneo et al. 2011). In 2001, a consortium of numerous state, federal, and international agencies, universities, and organizations was established to address the management and biology of the invasive *L. draba* (Hoary Cress Consortium, 2015). In field conditions, *L. draba* may be even more vigorous in the U.S. than in its indigenous settings of Europe (Cripps et al. 2009). *Lepidium latifolium* has been a serious invasive weed throughout the western U.S. since the 1980s (Francis and Warwick, 2007), and as early as 2005, began to invade New Mexico (Renz and Wilson, 2005). That species possesses significant vegetative propagule pressure with an extensive underground rhizomatous network (Francis and Warwick, 2007). The rhizome characteristics of *L. draba* and *L. alyssoides* have received comparatively little attention in the literature, although based on our observations, their rhizomes appear to display vegetative propagule potential just as for *L. latifolium*.

Lepidium alyssoides has received virtually no previous study, although its ability to become invasive under saline, alkaline, and sodic conditions has been clearly demonstrated (Picchioni et al. 2012 a, b). *Lepidium draba* (Lyons, 1998; Santa Margarita–San Luis Rey Weed Management Area, 2015) and *L. latifolium* (Francis and Warwick, 2007) are said to be "adapted to," "tolerate," or otherwise be "common on" saline and alkaline soils. *Lepidium latifolium* is "suited to" germinate in sodic conditions (Larson and Kiemnec, 2005). However, there is little or no quantitative data to support these statements about *L. draba* and *L. latifolium*. Lack of a quantitative database makes this taxon a good research model for soil water salinity and plant invasions in semiarid lands. It is important to note that, while *L. draba* and *L. latifolium* are well-recognized as alien and aggressive invaders (see above articles), *L. alyssoides* is indigenous to New Mexico (NRCS, 2015). Indigenous plant invasions are less common than non-

indigenous plant invasions (Randall, 1997), although in the southwestern U.S., loss of biotic integrity is associated with increased dominance of native invasive plant species (Herrick et al. 2010). Indigenous ruderal species may act like non-indigenous weeds and become invasive in response to human disturbance (Schwartz, 1997), and *L. alyssoides* appears to match that description. In fact, Brassicaceae members such as *Lepidium* spp. are largely ruderal in nature and may become highly competitive on a disturbed landscape (Chapin, 1980).

Research is needed to develop a better understanding of the conditions that cause *Lepidium* spp. to display invasive characteristics. Therefore, our objectives were to:

1) Evaluate growth and ion uptake of the potentially invasive *L. alyssoides* exposed to brackish irrigation solutions.
2) Compare growth and ion uptake of *L. alyssoides* with its invasive relatives, *L. draba* and *L. latifolium*, under brackish water irrigation; include growth analysis of two agricultural crop standards—salt-sensitive bean (*Phaseolus vulgaris* L.) and salt-tolerant cotton (*Gossypium hirsutum* L.)—under brackish water irrigation; and use the crop standards to disclose meaningful salt tolerance information on the *Lepidium* spp.
3) Assess seed propagule pressure of *L. alyssoides, L. draba,* and *L. latifolium* under brackish water irrigation through seed germination and vigor assays.

MATERIALS AND METHODS

Objective 1: *Lepidium alyssoides* Plant Salinity Responses

Seed Collection

Seeds of *Lepidium alyssoides* A. Gray var. *alyssoides* (mesa pepperwort) were collected in June 2012, from plants growing in semiarid landscapes of the northern Chihuahuan Desert near Las Cruces, NM. Three populations of *L. alyssoides*, spanning a land area of approximately 171 km^2, were sampled from the Las Cruces West Mesa (WM: W106°54', N32°16', and 1190 m elevation), the Interstate-10 freeway exit at the town of Mesquite, NM (MQ: W106°41', N32°10', and 1200 m elevation), and the Las Cruces East Mesa (EM: W106°44', N32°20', and 1290 m elevation). The three-population land area (Fig. 1) represents a minor fraction of the much larger Doña Ana County land area, and surrounds the city of Las Cruces that has attracted light industries, general manufacturing, technology-based companies, and a rapidly growing population as in other southwestern U.S. cities. Along with the growth has come land-use changes, human impacts, and increased likelihood of invasive plant encroachment, as previously discussed.

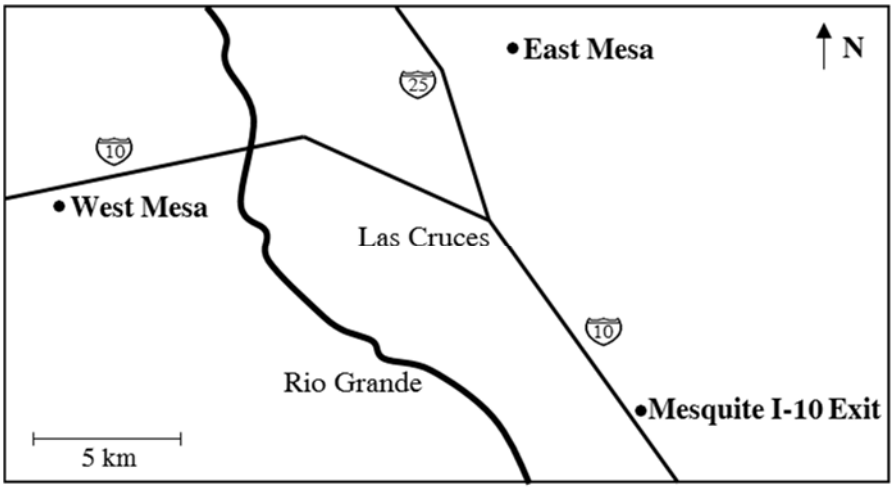

Figure 1. Map of southern New Mexico near Las Cruces showing the seed collection sites of the three populations of *L. alyssoides:* West Mesa (WM), Mesquite I-10 Exit (MQ), and East Mesa (EM).

What we are about to describe is appropriate to this methodological section and is symptomatic of the intensified land-use patterns in the semiarid southwestern U.S. Las Cruces merely serves as an example of the rapidly expanding urban populations. Describing the soils of the population collection

sites is not a straightforward task, especially when deciding whether to use the present tense, or the past tense. Human encroachment is occurring on these and many other sites of our expanding municipalities, on essentially a daily basis. Our case study is truly a lesson in vegetation dynamics in response to continuous and seemingly endless anthropogenic disturbances.

Each of the three *L. alyssoides* populations was highly visible and on disturbed Chihuahuan Desert shrubland sites (see *L. alyssoides* site photographs in Appendix A). The WM collection site was adjacent to the Las Cruces West Mesa Industrial Park. Since 2002, the site has been sprinkler-irrigated with saline–sodic industrial wastewater that is first passed through a secondary wastewater treatment plant (WWTP). The soil type of the WM site is a deep sand to at least 2 m and probably deeper. Sparing the details here, the reader may refer to Picchioni and others (2014) for more information about this site and potential for altering existing environmental mandates governing water reuse in New Mexico to prevent invasive plants such as *L. alyssoides.*

The MQ collection site was along the frontage road (state road FR 1037) at the southeast corner of the intersection of Interstate-10 (freeway) and county road CR B-59 (Mesquite freeway exit off of Interstate-10). This low-lying site was previously altered by clearing of shrubland vegetation, land grading, and road construction. The mouth of a concrete curb storm water diversion on the higher frontage road pointed directly into the path of the lower seed collection site. At this site, the surface soil seems to be continually changing due to replacement of the county road CR B-59 overpass in 2014, two years after the collection in 2012 noted previously. At the time of the MQ site seed collection (June 2012), the soil was sandy and gravelly to a depth of at least 20 cm. At this writing (currently May 2015), the entire area is bare ground, re-plowed and compacted by heavy equipment coincident to the recent overpass reconstruction, bearing even less of a resemblance to the surrounding shrubland than it did three years ago.

The EM collection site was on the raised bank of an intermittent effluent discharge stream below the Las Cruces East Mesa (tertiary) WWTP that became operational in 2010, and is located at the extreme east end of Lohman Avenue. The nearly shrubless bank was constructed from excavated soil, presumably from the stream construction. Effluent samples provided by the plant's manager in 2011 had an electrical conductivity (EC) of 2 dS m^{-1} and a sodium adsorption ratio (SAR) of 7. At the time of seed collection (June 2012), the EM site soil was sandy and gravelly to at least 20 cm depth, with *L. alyssoides* encroaching upon the bank. However, as recently as May 2015, *L. draba* had begun to occupy this site along with *L. alyssoides*, yet another example of *Lepidium* spp. encroachment upon disturbed semiarid landscapes.

At each of the three population sites, seeds were sampled (June 2012) from three to six randomly dispersed positions within an approximate 100 m^2 area heavily populated by *L. alyssoides*. Samples were

taken from the upper half of aboveground tissue and included stems, leaves, flowers, and seed-bearing fruit (silicles). The soil was also sampled to characterize broadly the edaphic conditions under which *L. alyssoides* proliferations were occurring, and the soil sampling positions matched the seed collection positions. One large cluster of aboveground tissue and two soil cores (2.5 cm wide by 20 cm deep) were collected and composited across the three to six sampling positions per population site, and saved for further use and analysis as described below.

Seed Cleaning

Vegetation samples were dried at room temperature for four months. After drying, silicles were separated from the rest of the vegetation by hand and then gently abraded on a rubbing board to break them open and release their seeds. Seeds were then passed through 1 and 2 mm sieves to screen out chaff. A seed blower (757 South Dakota, Seedburo Equipment Co., Des Plaines, IL) was used to finish cleaning seeds. Cleaned seed was then transferred to sealed watertight glass vials and stored at 4°C to await sowing.

Greenhouse Climate

Objective 1 was conducted in a climate-controlled A-frame greenhouse located at the New Mexico State University Fabian Garcia Science Center in Las Cruces, from March to August 2013. Climate data were collected and analyzed using a Watchdog 2475 Plant Growth Weather Station and SpecWare 9 Basic software (Spectrum Technologies, Inc., Aurora, IL). During the salt treatment period described below, maximum daytime temperature ranged from 25-38°C with a mean of 32°C. Minimum nighttime temperature ranged from 15-23°C with a mean of 20°C. Relative humidity ranged from 4-93% with a mean of 47%. Maximum photosynthetically active radiation (PAR) was 706 µmol m^{-2} s^{-1}. Daily light integral (DLI) ranged from 3-14 mol m^{-2} d^{-1} with a mean of 11 mol m^{-2} d^{-1}. A few low DLIs were caused by cloudiness so the DLI range of 3–14 is misleading. Extended durations of cloudiness in Las Cruces are rare and for this study, like any other study conducted in this area, the infrequent cloudy weather had essentially no impact on the mean DLI. A photoperiod consisting of 16 hours light and 8 hours dark was maintained throughout the duration of the study by operating high intensity discharge metal halide lamps from 5 to 8 AM, and from 5 to 9 PM. To conserve electricity and water for the cooling system, and to maintain summer daytime temperatures within appropriate limits, a nylon shade cloth to block 50% of the incoming solar radiation was installed atop the single layer corrugated polycarbonate roof, for the duration of the experiment.

Seed Sowing

Seeds were sown in the greenhouse on March 18, 2013 in 107-mL growing cells (3.8 cm width x 14 cm height; SC7 Ray Leach "Cone-tainers"; Stuewe and Sons, Inc., Tangent, OR) in pure coarse silica sand. Silica sand was selected because it is an inert and nutrient deficient medium, which allowed for more precise control of the root-zone mineral composition. Each cell contained 110 g of silica sand with a cotton ball plug to prevent sand from leaking through the bottom drainage holes. A 1-2 cm headspace remained at the top of each cell to allow for over-head irrigation. A total of 294 cells were prepared accordingly and arranged onto three trays each supporting 98 cells per population. Prior to sowing, the silica sand in each cell was acid-washed with 50 ml of 0.1 N sulfuric acid. After the acid wash, each cell was flushed with tap water until the mean leachate EC and pH from randomly selected cells dropped to levels similar to that of the tap water. Leachates were monitored using a TechPro II TPH1 sensor (Myron L Co., Carlsbad, CA). Seeds were then sown by hand in each cell at a depth of 1 cm. Three seeds were sown in each cell to ensure successful germination, but were later thinned so that only a single plant grew per cell.

Seedling Establishment

Seeds germinated in the greenhouse within seven days, and seedlings were established in the greenhouse under daily sub-irrigation in quarter-strength complete Hoagland's nutrient solution (Hoagland and Arnon, 1950) nutrient solution for approximately 6 weeks, at which time the plants averaged about 7.5 cm in height with several whorls of true leaves. Tap water was used as the water source (see below for tap water analysis). After establishment, plants were selected based on cell ("cone-tainer") weight and plant size to ensure uniform starting populations for saline irrigation treatments. For each of the three populations (WM, EM, and MQ), 63 plants were selected for a total of 189 plants (cells).

Experimental Layout and Design

The study was laid out as a split plot randomized completely (randomized complete block, RCB), with three blocks. Plant populations were whole plots and salt treatments (described below) were split plots. Blocking was necessary to account for greenhouse ventilation and temperature patterns, and to accommodate management and harvest tasks with the available personnel. The numerical average of three individual plants (cells) represented a single experimental unit (EU), which provided sufficient plant biomass at the end of the study for dry weight measurements and tissue mineral analyses. The EU's were randomized by an integer sequence random generator (Random.org, 2013).

Saline Irrigation Treatments

Saline irrigation treatments were applied in the greenhouse and prepared to evaluate specific effects of Na and Cl using three salts: NaCl, $CaCl_2$, and Na_2SO_4 (Table 1). The ECs of the saline irrigation solutions (2–7) met or exceeded the irrigation water salinity in a previous study (2.9 to 4.3 dS m^{-1}) that demonstrated invasiveness of *L. alyssoides* on a salt-affected Chihuahuan Desert shrubland (Picchioni et al. 2012a, b). Salt concentrations were calculated to equalize osmotic potential (Picchioni et al. 1989; Weast, 1985; U.S. Salinity Laboratory Staff, 1954) with each of the latter references corroborating isotonicity of the different salt concentrations. For each salt, -0.1 and -0.2 MPa isosmotic solutions were prepared (low and high salt concentrations, respectively), comprising the six saline irrigation treatments and their concentrations that are shown in Table 1. A total of seven salt treatments were included in the study: 0 mM salt (Control); 24 and 48 mM NaCl, 16 and 34 mM $CaCl_2$, and 17 and 37 mM Na_2SO_4. Salt treatments were prepared with tap water in 18.9-L volumes and stored in sealed buckets on-site. Greenhouse tap water salinity (0.6 dS m^{-1}) was included in each salt treatment and included (in meq L^{-1}) Na (2.8), Ca (2.4), Mg (1.0), Cl (0.5), SO_4 (4.0), and HCO_3 (1.8). The salt treatments were combined with complete Hoagland's nutrient solution at half-strength and at every irrigation (1.0 dS m^{-1}). All salts for Hoagland's nutrient solution and for salinization were laboratory analytical grade. Iron (for the nutrient solution) was supplied in chelated form as prescribed in Hoagland and Arnon (1950), as ferric ethylenediamine di-(o-hydroxyphenylacetate) (Sprint 138, Becker Underwood, Inc., Ames, IA).

Table 1. Composition and properties of the saline treatment irrigation solutions used.

		OP[z]	Concn.	dS m^{-1}		
Treatment	Salt	(MPa)	(mM)	EC[y]	EC[x]	SAR[w]
1 (Control)	- - -	- - -	- - -	- - -	1.6	1.2
2	NaCl	-0.1	23.8	2.3	3.9	11.7
3	NaCl	-0.2	47.9	4.6	6.2	22.3
4	$CaCl_2$	-0.1	16.5	3.4	5.0	0.6
5	$CaCl_2$	-0.2	33.8	6.1	7.7	0.4
6	Na_2SO_4	-0.1	17.1	3.3	4.9	16.2
7	Na_2SO_4	-0.2	36.7	5.8	7.4	33.5

[z]Osmotic potential.

[y]Electrical conductivity due to salt only.

[x]Electrical conductivity of treatment irrigation solution, including salt, half-strength Hoagland's complete nutrient solution, and tap water.

[w]Sodium adsorption ratio.

Saline Solution Irrigation

On the day of the first saline irrigation treatment, ten plants of each population were harvested and dried at 60°C to determine initial dry weight of shoots and roots. Saline irrigation was first applied on May 6, 2013, in an incremental fashion to prevent osmotic shock and to allow the plants to adjust osmotically. Treatments receiving salt were irrigated in step-wise increments of -0.05 MPa until the final salt concentrations were reached, which for -0.2 MPa, was on May 14. The saline solutions were applied overhead via 30-mL syringes, based on daily weights of the EU's. Irrigations were scheduled at 50% of total water storage depletion in the sandy growth medium. The 50% water depletion weight was the numerical "midpoint" weight between the theoretically dry cells in the absence of any water, and the completely moistened cells after irrigation and 10 min. drainage, for a particular growth stage (see "cell capacity" weights described below). The irrigation volume was double the difference between cell capacity and real-time cell weights in order to allow for both ET replenishment, and a targeted 50% LF.

Data Collection During the Experiment

Evapotranspiration (ET). Beginning at the first step-wise increment of saline irrigation, we recorded the daily weight (grams) of each EU. The ET was calculated by simply taking the difference between the cell weights (weight loss) on adjacent days. For days following an irrigation on the previous day, ET was calculated by the difference between the cell capacity weight (analogous to "field capacity" weight) and the lower cell weight on the following day. Cell capacity weights per EU (maximum weight 10 min. after irrigation and drainage) were re-evaluated throughout the duration of the study to account for increasing plant fresh weights due to growth. Adjusting the cell capacity weights enforced accurate volumes of saline irrigation solutions, constant leaching fractions, maintenance of a stable salt balance, and accurate ET assessments. Cumulative ET was determined by summation of daily ET, and plotted on a weekly basis. The final total ET over the duration of the experiment was also determined.

Growth Index (GI). At the beginning of saline irrigation treatment (first step-wise application), plant GI was recorded every two weeks and calculated as the average of individual plant height (measured from the sand level up to the highest naturally occurring part of the plant) and the individual plant diameter (average diameter of two opposite, equatorial measurements). The plant height and diameter measurements were recorded to the nearest half centimeter.

Leachate EC and Leaching Fraction (LF). When the final step-wise irrigation solution salinity was reached, leachates were collected every two weeks in plastic cups placed directly underneath the individual cells just prior to irrigations. The collected leachates were combined per EU and the volume and EC (dS m^{-1}) were recorded. The LF (%) was calculated per EU by dividing the leachate volume by the salt treatment irrigation volume, and multiplying by 100.

Termination, Harvest, and Sample Processing, Drying, and Storage

Objective 1 was terminated on August 5, 2013, after a duration of 91 days. The study was harvested and processed by blocking order. Harvesting and processing a single block required an 8-hr day and four employees. Blocks 1, 2, and 3 were harvested on August 5, 6, and 7, 2013, respectively. Photographs of the experiment on the days of saline treatment initiation (initial day) and termination (final day) are in Appendix B, Objective 1.

Aboveground Tissue Harvest and Processing. Aboveground tissue was cut at the sand level and rinsed in three successive reverse osmosis water baths. The EC of the water baths was monitored and when it exceeded 20 μS cm^{-1}, the water was discarded and replaced with a fresh supply with salinity at 10 μS cm^{-1}. The bulk tissue was then blotted dry and separated into leaf and stem tissues. Leaves were counted and the fresh weight of both leaves and stems were determined.

Belowground Tissue Harvest and Processing. Following the aboveground tissue, belowground tissue was then processed. Cells were split open with a single vertical incision using a razor blade to allow careful extraction of the belowground tissue. Loose sand was removed from the belowground tissue by hand followed by rinsing in reverse osmosis water baths as described above. Belowground tissue consisted of true roots and rhizomes (swollen underground stems) that could not be separated at harvest. For simplicity hereafter, the belowground tissues are referred to as "roots." In cases where new stems and leaves arose from belowground rhizomes, they were separated, washed, and pooled with the respective stem and leaf fractions for the fresh weight determinations noted previously. Belowground tissue was then blotted dry and the fresh weight was recorded.

Tissue Drying, Grinding, and Storage. After processing, all tissue samples were taken to complete dryness in a forced air drying oven at 60°C, and the dry weights were recorded. The dried samples were then ground in a Thomas Wiley Mini-Mill (Thomas Scientific, Swedesboro, NJ) to pass a 40-mesh screen. The ground tissues were stored in air-tight bags at room temperature to await mineral analysis.

Tissue and Soil Mineral Analysis

The ground plant tissue samples were thoroughly mixed, and 0.25-g subsamples were extracted using a MARS 5 microwave digestion system (CEM Corp., Matthews, NC) using the methods of Jones and others (1991) for Na determination by inductively coupled plasma atomic emission spectroscopy (Optima 4300V ICP-AES, Perkin Elmer, Shelton, CT). A second, 0.1-g subsample was subjected to 2% acetic acid extraction at room temperature (Jones et al. 1991) for determination of Cl on an auto-analyzer (AAII, Technicon Instruments, Tarrytown, NY). Bulked vegetation from the seed population collection sites (whole tops of combined leaves, stems, flowers, and fruit) were also ground and analyzed for Na and Cl as described above. The composite soil samples from the seed population collection sites were first

passed through a 2-mm sieve, and a single subsample per site was analyzed for Cl, soluble K, pH, EC, SAR, saturation percentage (SP), organic matter, NO_3-N, Olsen-P, and texture, all by the methods given online at NMSU SWAT laboratory (2015).

Statistical Analysis

At the termination of the experiment, the analysis of variance (ANOVA) was performed for final total ET, tissue dry weight, and tissue Na and Cl concentrations using PROC GLM in SAS (version 9.3, SAS Institute, Inc., Cary, NC). Normality of data was tested using Shapiro-Wilk, and means within subplots and within main plots were separated by Duncan's Multiple Range Test at an alpha of 0.05.

Objective 2: *Lepidium alyssoides, L. draba,* and *L. latifolium* Plant Salinity Responses

To gain insight for the degree of saline resistance of the three *Lepidium* spp. in question, especially in view of the protected (greenhouse) cultivation environment, three independent seedling growth experiments were conducted in 2014. Salt-tolerant *Gossypium hirsutum* L. (upland cotton) and salt-intolerant *Phaseolus vulgaris* L. (common bean) were used as known agricultural crop standards (Maas and Hoffman, 1977). The salinization period for the bean experiment (starting at the first stepwise increment of salinity) was February 18 to April 10, for the cotton experiment July 9 to August 21, and for the *Lepidium* spp. experiment April 24 to July 22. Methods for all experiments in Objective 2 were the same as those described in Objective 1 and in the same greenhouse, with exceptions noted below.

Seed Collection and Cleaning

Seed of *Lepidium draba* (L.) [= *Cardaria draba* (L.) Desv.] (whitetop) and *L. latifolium* L. (perennial pepperweed) were collected in July 2013 from plants growing prolifically in suburban agricultural areas near semiarid Los Lunas, NM (*L. draba*: W106°43', N34°43', and 1472 m elevation; and *L. latifolium*: W106°40', N34°49', and 1482 m elevation). The *L. draba* collection site was south of Los Lunas along a weedy fence row between a paved road and a small farm, while the *L. latifolium* collection site was north of Los Lunas along an equally weedy irrigation canal (Fig. 2). The site photographs of *L. draba* and *L. latifolium* (Appendix A) reveal the landscape alterations of water runoff from pavement, close proximity to managed farmland (*L. draba*), access to surface water, and soil disturbance (*L. latifolium*). The MQ population described previously served as the *L. alyssoides* representative for this objective. The *L. draba* and *L. latifolium* bulk vegetation, seed, and soils were sampled and handled in similar fashion as in the Objective 1 population site collection methods. The *Lepidium* vegetation was dried for three months

at room temperature, and then the seed was cleaned and stored as described above in Objective 1. Seed of *Gossypium hirsutum* L. (upland cotton, Acala 1517-99) and *Phaseolus vulgaris* L. (common bean, 'Contender') required no pretreatments prior to use.

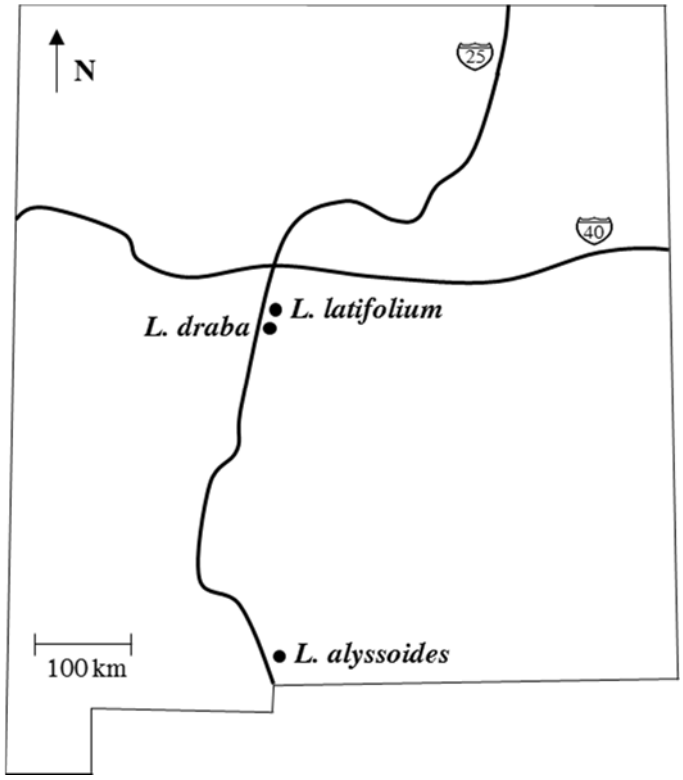

Figure 2. Map of New Mexico showing the seed collection sites of *L. alyssoides*, *L. draba*, and *L. latifolium*.

Greenhouse Climate

Each of the three independent experiments in Objective 2 mentioned previously was conducted in the same greenhouse described in Objective 1, from January to August 2014. As described previously, the photoperiod was held constant at 16 h, and the 50% shade cloth was installed March 1 and remained in place atop the roof through the duration of the three experiments.

For the *Lepidium* spp. experiment, during the duration of salt treatment described below, the maximum daytime temperature ranged from 24-34°C with a mean of 27°C. Minimum nighttime temperature ranged from 15-22°C with a mean of 18°C. Relative humidity ranged from 8-85% with a mean of 49%. Maximum (PAR) was 717 µmol m^{-2} s^{-1}. Daily light integral (DLI) ranged from 6-16 mol m^{-2} d^{-1} with a mean of 13 mol m^{-2} d^{-1}.

During the saline treatment application period for the bean experiment, maximum daytime temperature ranged from 24-34°C with a mean of 27°C. Minimum nighttime temperature ranged from

14-16°C with a mean of 15°C. Relative humidity ranged from 7-66% with a mean of 32%. Maximum PAR was 2032 μmol m^{-2} s^{-1} recorded in late February prior to shade cloth installation. The DLI ranged from 9-39 mol m^{-2} d^{-1} (high DLI readings recorded in late February) with a mean of 24 mol m^{-2} d^{-1}.

For the cotton study, the climatic conditions during the saline treatment application period were as follows: Maximum daytime temperature ranged from 26-31°C with a mean of 28°C. Minimum nighttime temperature ranged from 20-23°C with a mean of 21°C. Relative humidity ranged from 42-88% with a mean of 66%. Maximum PAR was 779 μmol m^{-2} s^{-1}. The DLI ranged from 6-15 mol m^{-2} d^{-1} with a mean of 12 mol m^{-2} d^{-1}.

Seed Sowing

Seeds of all species were sown in the greenhouse on January 27, 2014. Cotton was reseeded later in the year (June) due to poor germination in January caused by shallow seeding depth and suboptimal temperatures. Each cell contained 100 g of sand and two cotton ball plugs to prevent sand from leaking from the bottom drainage holes. A 1-2 cm headspace still remained at the top of each cell, the same as Objective 1. In January, a total of 280 sown cells were prepared and arranged onto five trays, each holding 56 cells per species, which for cotton, was repeated in June. The silica sand growing medium was not acid-washed but was instead simply rinsed with tap water flushes as described in Objective 1, until the leachate EC and pH dropped to tap water levels. Seeds of the *Lepidium* spp. were sown and seedlings thinned as described in Objective 1, while single seeds of bean and cotton were sown to a depth of 3 to 4 cm.

Seedling Establishment

Seeds of all species had germinated in the greenhouse within 10 days or less under daily sub-irrigation in tap water, after which time seedlings were raised up and established using quarter-strength Hoagland's nutrient solution as described in Objective 1. Seedling establishment varied in duration due to different growth rates of each species. Seedling establishment and selection procedures followed those of Objective 1. For all five species, 27 plants were selected for uniformity. A foliar insecticidal spray solution of imidacloprid (Mallet 2F T&O, Nufarm Americas, Inc., Burr Ridge IL) combined with bifenthrin (Talstar GH, FMC Corp., Philadelphia, PA) at the rates of 126 μL and 3 mL per L tap water, respectively, was used to control thrips and aphids on all species. A 0.5-L solution was prepared for each of three foliar spray applications made in April, June, and July 2014.

Experimental Layout and Design

Experimental design for the *Lepidium* spp. experiment remained the same as in Objective 1 including blocking, except that salt treatments were whole plots and plant species were split plots. Bean and cotton were laid out as single-factor RCBs and with three blocks. Bean, cotton, and *Lepidium* spp. were separated into three independent experiments in time, to accommodate the available personnel and because of the difficulty in studying vastly different taxa at equivalent growth stages. Methods for all three studies were identical, and all of the studies were completed in the same greenhouse.

Saline Irrigation Treatments

Along with the non-saline (control) treatment, two salinized treatments consisted of the single salt, NaCl. The NaCl concentrations (Table 1) and nutrient solution management were identical to those in Objective 1. Specifically, the treatments were as follows: 0 mM NaCl (Control) and 24 and 48 mM NaCl (-0.1 and -0.2 MPa, respectively).

Saline Solution Irrigation

On the day of the first saline irrigation treatment, ten plants of each *Lepidium* spp. and nine plants each of bean and cotton were harvested and dried at 60°C to determine initial dry weight of shoots and roots. All irrigation methods were identical for bean, cotton, and *Lepidium* spp. experiments, and as previously outlined in Objective 1, except that irrigation frequency was daily and not based on 50% total water storage depletion as in Objective 1. Saline solutions were first applied gradually, increasing in step-wise fashion and up to the final concentrations reached in Table 1, exactly as described in the saline solution irrigation methods in Objective 1. The first and final step-wise saline irrigation treatments were, respectively, as follows: February 18 and 24 (bean); April 24 and May 1 (*Lepidium* spp.); and July 9 and 16 (cotton). For ET replenishment and maintenance of a constant LF, the necessary (calculated) volumes of salt treatment irrigations were applied daily, based on gravimetrical assessments of both water depletion replacement and 50% LF needs, and per the three-cell EU's. Saline treatment irrigation volumes were calculated by multiplying the difference between the EU's cell capacity weight and the current cell weight by two, in order to allow for a targeted 50% leaching fraction. Cell capacity weights were updated as necessary, as described in Objective 1. After May 30 in the *Lepidium* spp. experiment, salt treatment irrigations to replace ET and provide a 50% LF were applied twice daily until termination of the study, and the total water storage depletion never exceeded 50%. No such adjustment was necessary for the bean and cotton experiments.

Data Collection During the Experiments

Throughout the duration of Objective 2, weekly cumulative and final ET, GI (*Lepidium* spp. only), leachate EC and LF leaching fraction along with cell capacity weights, were determined as described in Objective 1. Leachate collection intervals were 7 to 11 days for bean, 2 weeks for *Lepidium* spp., and 13 to 20 days for cotton.

Termination, Harvest, and Sample Processing, Drying, Grinding, Storing, and Mineral Analysis

Photographs of all three experiments on the days of saline treatment initiation (initial day) and termination (final day) are available in Appendix B, Objective 2. Termination dates for the bean and cotton experiments were April 10 (51 days treatment) and August 21 (43 days treatment), respectively. On those termination dates, the tissues of all three blocks were harvested and fractionated for determining only dry matter production. For the *Lepidium* spp. experiment, the termination date was July 22 (89 d treatment) and blocks 1, 2, and 3 were harvested in order on July 22, 23, and 24, respectively, with harvesting, processing, drying, grinding, storage, and mineral extraction and analysis methods remaining the same as described in Objective 1 except that, in addition, fresh leaf area was measured using a LI-3100C area meter (LI-COR Biosciences, Lincoln, NE). Once again, the belowground *Lepidium* spp. tissues were pooled (roots plus rhizomes) and for simplicity going forward, we will designate these tissues as "roots." Pooling these tissues, like in Objective 1, was unavoidable since it was not possible to physically separate them at termination. Nonetheless, rhizome tissues set these weedy species apart from most of the agronomic species such as bean and cotton that do not have such high belowground vegetative propagule pressure. Thus, pooling the tissues into a combined belowground biomass fraction is appropriate for weedy species, although we acknowledge this biological assessment limitation in our methods that also applies to Objective 1. For the *L. draba* and *L. latifolium* seed collection sites, the bulked vegetation and composited soil samples were processed and analyzed identically to the Objective 1 procedures reported earlier.

Statistical Analysis

At termination of each of the three experiments, the final total ET and tissue dry weights, and for *Lepidium* spp. only, tissue Na and Cl concentrations, were analyzed by ANOVA using SAS software as described in Objective 1. The ANOVA was applied independently for each of the bean, cotton, and *Lepidium* spp. studies, all laid out as RCBs in the greenhouse. The *Lepidium* spp. experiment was a split plot as previously mentioned, and the bean and cotton experiments were analyzed as simple one-factor ANOVAs. Mean separation was as described in Objective 1.

ET and Growth Comparisons between Bean, Cotton and *Lepidium* spp.

During the Objective 2 experiments, growing degree days (GDDs, i.e., "heat units") were calculated as daily mean greenhouse air temperature (°C) minus a base temperature of 10°C. In the high salinity treatment (-0.2 MPa NaCl), bean shoots had expressed severe necrosis by April 9 (one day prior to the termination of its experiment), corresponding to 506 GDDs. Therefore, we evaluated the cumulative, total ET of all three experiments up to 506 to 508 GDDs. Those GDDs corresponded to 1, 8, and 43 days prior to the termination of saline irrigation on bean, cotton, and *Lepidium* spp., respectively. We restrict our discussion on ET and dried biomass (see later in report) to comparisons within-species and across the salt levels. However, (see later), we normalized the dry matter productivity (total plant biomass) as percentage of the non-saline (control) solution in similar experimental conditions, to allow for the broader assessment of the *relative salt tolerance of the different species* under our greenhouse conditions, and therefore, to gain insight for the degree of *Lepidium* spp. saline tolerance under protected cultivation.

Objective 3: *Lepidium alyssoides, L. draba,* and *L. latifolium* Seed Germination Salinity Responses

Seed Germination

On October 21, 2014, seeds of *L. alyssoides, L. draba, and L. latifolium* (from the same populations described in Objective 2 previously) were placed in petri dishes lined with blotting paper (9 cm diameter, Anchor Steel Blue Seed Germination Blotter, Anchor Paper Co., Saint Paul, MN). The blotting paper was pre-soaked with 5 mL of the same saline treatment solutions that were used in Objective 2, except for the omission of Hoagland's nutrient solution and the use of deionized water at 8 μS cm^{-1}. Consequently, the two NaCl treatments used in the present study (-0.1 MPa and -0.2 MPa) registered lower ECs (2.7 and 5.2 dS m^{-1}, respectively) than their greenhouse study counterparts that included tap water and the nutrient solution (Table 1). Fifty seeds per species were placed in each dish. Dishes were then sealed with parafilm and further sealed inside plastic zip bags to minimize evaporation loss of the treatment solutions. Dishes were completely randomized with four, single-dish replications and then placed into a seed germination chamber (GR41VL, Percival Scientific, Inc., Perry IA). A photograph of the seed germination dish layout is available in Appendix B, Objective 3. The growth chamber was programmed to provide a 16-hour photoperiod with fluorescent lighting at 40 μmol m^{-2} s^{-1} and a day/night temperature of 26°C/15°C, to be consistent with the greenhouse photoperiod and temperature conditions that we recorded during the late January to early February 2014 *Lepidium* spp. seed germination phase of Objective 2.

Seeds were inspected daily at approximately noon, for germination (visible white radicle). On a daily basis, germination was recorded and germinated seeds removed from each dish. Dishes were then re-sealed and placed back into the chamber according to their randomization. The 2-week study was terminated on November 4. Remaining (non-germinated) seeds were tested for viability using a 0.5% tetrazolium stain procedure established in the Association of Official Seed Analysts and the Society of Commercial Seed Technologists (Miller and Peters, 2010). The number of viable seed that had not germinated was counted in the 50-seed total. The number of non-viable (dead) seed was deducted from the 50-seed total to provide the final assessments of percentage germination and mean germination time (see these response variables noted below).

Seed Vigor

On January 16, 2015, seeds of three *Lepidium* spp. were placed in petri dishes as described above, except that only ten seeds were placed in each dish. Dishes were tilted at an approximate 60° angle to promote straight radicle growth. A photograph of the seed vigor dish layout is available in Appendix B, Objective 3. After seven days (January 23), dishes were removed from the chamber and radicle lengths were scanned and measured using ImageJ software (National Institutes of Health, Bethesda, MD).

Response Variables and Statistical Analysis

Seed Germination. Final percentage germination at two weeks and mean germination time were calculated after the methods in Ranal and others (2009). Datasets were statistically analyzed as described in Objective 2 for *Lepidium* spp.

Seed Vigor. Final radicle length (7 d) was measured in mm and statistically analyzed as described above for seed germination.

RESULTS AND DISCUSSION

Objective 1: *Lepidium alyssoides* Plant Salinity Responses

Soil and Vegetation Analysis of the Seed Collection Sites

The texture of each soil from the *L. alyssoides* WM, MQ, and EM sites was a sand, with each having a low saturation percentage of 15–18 and low organic matter from 0.4–1.2% (Table 2). The soils were non-saline with soil saturation extract EC ranging from 1.6–2.0 dS m^{-1} and Cl from ≈ 4–12 meq L^{-1}. The soils from the MQ and EM sites were slightly basic and non–sodic, with soil saturation extract pH of 7.2 and soil saturation extract SAR ranging from 0.6–1.7, while the soil from the WM site was more basic (pH 7.9) with SAR of 12.5, which is at the sodic level (SSSA, 2008). The higher pH and SAR in the WM soil may be attributed to an earlier study, when at this location, saline–sodic, alkaline, treated wastewater was land-applied from 2002–2006 (Picchioni et al. 2012a). At all sites, the soil NO_3–N and Olsen–P concentrations were low, and soluble K moderate to sufficient on most agricultural crop standards (personal communication, R.P. Flynn, 2015).

Table 2. Soil characteristics of the seed collection sites from the three populations of *L. alyssoides* (WM, MQ, and EM). The pH, EC, SAR, and Cl were determined in the soil saturation extract.

		EC		Cl			mg kg^{-1}		
Population	pH	(dS m^{-1})[z]	SAR[y]	(meq L^{-1})	SP (%)[x]	OM (%)[w]	NO_3-N	Soluble K	Olsen-P
WM	7.9	1.7	12.5	6.3	18.1	0.4	3.9	81.0	7.2
MQ	7.2	1.6	1.7	4.1	15.8	1.2	17.3	76.5	9.4
EM	7.2	2.0	0.6	12.4	15.3	0.4	4.6	51.8	5.2

[z]Electrical conductivity.

[y]Sodium adsorption ratio.

[x]Saturation percentage.

[w]Organic matter.

Aboveground vegetation of *L. alyssoides* from each of the three collection sites had Na and Cl concentrations that ranged from 0.01–0.13 and 0.56–0.94 percent of dry weight, respectively (data not shown). Of these values, the WM population had the highest Na and Cl concentrations.

Leachate Characteristics throughout Study Duration

Throughout the duration of the study, there was some variation in leaching fractions (LF) within given salt treatments, with an overall range of ≈38–50% for all treatments and populations (Table 3). The control treatment across all populations received the lowest LF, ranging from ≈37–39%. The LFs of the -0.1 MPa (even-numbered) treatments averaged slightly lower than those of the -0.2 MPa (odd-numbered) treatments. These differences were likely caused by the increased ET demand of the control plants (and plants growing in -0.1 MPa treatments; see below), speeding the depletion of root-zone water and allowing less water to leach through the cells.

Table 3. Leachate characteristics of the various saline irrigation treatments from the three populations of *L. alyssoides* (WM, MQ, and EM). Each value is presented as the mean ± s.d. of six biweekly measurements taken throughout the study. Each measurement was the average of three replications, each comprised of three cells. For treatment composition and properties, see Table 1.

	WM		MQ		EM	
Treatment	EC (dS m^{-1})[z]	LF (%)[y]	EC (dS m^{-1})	LF (%)	EC (dS m^{-1})	LF (%)
1 (Control)	2.5 ± 0.4	38.3 ± 7.4	2.4 ± 0.5	39.3 ± 7.7	2.6 ± 0.7	37.7 ± 4.0
2	7.7 ± 1.3	44.7 ± 3.1	8.2 ± 1.3	42.4 ± 3.3	8.4 ± 1.5	41.9 ± 4.3
3	13.1 ± 1.4	48.6 ± 6.0	13.1 ± 1.1	48.5 ± 3.6	13.9 ± 1.5	45.5 ± 5.5
4	9.2 ± 1.7	41.9 ± 1.9	9.4 ± 1.5	43.4 ± 3.8	9.8 ± 1.9	40.4 ± 2.1
5	16.3 ± 1.7	44.4 ± 6.4	16.5 ± 2.1	46.5 ± 6.0	16.2 ± 1.5	48.0 ± 5.4
6	9.2 ± 1.0	42.0 ± 2.0	9.2 ± 1.2	44.9 ± 4.1	9.4 ± 1.3	43.1 ± 3.0
7	14.7 ± 1.4	49.3 ± 4.8	15.1 ± 1.7	50.0 ± 5.8	15.9 ± 1.0	46.7 ± 2.6

[z]Electrical conductivity.

[y]Leaching fraction.

Despite the lower leaching fractions experienced by the control plants and those under the -0.1 MPa treatments, leachate EC of these treatments did not increase during the study. In fact, there was relatively minimal leachate EC variation, and thus a steady-state salt balance was maintained for all treatments and populations (Table 3). The importance of leaching has long been recognized for preventing the buildup of soluble salts introduced by irrigation water (Ayers and Westcot, 1985). Leachate EC was lowest in the non-saline control treatment. Leachate EC in the -0.1 MPa salt treatments (2, 4, and 6) was moderately high and ranged from 7.7–9.8 dS m^{-1}. As expected, leachate EC in the -0.2 MPa salt treatments (3, 5, and 7) was incrementally higher and ranged from 13.1–16.5 dS m^{-1}. In greenhouse potting substrates, leachate ("PourThru") EC is about 30% higher than the EC from a corresponding substrate saturation extract (Cox, 2005), which is the standard soil salinity metric for assessing crop salt tolerance (Maas and Hoffman, 1977). Given this relationship, leachate EC levels in all

saline treatments (2–7) would correspond to saturation extract salinities of ≈ 6–13 dS m^{-1} that would cause severe injury and growth suppression to most crop plants (Ayers and Westcot, 1985). Since this study was investigating the apparent salt tolerance of an opportunistic invasive species, *L. alyssoides*, these EC levels were appropriate. Leachate ECs of the NaCl treatments (2–3) were consistently lower than those of the $CaCl_2$ and Na_2SO_4 treatments (4–5 and 6–7, respectively, Table 3). This difference followed the inherent conductance properties of these salts under isosmotic conditions (Table 1).

Growth Index (GI) Measurements throughout Study Duration

At times, GI means were somewhat variable, depending on treatment and population (Figure 3). Several individual WM and MQ means seemed to vary the most, especially during the last four weeks. Because GI was hand-measured, some variability was expected. For all treatments and populations, GI steadily increased throughout most of the study but subsided during the final two to four weeks. Growth capacity of the belowground tissues was most likely reached during the 12th week due to the limitation of the cell size, as excavation at the termination of the study revealed compressed underground structures. The control treatment appeared to have the highest GI throughout the study compared to all other treatments, and for all populations. In addition, GI of the salt treatments appeared to trend according to osmotic potential, with the -0.1 MPa salts providing higher numerical GI averages than the -0.2 MPa salts for all populations, but especially for MQ. Within each level of osmotic potential, no salt solution appeared to reduce GI more than others, except for possibly -0.2 MPa Na_2SO_4 (Treatment 7, with the highest Na of all treatments), and only within the WM population.

Evapotranspiration (ET): Cumulative and Final Total

Cumulative ET per treatment steadily increased throughout the duration of the study and was similar across all populations (Fig. 4), with no significant main effect of population or of the population × treatment interaction on the final total ET (P = 0.0711 and 0.5126, respectively; final totals in Table 4). Thus, there was similar physiological behavior between the three *L. alyssoides* populations regarding salinity effects on water use. Saline treatment main effect on final total ET was highly significant ($P < 0.0001$). The control plants had the highest ET throughout the study and ended with the highest final total ET of all treatments. Salt-induced reduction in cumulative ET became apparent as early as 5–6 weeks after initiating treatments, with incremental effects of salinity (-0.1 MPa to -0.2 MPa) appearing at about 6–8 weeks, depending on population (Fig. 4). The incremental salinity effect was reflected in final total ET, and for each population (i.e., compare treatments 2 with 3, treatments 4 with 5, etc.; Table 4). Within none of the populations were there differences in final total ET between the three salts at a given osmotic

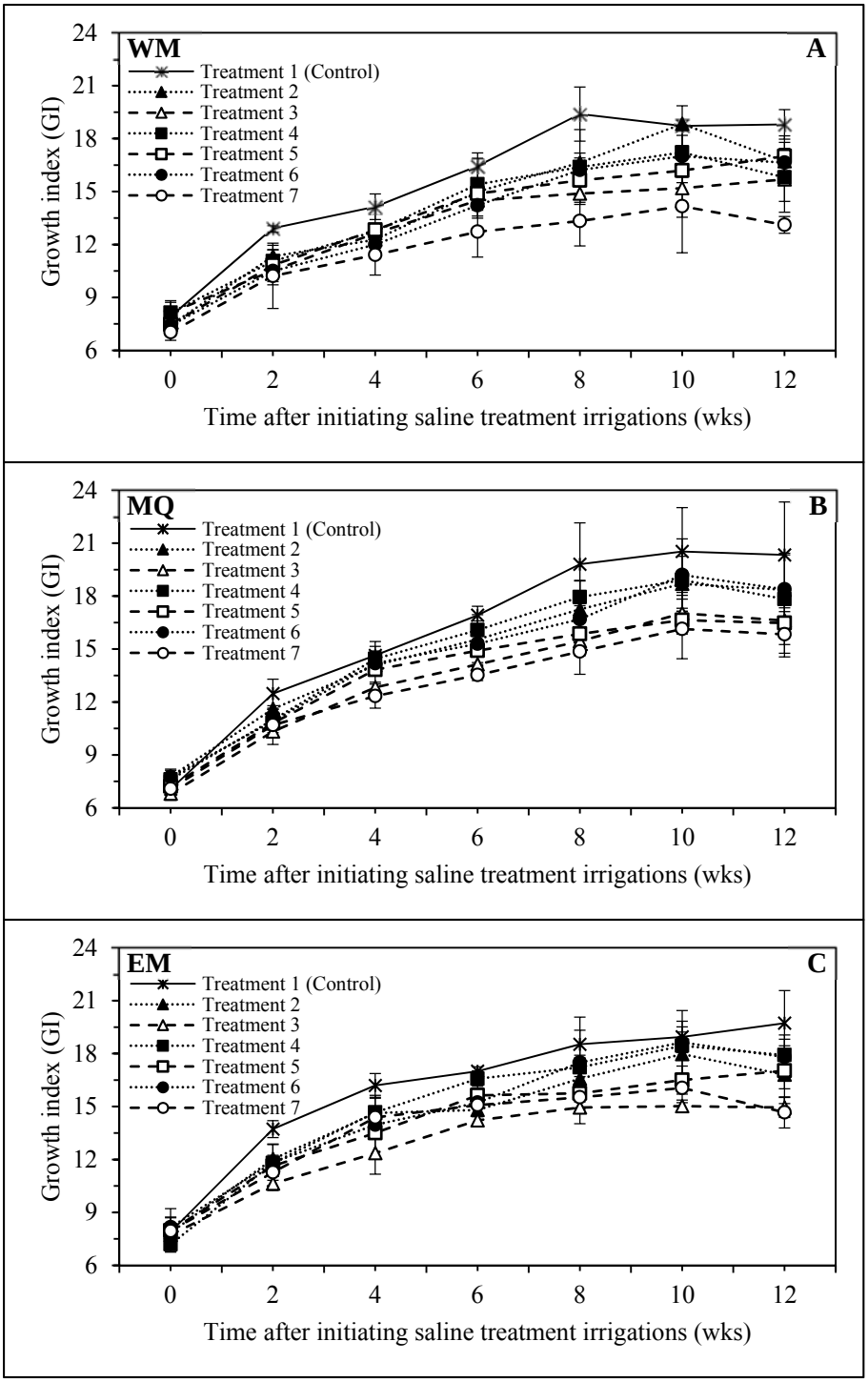

Figure 3. Biweekly growth index (GI) under various saline irrigation treatments of the three populations of *L. alyssoides*: WM (A), MQ (B), and EM (C). Each point is presented as the mean ± s.d. of three replications, each comprised of three cells. Treatments 2 and 3 (NaCl), 4 and 5 ($CaCl_2$), and 6 and 7 (Na_2SO_4). Closed symbols represent osmotic potential of -0.1 MPa; open symbols represent osmotic potential of -0.2 MPa. Further treatment details in Table 1.

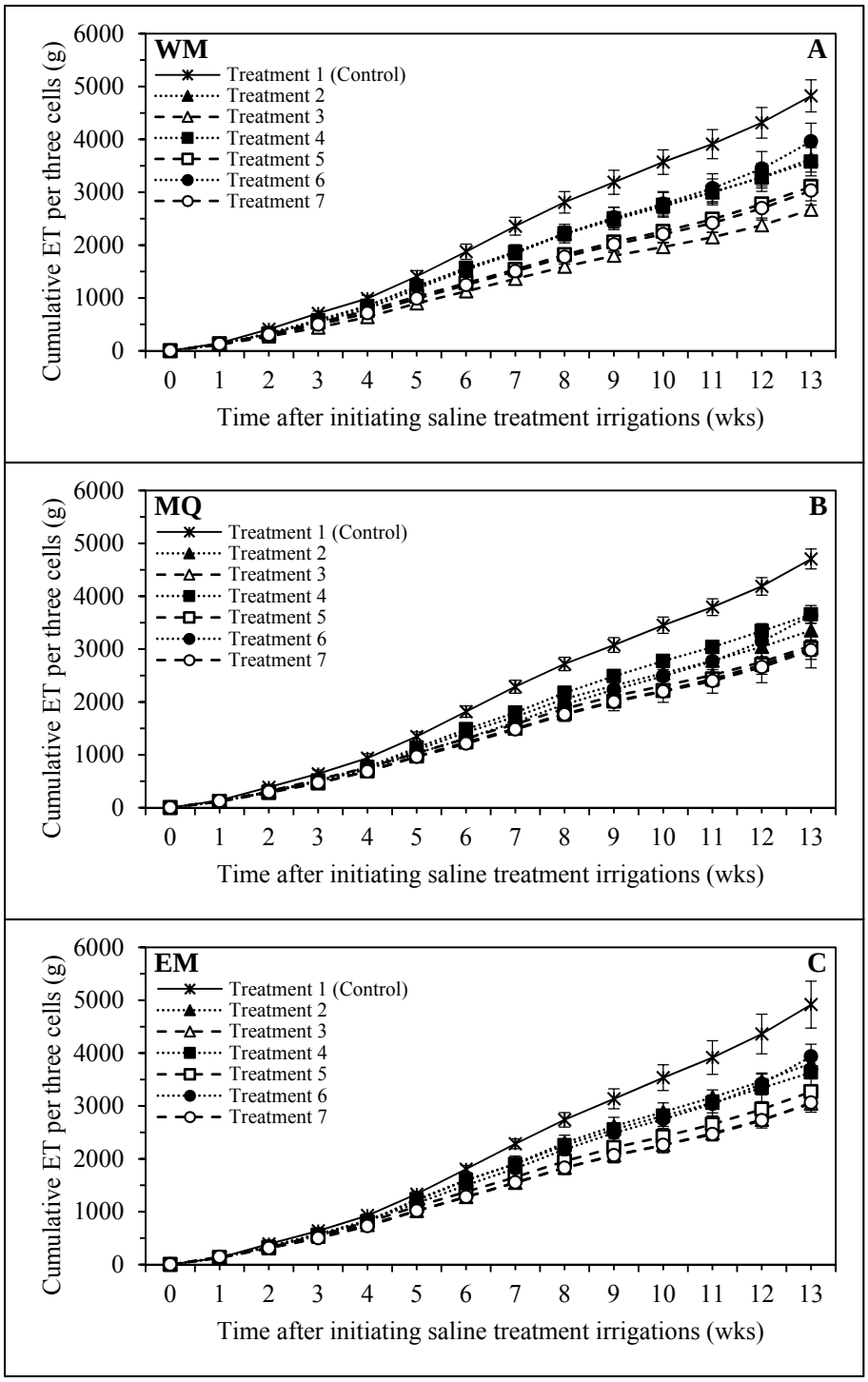

Figure 4. Weekly cumulative evapotranspiration (ET) under various saline irrigation treatments of the three populations of *L. alyssoides*: WM (A), MQ (B), and EM (C). Each point is presented as the mean ± s.d. of three replications, each comprised of three cells. Treatments 2 and 3 (NaCl), 4 and 5 ($CaCl_2$), and 6 and 7 (Na_2SO_4). Closed symbols represent osmotic potential of -0.1 MPa; open symbols represent osmotic potential of -0.2 MPa. Further treatment details in Table 1.

potential. This indicates a lack of salt specificity on ET reduction under these conditions, such as high Cl in treatments 4 and 5, or high Na in treatments 6 and 7.

Table 4. Final total evapotranspiration (ET) under various saline irrigation treatments of the three populations of *L. alyssoides* (WM, MQ, and EM) in grams per three cells. Each value is presented as the average of three replications, each comprised of three cells. For treatment composition and properties, see Table 1.

Treatment	WM	MQ	EM
1 (Control)	4822 A	4704 A	4917 A
2	3631 B	3354 BC	3806 B
3	2672 C	3066 C	3045 D
4	3577 B	3656 B	3632 BC
5	3107 C	3018 C	3269 CD
6	3961 B	3643 B	3935 B
7	3031 C	2979 C	3064 D

Within populations, means followed by different letters indicate a significant difference according to Duncan's Multiple Range Test ($P < 0.05$).

Dry Weight (DW) of Leaf, Stem, Root, and Total Plant

At the initiation of salt treatments and across the populations, average total root DW ranged from 0.21–0.30 g per three seedlings, and average total shoot DW (leaves plus stems) ranged from 0.43–0.50 g per three seedlings. At termination of the study, for the DWs of leaf, stem, root, and total plant (TDW), the treatment main effect was highly significant ($P < 0.0001$), with generally incremental salinity effects as noted previously. There was no significant interaction between population and treatment, for any of these response variables ($P \geq 0.1035$). For leaf and root DW, and TDW, there was no significant population main effect ($P \geq 0.1803$) and therefore these data were pooled across populations (Table 5). However, there was a significant population main effect on stem DW to be discussed later.

Leaf DW was highest for the control treatment and treatment 4 (Table 5). This may indicate that, at -0.1 MPa, $CaCl_2$ (high Cl but low Na proportion in treatment 4) was less deleterious to leaf biomass in these *L. alyssoides* populations than were Na_2SO_4 (low Cl but high Na proportion in treatment 6) and NaCl (moderately high Na plus Cl proportions in treatment 2). Leaf DW was lowest in treatment 7 (Na_2SO_4 at -0.2 MPa), which had the highest treatment solution Na concentration. In most crop species, Na may cause toxicity before Cl does (Munns and Tester, 2008) although in *Lepidium* spp., data are limited.

Table 5. Leaf and root tissue dry weight and total dry weight (TDW) of *L. alyssoides* in grams per three cells. Populations were pooled due to a lack of significance ($P \geq 0.2331$). For treatment composition and properties, see Table 1.

Treatment	Leaf	Root	TDW
1 (Control)	6.38 A	3.98 A	13.11 A
2	4.30 BC	2.73 B	8.21 C
3	3.33 D	1.90 C	5.96 D
4	5.73 A	2.85 B	9.65 B
5	3.67 CD	1.63 C	6.02 D
6	4.90 B	3.02 B	9.41 B
7	2.61 E	1.85 C	5.32 D

Within tissues, means followed by different letters indicate a significant difference according to Duncan's Multiple Range Test ($P < 0.05$).

At termination, 59% of all experimental units had rhizomes of various numbers and at various stages of budding, either within the sand medium or rising above the surface of the sand (data not shown). Because belowground rhizome biomass could not be determined (roots and rhizomes pooled together), we were unable to discern population or treatment effects on rhizome development, although when examining all of the experimental units, there was a trend for lower frequency of rhizome sightings in the highest (-0.2 MPa) salt treatments. Further research is needed to determine salt effects on *L. alyssoides* rhizome production.

Root DW (Table 5) was highest for the control treatment, followed by the -0.1 MPa treatments (2, 4, and 6), and finally by the -0.2 MPa treatments (3, 5, and 7). Root DW did not differ between the salts within each level of osmotic potential, which may indicate osmotic effects on the plants.

The TDW was highest for the control treatment, with reductions in TDW for all of the salt treatments (Table 5). For TDW, once again, there were incremental declines with each increase in treatment solution salinity, and for all salts. There was little or no indication of specific Na or Cl effects on the TDW reductions, although for the -0.1 MPa treatments, TDW was lowest in treatment 2, which had moderately high Na plus Cl proportions. Of the TDW showing in Table 5, 49–61% was in leaves, with 27–36% in roots (averaged across the treatments). Thus, quantitatively, salt effects on TDW were largely associated with leaf and root growth reduction.

For stem DW (Table 6), there was a significant population main effect ($P = 0.0015$), along with the treatment effect previously noted. Stem DW of all of the populations was reduced by saline irrigation, but not incrementally (-0.1 MPa to -0.2 MPa) in the WM and EM populations. The stem DW of the WM

population was the lowest of the three populations in treatments 3 and 4 (NaCl at -0.2 MPa and $CaCl_2$ at -0.1 MPa, respectively), and the EM population stem DW was highest among the populations in treatment 7 (Na_2SO_4 at -0.2 MPa). It seems unlikely that the three populations of *L. alyssoides* could be distinguished by stem weight variation in these conditions, but rather, since the stem DW was the smallest fraction of TDW (14% on average), experimental variability may be the cause for the population differences detected.

Table 6. Stem tissue dry weight of the three populations of *L. alyssoides* (WM, MQ, and EM) in grams per three cells. For treatment composition and properties, see Table 1.

Treatment	WM	MQ	EM
1 (Control)	2.06 A a	2.56 A a	3.64 A a
2	0.99 B a	1.19 BC a	1.36 B a
3	0.49 B b	0.76 C ab	0.94 B a
4	0.65 B b	1.46 B a	1.11 B a
5	0.64 B a	0.76 C a	0.77 B a
6	1.03 B a	1.53 B a	1.92 B a
7	0.78 B b	0.76 C b	1.03 B a

Means followed by different letters indicate a significant difference according to Duncan's Multiple Range Test ($P < 0.05$); uppercase within columns, lowercase within rows.

Na and Cl Concentrations in the Leaf, Stem, and Root Tissues

For Na and Cl concentrations in leaves, stems, and roots, the treatment main effect was highly significant ($P \leq 0.0001$). There was no significant population main effect on leaf and stem Na or Cl concentrations ($P \geq 0.0992$) and therefore those data were pooled across the populations (Tables 7 and 8). However, there was a significant population effect on root Na and Cl concentrations. That population effect was attributed to marginally higher root Na and Cl concentrations in WM with high $CaCl_2$ (treatment 5), and marginally higher root Cl concentrations in WM with low NaCl (treatment 2), as compared with MQ and EM. There was no treatment x population interaction on Na and Cl concentrations in any of the tissues ($P \geq 0.2935$), except for root Cl concentration ($P = 0.0423$). Root Cl increased throughout the NaCl concentration range (treatments 2–3) in MQ and EM, but not in WM. In addition, root Cl concentrations in WM increased incrementally with increasing $CaCl_2$ concentration, although not in MQ and EM (treatments 4–5).

Table 7. Na concentration (% of DW) of leaf, stem, and root tissues from the three populations of *L. alyssoides* (WM, MQ, and EM) subjected to various saline irrigation treatments. Populations were pooled within leaf and stem tissues due to a lack of significance ($P \geq 0.0992$). Each value is presented as the mean of three replications, each comprised of three cells. For treatment composition and properties, see Table 1.

			Root		
Treatment	Leaf	Stem	WM	MQ	EM
1 (Control)	0.12 C	0.17 D	0.23 C a	0.28 BCD a	0.17 D a
2	3.00 B	0.82 C	0.72 B a	0.68 AB a	0.55 BC a
3	4.20 A	1.02 AB	0.80 AB a	0.87 A a	0.73 AB a
4	0.14 C	0.17 D	0.21 C a	0.19 CD a	0.24 CD a
5	0.05 C	0.15 D	0.22 C a	0.15 D b	0.15 D b
6	2.66 B	0.90 BC	0.71 B a	0.59 ABC a	0.93 A a
7	3.83 A	1.09 A	0.97 A a	0.97 A a	0.86 AB a

Within tissues, means followed by different letters indicate a significant difference according to Duncan's Multiple Range Test ($P < 0.05$); uppercase within columns, lowercase within rows.

Table 8. Cl concentration (% of DW) of leaf, stem, and root tissues from the three populations of *L. alyssoides* (WM, MQ, and EM) subjected to various saline irrigation treatments. Populations were pooled within leaf and stem tissues due to a lack significance ($P = 0.3080$). Each value is presented as the mean of three replications, each comprised of three cells. For treatment composition and properties, see Table 1.

			Root		
Treatment	Leaf	Stem	WM	MQ	EM
1 (Control)	0.93 C	0.17 C	0.19 C a	0.20 C a	0.14 C a
2	4.86 B	0.60 A	0.74 A a	0.52 B ab	0.42 B b
3	5.24 AB	0.68 A	0.77 A a	0.82 A a	0.60 A a
4	5.91 A	0.46 B	0.58 B a	0.53 B a	0.48 AB a
5	5.56 AB	0.61 A	0.73 A a	0.48 B b	0.48 AB b
6	0.60 C	0.13 C	0.14 C a	0.13 C a	0.16 C a
7	0.65 C	0.09 C	0.15 C a	0.14 C a	0.13 C a

Within tissues, means followed by different letters indicate a significant difference according to Duncan's Multiple Range Test ($P < 0.05$); uppercase within columns, lowercase within rows.

In general, Na and Cl concentrations were significantly higher in tissues of plants receiving Na or Cl-containing solutions, respectively ($P \leq 0.05$; Tables 7 and 8). There were numerically lower Na concentrations in stems and roots than in leaves for treatments with Na salts (treatments 2, 3, 6, and 7). However, this was not the case for non-Na control and $CaCl_2$ treatments 1, 4, and 5, for which stem and root Na concentrations were numerically higher than in leaves. The latter observation resembles a shoot Na "exclusion" response expressed by salt-sensitive plants (Yeo et al. 1977; Läuchli et al. 1971: Jacoby, 1964), but only under the low-Na conditions in our study (see below for high-Na conditions). Within all treatments, Cl concentrations were lowest in stems and roots, and highest in leaves. For stems and roots, in only a minority of paired comparisons within specific salt treatments at -0.1 MPa and -0.2 MPa (treatments 2–3, 4–5, and 6–7) was there an incremental increase in Na or Cl concentrations.

In leaves, Na and Cl accumulated to high levels in treatments 2, 3, 6, and 7 (Na), and in treatments 2, 3, 4, and 5 (Cl). Leaf Na concentrations were higher in -0.2 MPa Na treatments (3 and 7) than in the -0.1 MP Na treatments (2 and 6) (Table 7). At each osmotic level (-0.1 MPa and -0.2 MPa), leaf Na did not differ between the NaCl and Na_2SO_4 treatments. Unlike leaf Na, leaf Cl concentrations did not differ between the -0.1 MPa and -0.2 MPa NaCl and $CaCl_2$ treatments 2–3 and 4–5, respectively (Table 8).

The leaf Na concentrations in the NaCl and Na_2SO_4 treatments (2.7–4.2%), and the leaf Cl concentrations in the NaCl and $CaCl_2$ treatments (4.9–5.9%), are exceptionally high on agricultural standards in that many crop species would express severe leaf necrosis at even much lower leaf Na and Cl concentrations (Ayers and Westcot, 1985). This was not the case with *L. alyssoides*, even with the high leaf Na and Cl concentrations. At the time of termination, there was no indication of leaf injury in any of the salt treatments. The combined Na and Cl concentrations in leaves of treatment 3 (high NaCl) approached 10% of leaf DW, which is on the order of halophyte concentrations (Miyamoto et al. 1996; Glenn et al. 1994). Most halophytes are ion "includers" and store Na and Cl in leaf vacuoles as energy-efficient osmotica for maintaining turgor pressure and water uptake in high saline conditions (Flowers et al. 1977 and 2015; Greenway and Munns, 1980; Munns and Tester, 2008). If *L. alyssoides* is accumulating such high Na and Cl concentrations in the leaf tissue, still actively growing, and transpiring (as shown), then there is evidence that this plant species has halophyte characteristics. However, high leaf Na and Cl would be deposited to the ground through the annual shedding of leaves as decaying, recalcitrant (high salt) litter, thereby altering the ecosystem by governing the species pool to its own favor and to the detriment of other, salt-sensitive plant species. Francis and Warwick (2007) had this suspicion about "high"-Na litter deposition by the related exotic invasive, *L. latifolium*. They cited Blank and Young (2002), who presented no confirmatory data on high-Na deposition by this species to the levels that we report. By dominating a vegetation community (as observed in Picchioni et al. 2012b), *L.*

alyssoides would be essentially monopolizing the soil water supply at the expense of desired vegetation, and this should be of concern to water and land managers in the semiarid southwest.

Overview of Population and Isosmotic Salt Effects on ET, Growth, and Ion Concentrations

Plants and their progeny become adapted to local habitat characteristics, and evidence is available for "edaphic" (soil-related) ecotypes within a given plant species, such as *L. alyssoides* in our case (Epstein and Bloom, 2005 and references cited within). In our study, there was little or no population effect on plant ET and growth, and the Na and Cl concentrations of the three populations, within each tissue and salt treatment, were broadly similar. Lack of population effect may be due to the relatively small geographic range of the population sites, although the WM site had a sodic soil while the soils at the EM and MQ sites were non-sodic. There was no evidence to suggest that the WM population was "preconditioned," or had a greater "fitness" to perform best in any of the high SAR treatments 2, 3, 6, and 7. In field conditions (Picchioni et al. 2012b), the WM population aggressively colonized a site where shallow depth soil saturation extract SAR increased from 15 to 35 over a 3–yr period, essentially becoming a monotypic stand that replaced six other indigenous herbaceous species. However, as shown by the EM and MQ population characteristics—soils, plant ET, and plant growth—*L. alyssoides* appears to be an adaptive and resilient species with respect to soil sodicity, and may aggressively colonize both sodic and non-sodic sites. That is, this species may alter vegetation diversity and the soil water supply under a wide range of soil sodicity and, thus, over potentially wide geographic areas.

Comparisons between isosmotic treatment solutions of different salts may reveal the relative importance of adverse water relations (osmotic effects) and toxic effects of ion excess, particularly Na and Cl (Greenway and Munns, 1980). Isosmotic salt experimental designs are not without pitfalls, because at high salinity, delineation of osmotic and specific ion effects may not always be clear (Munns and Tester, 2008). Nevertheless, with a few exceptions of marginal but significant high-Na salt treatment effects mentioned previously, our data suggest that plant ET and growth were largely controlled by the osmotic potential of these different treatment solutions. Comparing the high NaCl solution treatment 3 with its counterpart isosmotic solution treatments 5 and 7, there were no additional ET or growth suppressions with leaf Na plus Cl concentrations (additive plant stresses in treatment 3) reaching 9–10% of dry weight (Tables 5–8). Even after pooling the data across the three populations to triple the number of observations and statistical power of the mean separations, we were simply unable to detect any more leaf mortality or any more of a decline in leaf dry weight, root dry weight, or TDW in treatment 3 than in treatments 5 and 7 (Table 5), the latter of which did not nearly result in such high combined leaf Na and Cl concentrations as 9–10% in treatment 3. The exceptionally high salt accumulation raises striking possibilities for how this species may manage its leaf Na and Cl through cellular compartmentation

processes (Harvey et al. 1976; Zhang et al. 2001), use these electrolytes beneficially for the maintenance of turgor (Flowers et al. 1977), deplete the soil water supply, deposit recalcitrant litter to the ground, and effectively mandate the conditions over a vegetation site to favor its own existence at the expense of other species.

Objective 2: *Lepidium alyssoides, L. draba,* and *L. latifolium* Plant Salinity Responses

Soil and Vegetation Analysis of the Seed Collection Sites

Soils from the *L. alyssoides* (MQ) population site (reported and discussed in Objective 1) and the *L. latifolium* collection site had low saturation percentages ranging from ≈16–18, and low organic matter, from 0.8–1.2% (Table 9), and both soils were a sand. Soil from the *L. draba* seed collection site had a higher saturation percentage of 29 and around twice the organic matter (2.2%) as compared with the other two soils, and was a loam. None of the three soils were saline with soil saturation extract ECs ranging from 1.6–2.4 dS m^{-1}, and Cl from 4.1–6.5 meq L^{-1}. The soils were slightly basic with pH ranging from 7.2–7.4, and non-sodic with soil saturation extract SAR no higher than about 2. The *L. draba* collection site was the most fertile with NO_3-N, Olsen-P, and soluble K concentrations moderate to sufficient, while at the other two sites, low to moderate on most agricultural crop standards (personal communication, R.P. Flynn, 2015). The aboveground vegetation of all three *Lepidium* spp. had Na and Cl concentrations ranging from 0.02–0.08%, and from 0.23–0.56% of dry weight, respectively (data not shown).

Table 9. Soil characteristics of the seed collection sites of *L. alyssoides*, *L. draba*, and *L. latifolium*. The pH, EC, SAR, and Cl were determined in the soil saturation extract.

		EC		Cl			mg kg^{-1}		
Species	pH	(dS m^{-1})[z]	SAR[y]	(meq L^{-1})	SP (%)[x]	OM (%)[w]	NO_3-N	Soluble K	Olsen-P
L. alyssoides[v]	7.2	1.6	1.7	4.1	15.8	1.2	17.3	76.5	9.4
L. draba	7.2	2.4	1.1	6.5	29.1	2.2	24.1	155.0	20.9
L. latifolium	7.4	2.1	1.0	5.3	18.1	0.8	5.7	107.0	12.3

[z]Electrical conductivity.

[y]Sodium adsorption ratio.

[x]Saturation percentage.

[w]Organic matter.

[v]For convenience, copied from Table 2 (Objective 1), MQ population.

Results and Discussion Pertaining to Three Different Experiments

The reader is reminded that in this objective, three separate experiments were conducted, all in similar experimental conditions and in the same greenhouse, but at different times during 2014 (please see Objective 2 materials and methods for detail). The three experiments involved the following plant species: 1) three *Lepidium* spp., 2) bean, and 3) cotton. In cases of response variables involving all three experiments, we will discuss the results on *Lepidium* spp. first since they were the primary focus of our project.

Leachate Characteristics throughout Experimental Durations

Lepidium spp. Throughout the study, leaching fractions (LF) ranged from ≈37–44% across all treatments and species (Table 10). All treatments received equivalent LFs throughout the study, in contrast to Objective 1 (see previously reported data). That difference may be attributed to the irrigation scheduling adjustment in Objective 2 reported earlier.

Table 10. Leachate characteristics of the saline irrigation treatments from *L. alyssoides*, *L. draba*, and *L. latifolium*. Each value is presented as the mean ± s.d. of six biweekly measurements taken throughout the study. Each measurement was the average of three replications, each comprised of three cells. For treatment composition and properties, see Table 1.

	L. alyssoides		*L. draba*		*L. latifolium*	
Treatment	EC (dS m^{-1})[z]	LF (%)[y]	EC (dS m^{-1})	LF (%)	EC (dS m^{-1})	LF (%)
1 (Control)	2.2 ± 0.4	42.8 ± 8.6	2.1 ± 0.3	42.1 ± 3.5	2.2 ± 0.3	44.1 ± 3.6
2	8.4 ± 1.0	39.7 ± 8.5	8.5 ± 0.7	37.1 ± 5.6	8.3 ± 0.8	41.6 ± 3.4
3	13.4 ± 2.1	44.0 ± 8.9	12.6 ± 1.9	42.4 ± 8.3	13.5 ± 1.5	41.4 ± 4.4

[z]Electrical conductivity.

[y]Leaching fraction.

Maintenance of a steady-state salt balance during this study is indicated by the minimal variation in leachate EC per treatment (Table 10). Moreover, the leachate ECs for *L. alyssoides* in saline treatments 2 and 3 of Objectives 1 and 2 were within 2% of one another, reflecting high reproducibility in this experimental system. Within each treatment, leachate EC varied little (if at all) between the species. The leachate EC was lowest in the non-saline control treatment (2.1–2.2 dS m^{-1}). Leachate EC was moderately high in treatment 2 (NaCl at -0.1 MPa) and ranged from 8.3–8.5 dS m^{-1}. Leachate EC increased a step higher with the -0.2 MPa saline treatment, to 12.6–13.5 dS m^{-1}. Averaged across the species, the leachate EC of treatments 2 and 3 correspond to saturation extract salinities of ≈6–10 dS m^{-1} given the relationship between leachate ("PourThru") EC and soil medium saturation extract EC, as

explained in Objective 1. Growth, yield, and even survival of many crop species are severely restricted at this level of salinity (Ayers and Westcot, 1985). For the *Lepidium* spp., high salinity was necessary for establishing a range of growth responses so as to provide scientifically credible salt tolerance information, which is heretofore lacking.

Bean. The LFs ranged from 44–54% for all treatments throughout the study (data not shown). These values were slightly higher than those for *Lepidium*, resulting in lower leachate ECs that, when averaged across six measurements spaced 7–11 days apart, were 1.9 $\pm$ 0.1, 8.0 $\pm$ 1.2, and 10.6 $\pm$ 2.2 dS m^{-1} in treatments 1, 2, and 3, respectively. These averages were 80–96% of those for the three-species *Lepidium* averages. For treatments 1 and 2, (control and NaCl at -0.1 MPa, respectively), leachate EC varied by less than 15% from those of *Lepidium* at analogous treatments (see footnote of Table 16). The average LF for treatment 3 on bean (NaCl at -0.2 MPa) was relatively high (54%) which, in turn, produced a leachate EC that averaged 20% lower than the *Lepidium* treatment 3 average. Therefore, caution is raised on the bean ET and growth responses in treatment 3 (discussed below), specifically that bean salt tolerance may be overestimated when compared to *Lepidium* ET and growth response in treatment 3. We will focus our growth comparisons of bean and *Lepidium* on treatment 2 because of the nearly identical leachate ECs recorded for these independent experiments.

Cotton. The LFs ranged from 40–48% for all treatments throughout the study (data not shown). Leachate ECs averaged across two measurements taken 2–3 weeks apart were 2.2 $\pm$ 0.1, 8.1 $\pm$ 0.4, and 16.4 $\pm$ 0.3 dS m^{-1} for treatments 1, 2, and 3, respectively. For treatments 1 and 2 (control and NaCl at -0.1 MPa, respectively), cotton leachate ECs varied by less than 4% from those of the three-species *Lepidium* averages. However, treatment 3 leachate EC (NaCl at -0.2 MPa) was 24% higher than that of the three-species *Lepidium* average. The high cotton leachate EC in treatment 3 corresponded the lowest LF of that experiment (40%). Therefore, caution is necessary for the ET and growth responses of cotton at the treatment 3 level, namely that cotton salt tolerance may be underestimated when compared to treatment 3 responses of *Lepidium*. Similar to the situation with bean, we will primarily make relative comparisons between cotton and *Lepidium* spp. at the treatment 2 level for which the average leachate ECs in both of these separate experiments were nearly identical.

Growth Index (GI) throughout Study Duration

For *L. alyssoides*, GI in all treatments generally increased throughout most of the study and appeared to plateau during the final two weeks (Fig. 5). There was high variability in *L. alyssoides* GI and thus no apparent distinction between the different treatments. By contrast, GI for both *L. draba* and *L. latifolium* appeared to trend according to treatment solution osmotic potential, with the controls (treatment 1) having the highest GI, and step-wise reductions in saline treatments 2 and 3 (NaCl at -0.1 MPa and -0.2 MPa,

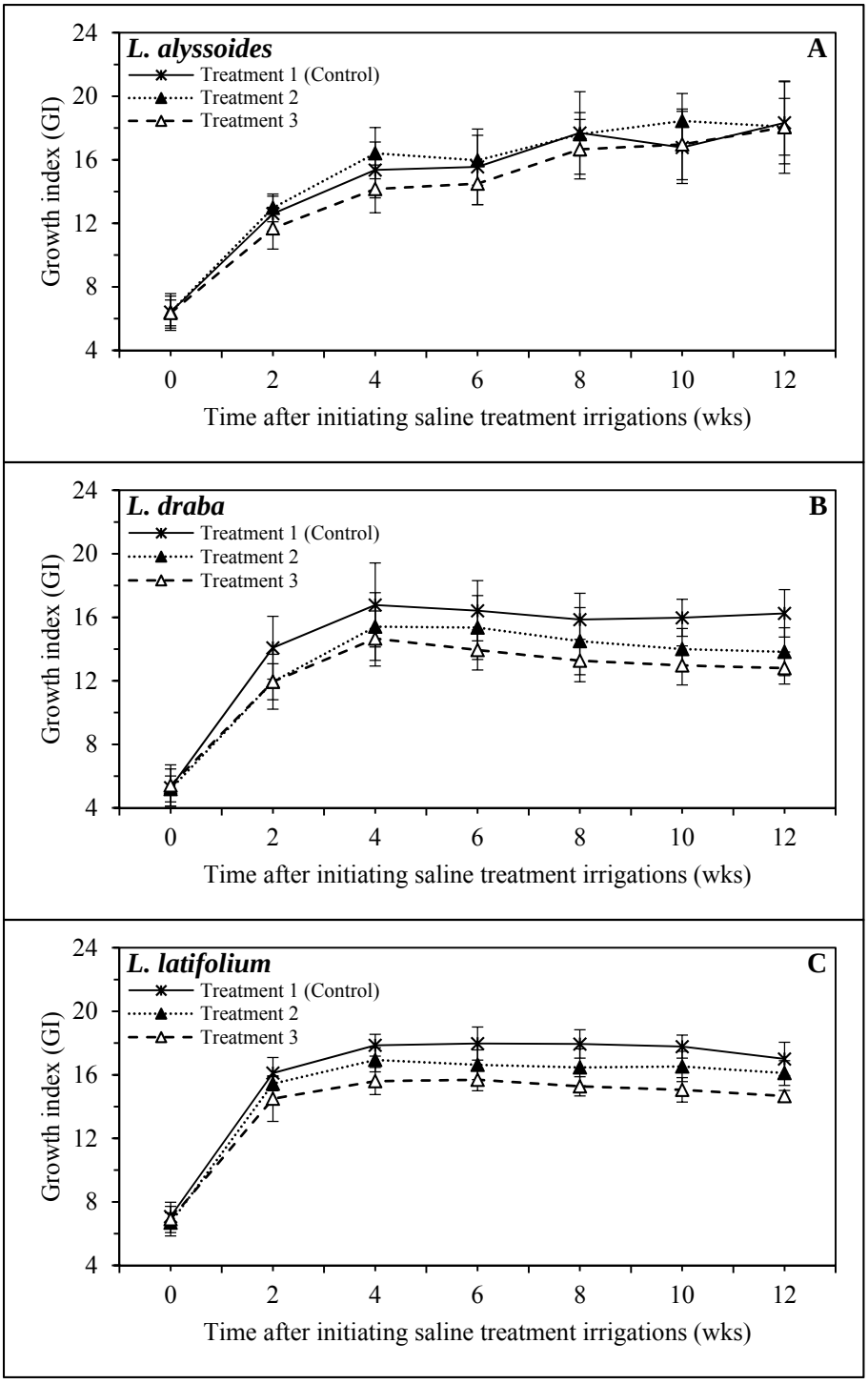

Figure 5. Biweekly growth index (GI) under saline irrigation treatments of *L. alyssoides* (A), *L. draba* (B), and *L. latifolium* (C). Each point is presented as the mean $\pm$ s.d. of three replications, each comprised of three cells. For treatment composition and properties, see Table 1.

respectively) that were apparent as early as 2–4 weeks. Both *L. draba* and *L. latifolium* expressed a limit in GI as early as 4 weeks which may be attributed to the limited size of the growing cells. During the first 2 weeks, GI of *L. latifolium* appeared to be the most rapid of the three species. Relatively high initial growth rate during seedling establishment, even under saline conditions, could be a contributing factor to aggressive invasions by *L. latifolium*. The relatively minimal variability (small standard deviations) for the individual GI means of *L. latifolium* is noteworthy in light of Gaskin and others (2013), who stated that there is low genetic diversity within North American introduced populations of this species.

Evapotranspiration (ET): Cumulative and Final Total

Lepidium spp. For each species and treatment, weekly cumulative ET increased steadily throughout the study (Fig. 6), and the curves had a sigmoidal pattern that, for the *L. alyssoides* MQ population, differed from Objective 1 (compare Fig. 6A to Fig. 4B). In the present Objective 2 (Fig. 6), the control plants had the highest ET throughout the study, with incremental effects of salinity (-0.1 MPa, -0.2 MPa) appearing at about 6–8 weeks, much like in Objective 1 (Fig. 4). For the final total ET (Table 11), there was no significant interaction between treatment and species ($P = 0.9123$) but highly significant treatment and species main effects ($P = 0.0200$ and $P < 0.0001$, respectively). *Lepidium latifolium* had the highest final total ET in all three treatments and *L. alyssoides* had the lowest, with *L. draba* intermediate. As with weekly cumulative ET, incremental effects of salinity (-0.1 MPa and -0.2 MPa) were also observed in the final total ET, although for *L. draba*, only between treatments 1 and 3 (control and NaCl at -0.2 MPa).

Bean. Weekly cumulative ET was highest for the control plants and steadily increased during the study (Fig. 7). Salinity of treatments 2 and 3 reduced ET within 2 weeks. Subsequently, ET in treatments 2 and 3 progressively diverged from that of the control treatment up to termination, at which point there was a substantial difference between the control and treatment 2, followed by a smaller incremental reduction between treatments 2 and 3. Cumulative ET of treatment 3 plants began to subside during the final two weeks, during which time we observed severe shoot necrosis and dieback in this treatment.

Cotton. The cotton experiment required only 5 weeks to reach 500 GDD as compared with 6.5–7 weeks for the *Lepidium* spp. and bean experiments. The shorter cotton duration was under warmer conditions (higher average greenhouse temperatures) of early to mid-summer, as compared with the cooler conditions of early spring to early summer for *Lepidium*, and winter to early spring for bean. Like bean, weekly cumulative ET of cotton was highest for the non-saline control plants and their ET steadily increased throughout the study (Fig. 8). Salt-suppressive effects of treatments 2 and 3 became evident as early as 2 weeks and those effects progressively increased throughout the remainder of the study. By 5 weeks (500 GDD), there was a relatively large ET reduction in treatment 2 below the control, but only a marginally incremental reduction at treatment 3. In contrast to bean, cumulative cotton ET of treatment 3

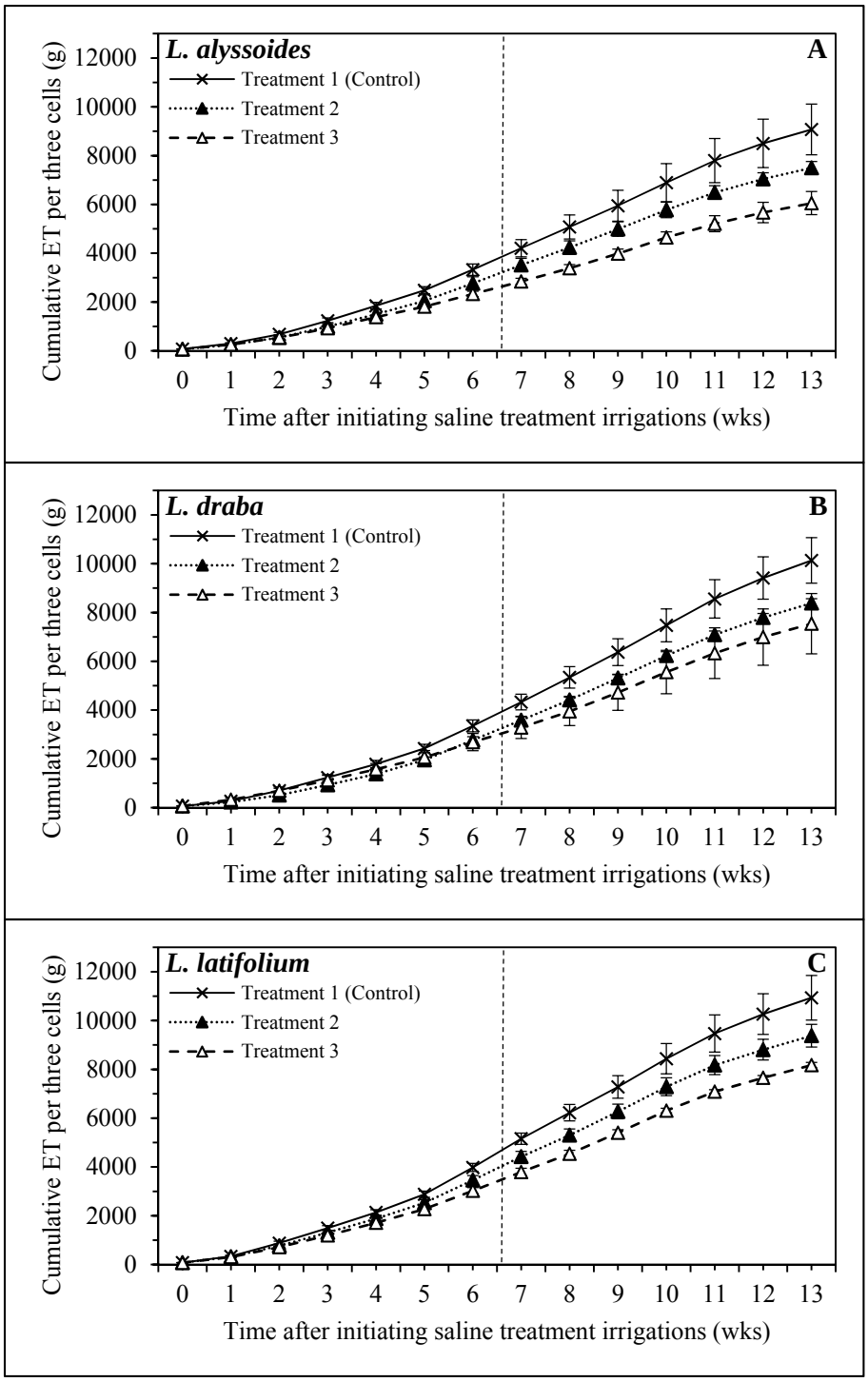

Figure 6. Weekly cumulative evapotranspiration (ET) under saline irrigation treatments of *L. alyssoides* (A), *L. draba* (B), and *L. latifolium* (C). Each point is presented as the mean $\pm$ s.d. of three replications, each comprised of three cells. The vertical dashed line represents the time at which approximately 500 growing degree days (GDD, heat units) were accumulated, for equal comparisons to bean and cotton. Further treatment details in Table 1.

Table 11. Final total evapotranspiration (ET) under saline irrigation treatments of *L. alyssoides*, *L. draba*, and *L. latifolium*, in grams per three cells. Each value is presented as the average of three replications, each comprised of three cells. For treatment composition and properties, see Table 1.

Treatment	*L. alyssoides*	*L. draba*	*L. latifolium*
1 (Control)	9073 A b	10133 A ab	10935 A a
2	7500 B c	8387 AB b	9378 B a
3	6056 C b	7543 B ab	8174 C a

Means followed by different letters indicate a significant difference according to Duncan's Multiple Range Test ($P < 0.05$); uppercase within columns, lowercase within rows.

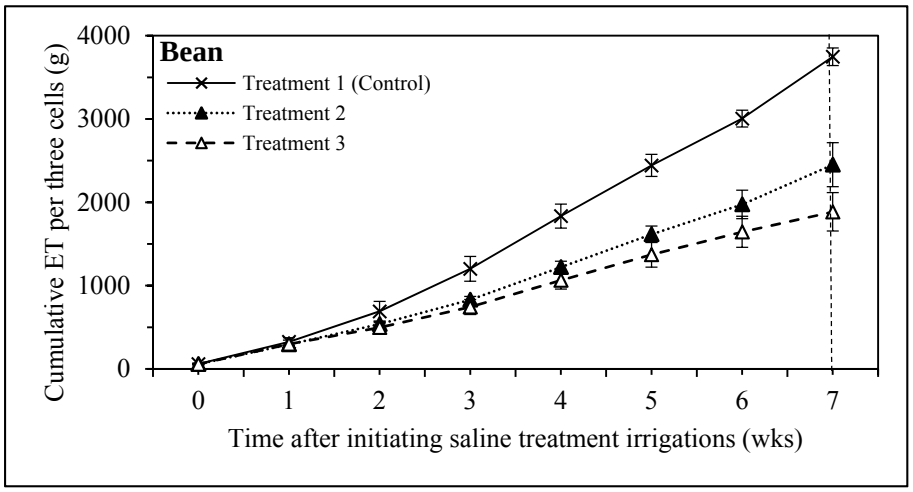

Figure 7. Weekly cumulative evapotranspiration (ET) of bean under saline irrigation treatments. Each point is presented as the mean ± s.d. of three replications, each comprised of three cells. The vertical dashed line represents the time at which approximately 500 growing degree days (GDD, heat units) were accumulated, for equal comparisons to *Lepidium* and cotton. Treatment details in Table 1.

did not subside or "tail-off" even after 500 GDD at 6 weeks, and there was no shoot necrosis at all in treatment 3.

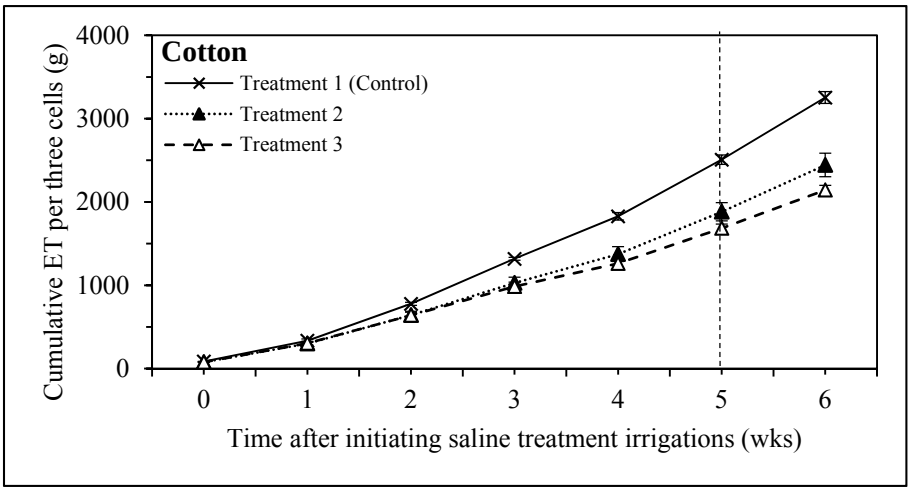

Figure 8. Weekly cumulative evapotranspiration (ET) of cotton under saline irrigation treatments. Each point is presented as the mean ± s.d. of three replications, each comprised of three cells. The vertical dashed line represents the time at which approximately 500 growing degree days (GDD, heat units) were accumulated, for equal comparisons to *Lepidium* and bean. Treatment details in Table 1.

Bean, Cotton, and *Lepidium* Cumulative ET Comparisons at 500 GDD. There was a significant treatment main effect on the total cumulative bean and cotton ET at 500 GDD ($P = 0.0022$ and 0.0007, respectively). Total bean and cotton ET in treatments 2 and 3 were significantly lower than that of their controls (Table 12). However, in either species, total ET in treatments 2 and 3 did not differ significantly, reflecting the relatively small incremental suppressions that were visually noted in Figs. 7–8. The lack of incremental salt effect in treatment 3 can be traced back to most of the experimental durations when there were relatively small accrued water deficits (the area between treatment 2 and 3 curves in Figs. 7–8), especially for cotton. In marked contrast, the significant salt suppression of treatment 2 (Table 12) resulted from the much larger accrued water deficits (the area between treatment 1 and 2 curves in Figs. 7–8).

Considering the ET at 500 GDD for all species and experiments together (*Lepidium*, bean, and cotton), we expressed the cumulative treatment 2 and 3 ET averages as percentages of the applicable species control treatment averages. As expected, bean ranked the lowest in both treatments 2 and 3 (65% and 50% of its control, respectively). Cotton ranked in the middle (75% and 67% of its control, respectively), and the *Lepidium* spp. ranked the highest (83–86%, and 69–78% of their controls, respectively). Restricting the relative ET comparisons to treatment 2 (rationale noted previously), the ability of the *Lepidium* spp. to maintain water uptake under saline conditions exceeded that of even

salt-tolerant cotton under similar experimental conditions.

Table 12. Total evapotranspiration (ET) at approximately 500 growing degree days (GDD, heat units) of bean and cotton under saline irrigation treatments, from independent experiments. Each value is presented as the average of three replications, each comprised of three cells. For treatment composition and properties, see Table 1.

Treatment	Bean	Cotton
1 (Control)	3747 A	2508 A
2	2451 B	1883 B
3	1886 B	1685 B

For each species (within columns), means followed by different letters indicate a significant difference according to Duncan's Multiple Range Test ($P < 0.05$). Species statistically tested independent of each other.

Dry Weight (DW) of Leaf, Stem, and Root Tissues, and Total Plant DW (TDW)

Lepidium spp. At the initiation of saline irrigation, the total root DW ranged from 0.21–0.51 g per three seedlings, and the total shoot DW (leaves plus stems) ranged from 0.36–0.43 g per three seedlings across the three species. At termination of the study, there were significant main effects of treatment and species on leaf DW, root DW, and TDW ($P \leq 0.0228$). However, only the species main effect was significant for stem DW ($P < 0.0001$). Species interacted with the treatment on stem and root DW, and on TDW ($P \leq 0.0421$), but not on leaf DW ($P = 0.6514$).

Leaf DW of all species was highest in the control treatment (Table 13). There were no declines in leaf DW of *L. alyssoides* and *L. draba* from treatment 2 to treatment 3. However, there was a reduction in leaf DW for *L. latifolium* from treatment 2 to 3. Although leaf DW among the species did not differ at treatments 2 and 3, *L. draba* had the highest leaf DW in the control treatment.

On average, stems comprised only 3–10% of TDW, depending on species (Table 13). Regardless of the treatment, *L. draba* had the lowest stem DW of all species. The significant treatment x species interaction on stem DW mentioned previously was largely the result of an increase in stem DW of *L. latifolium* and a decrease in stem DW of *L. alyssoides*, from treatment 1 to treatment 2.

At termination, essentially all of the 3-cell experimental units had rhizomes of various numbers and at various stages of budding within the sand medium or rising above the sand surface (data not shown). In addition, rhizomes of *L. latifolium* were substantially thickened, and this was reflected in the highest "root" DW of all the species (Table 13). Even before termination of the study, there were numerous cells of *L. latifolium* that had begun to split longitudinally, which provided us with a visual

Table 13. Leaf, stem, and root tissue dry weight and total dry weight (TDW) of *L. alyssoides*, *L. draba*, and *L. latifolium*, in grams per three cells. For treatment composition and properties, see Table 1.

	Leaf			Stem			Root			TDW		
Treatment	*L. alyssoides*	*L. draba*	*L. latifolium*	*L. alyssoides*	*L. draba*	*L. latifolium*	*L. alyssoides*	*L. draba*	*L. latifolium*	*L. alyssoides*	*L. draba*	*L. latifolium*
1 (Control)	12.35 A ab	14.10 A a	11.54 A b	2.58 a	0.66 b	1.98 a	10.12 A c	19.13 A b	30.67 A a	25.05 A c	33.89 A b	44.20 A a
2	11.53 AB a	11.05 B a	10.50 B a	2.06 b	0.92 c	3.10 a	8.44 A b	12.32 B b	21.89 B a	22.03 A b	24.30 B b	35.49 B a
3	9.53 B a	10.10 B a	9.07 C a	1.77 ab	0.80 b	2.77 a	5.02 B b	8.98 B b	18.80 C a	16.32 B c	19.88 C b	30.64 C a

Within tissues, means followed by different letters indicate a significant difference according to Duncan's Multiple Range Test ($P < 0.05$); uppercase within columns, lowercase within rows.

analogy of its "propagule pressure" (photos available from authors). Across all treatments, *L. latifolium* "root" DW comprised 66% of TDW, whereas "root" DW of *L. draba* averaged 50% of TDM, and "root" DW of *L. alyssoides* averaged only 38% of TDW.

Root DW of *L. draba* and *L. latifolium* declined at the treatment 2 salinity level, whereas that of *L. alyssoides* declined only at the highest salinity level of treatment 3, leading to the treatment x species interaction noted above. In treatment 3, there was an additional decline in *L. latifolium* root DW but not in *L. draba* root DW, which also contributed to the interaction.

The control treatment provided the highest TDW for all species, with incremental salinity effects at -0.1 MPa and -0.2 MPa that were lacking only at -0.1 MPa for *L. alyssoides* (Table 13), leading to the treatment x species interaction reported previously. For all treatments, *L. latifolium* had the highest TDW, while *L. alyssoides* had the lowest TDW except at the treatment 2 salinity level, within which TDW of *L. alyssoides* did not differ from that of *L. draba*.

Bean. At the start of saline irrigation, the total root DW averaged 0.54 g per three seedlings, and the total shoot DW (leaves plus stems) averaged 0.55 g per three seedlings. By the end of the study, numerous bean plants had formed immature fruiting pods that were harvested and weighed along with the other tissues. At this time, there was a significant treatment effect on leaf, stem, and root DW, and on TDW ($P \leq 0.0119$) (Table 14). However, there was no significant treatment effect on fruit DW ($P = 0.2523$) even though the numerical averages declined with salinity level. Fruit DW was highly variable in treatments 1 and 2. Disregarding fruit, the DWs were significantly highest in the control treatment with incremental reductions in saline treatments 2 and 3, except for stem DW that declined only at treatment 2.

Table 14. Fruit, leaf, stem, and root tissue dry weight, and total dry weight (TDW) of bean in grams per three cells. For treatment composition and properties, see Table 1.

Treatment	Fruit	Leaf	Stem	Root	TDW
1 (Control)	3.90	5.88 A	3.26 A	3.21 A	16.24 A
2	2.96	3.47 B	1.59 B	1.85 B	9.88 B
3	0.38	2.00 C	0.80 B	0.64 C	3.81 C

Within tissues (columns), means followed by different letters indicate a significant difference according to Duncan's Multiple Range Test ($P < 0.05$).

Cotton. At the start of saline irrigation, the total root DW averaged 0.39 g per three seedlings, and the total shoot DW (leaves plus stems) averaged 1.00 g per three seedlings. By the time this experiment was terminated, there were significant treatment effects on leaf and stem DW, and on TDW ($P \leq 0.0110$), but

not on root DW ($P = 0.0889$) (Table 15). Aside from the roots, the DWs were highest in the controls, declined with treatment 2, but declined no further with highest salinity treatment 3.

Table 15. Leaf, stem, and root tissue dry weight, and total dry weight (TDW) of cotton in grams per three cells. For treatment composition and properties, see Table 1.

Treatment	Leaf	Stem	Root	TDW
1 (Control)	6.02 A	5.21 A	3.45	14.67 A
2	4.99 B	3.52 B	2.84	11.36 B
3	4.53 B	3.31 B	2.36	10.19 B

Within tissues (columns), means followed by different letters indicate a significant difference according to Duncan's Multiple Range Test ($P < 0.05$).

Bean, Cotton, and *Lepidium* Dry Matter Production Comparisons. For the destructive harvest at termination of the Objective 2 experiments, the TDW as percentage of control (treatment 1) is presented for each species in Table 16. The foregoing discussion is only broadly based on the numerical averages in three independent experiments under similar conditions but at different times during 2014. Thus, statistical analyses were not appropriate. In saline treatment 2, TDW as a percentage of control was lowest in salt-sensitive bean and considerably higher in salt-tolerant cotton, as expected. Bearing in mind the focus of this discussion—saline treatment 2—and considering all of the *Lepidium* spp. in that treatment, their TDW as a percentage of control was more similar to that of cotton (treatment 2) than it was to bean (treatment 2), and in the cases of *L. alyssoides* and *L. latifolium*, even somewhat higher than that of cotton. For saline treatment 3, a similar trend was observed in that TDW as a percentage of control was substantially reduced in bean but was only marginally reduced in cotton, again consistent with the differential salt tolerance of those crop species. For all three *Lepidium* spp., TDW in treatment 3 (percent of control) declined similar to cotton. Of the three *Lepidium* spp., *L. draba* had the lowest relative TDW for both saline treatments 2 and 3, but it was still much higher than that of bean.

It is important to reiterate some important facts pertaining to the leachate salinities recorded in these three experiments. The average leachate EC for all species during their respective experiments was markedly similar in both the control and saline solution 2 treatments (see Table 10 and the footnote in Table 16). That is, across all three experiments, leachate salinity of the controls ranged from 1.9–2.2 dS m^{-1}, and for saline solution 2 treatment, leachate salinity ranged from 8.0–8.4 dS m^{-1} (highest in *Lepidium* spp. averaged across those three species). However, for saline treatment 3, the average leachate EC for bean (10.6 dS m^{-1}) was lower, and the average leachate EC for cotton (16.4 dS m^{-1}) was higher than that of the *Lepidium* spp. (13.2 dS m^{-1} averaged across the three species). This may have caused the salt

tolerance to have been overestimated for bean and underestimated for cotton, for this specific treatment. Thus, TDW comparisons in treatment 3 as a percentage of controls should be made with caution.

Table 16. Total plant dry weight (TDW) as percent of control (Treatment 1) for *L. alyssoides*, *L. draba*, *L. latifolium*, bean, and cotton. For treatment composition and properties, see Table 1.[z]

Treatment	*L. alyssoides*	*L. draba*	*L. latifolium*	Bean	Cotton
1 (Control)	100	100	100	100	100
2	88	72	80	61	77
3	65	59	69	23	69

[z]For average leachate ECs of *Lepidium* spp., see Table 10. For bean, the average leachate ECs for treatments 1, 2, and 3, respectively, were: 1.9 $\pm$ 0.1, 8.0 $\pm$ 1.2, 10.6 $\pm$ 2.2 dS m^{-1} (averaged across six measurements, taken at 7 to 11-day intervals. For cotton, the respective leachate EC averages were: 2.2 $\pm$ 0.1, 8.1 $\pm$ 0.4, and 16.4 $\pm$ 0.3 dS m^{-1} (averaged across two measurements, taken at 2 to 3-week intervals).

We were somewhat surprised to find that for cotton in saline solution treatment 2, our greenhouse conditions produced a marked growth suppression at a salinity level that was probably lower than the published cotton salinity threshold at 7.7 dS m^{-1} in a field's soil saturation extract, which seems to be the accepted threshold for this crop species over the many years (Maas and Hoffman, 1977). Given the relationship between leachate ("PourThru") EC and the EC in the medium saturation extract of pot studies that was previously discussed, the average leachate EC of cotton in saline solution 2 (8.1 dS m^{-1}) would correspond to a saturation extract salinity of ≈6.2 dS m^{-1}. We would expect higher salt tolerance thresholds under the relatively low greenhouse evaporative demand conditions than we would under outdoor semiarid conditions with the lower relative humidity that has long been known to accentuate salinity effects on plant growth and yield (Greenway and Munns, 1980; Bernstein, 1975). Additionally, in the outdoor semiarid environment, the higher daytime temperatures, higher light intensity, and dry winds should be expected to further exacerbate salinity stress. In nearby semiarid El Paso, TX, plant salt tolerance in greenhouse conditions may be as much as two to three times higher than that in outdoor saline lysimeter conditions (personal communication, S. Miyamoto, 1989). Our greenhouse conditions provided a fair and realistic assessment of cotton salt tolerance, which was an important consideration for providing credible information on *Lepidium* salt tolerance under protected cultivation. The findings should encourage others to raise the level of related vegetation science reporting beyond casual and anecdotal observations, since as we show here with hard facts, these *Lepidium* spp. are indeed salt tolerant on agricultural crop terms.

Na and Cl Concentrations in Leaf, Stem, and Root Tissues

There were no significant treatment x species interactions on Na or Cl concentrations in leaves, stems, and roots ($P \geq 0.1054$), except for leaf Na concentration ($P = 0.0237$) and for stem Cl concentration ($P = 0.0180$). The latter interactions were largely attributed to the greater magnitude of increases in *L. draba* leaf Na and stem Cl concentrations from treatment 1 to 2, as compared to smaller increases in leaf Na and stem Cl in *L. alyssoides* and *L. latifolium* from treatment 1 to 2. There were significant main effects of treatment and species for Na and Cl concentrations in all tissues ($P \leq 0.0052$), except that for stem Na concentration, there was no significant species main effect ($P = 0.1593$).

For each of the *Lepidium* spp. and in all of their tissues, Na and Cl concentrations increased significantly with increasing salinity, although the effect was not additive across the treatment levels for all species x tissue combinations ($P \leq 0.05$; Tables 17 and 18). In the non-saline control treatment, this *L. alyssoides* population (MQ) had numerically higher root and stem Na concentrations than leaf Na concentration as previously reported in Objective 1. In the very same treatment, however, the leaf Na concentrations of *L. draba* and *L. latifolium* (four to five times those of *L. alyssoides*) exceeded their own root and stem Na concentrations by two to seven times (Table 17). Further, in treatment solution 2, *L. latifolium* had the lowest root Na concentration of all species. Roots and stems are known to serve as Na retention organs, thus limiting Na transport to leaves (Picchioni et al. 1990). While these findings may indicate differential Na exclusion properties between these species in low-saline conditions (see Objective 1 for further discussion), additional research is needed to separate underground tissues in these *Lepidium* spp. in order to discern the relative importance of true roots and rhizomes in Na retention.

In both saline treatments 2 and 3 and all species, Na concentrations were lower in stems and roots than in leaves. With increasing salinity, stem Na concentrations increased more noticeably than in roots, reaching or exceeding 1% of DW. Considering the Na concentrations and DW distribution across the tissues of these three species, leaves became the primary Na accumulation site as salinity was increased. *Lepidium draba* and *L. latifolium* had higher leaf Na concentrations than did *L. alyssoides* in the salinized treatments, although the leaf Na concentration of *L. alyssoides* MQ in treatment 3 of the present study ($2.6 \pm 0.5\%$) was lower than it was in Objective 1 ($4.1 \pm 0.1\%$ in treatment 3). Perhaps a larger picture to emerge from this study is that the leaf Na concentrations reported in Table 17, particularly in *L. draba* and *L. latifolium* (4.5–5.5%), are extremely high on agricultural crop standards, as discussed in Objective 1.

For all species and in all treatments, Cl concentrations were higher in leaves than in stems and roots (Table 18). Even in the control treatment, leaf Cl ranged from 1.1–1.6%, which is high when considering the fact that the sole Cl source in that treatment (tap water) was only at 0.5 meq L^{-1}. Compared to *L. draba* and *L. latifolium*, *L. alyssoides* tended to have low stem Cl across all treatments and low root and leaf Cl in treatments 1 and 2. As salinity was increased for *L. draba* and *L. latifolium*,

Table 17. Na concentration (% of DW) of leaf, stem, and root tissues from *L. alyssoides*, *L. draba*, and *L. latifolium* subjected to saline irrigation treatments. Each value is presentred as the mean of three replications, each comprised of three cells. For treatment composition and properties, see Table 1.

	Leaf			Stem			Root		
Treatment	*L. alyssoides*	*L. draba*	*L. latifolium*	*L. alyssoides*	*L. draba*	*L. latifolium*	*L. alyssoides*	*L. draba*	*L. latifolium*
1 (Control)	0.13 B c	0.50 B b	0.66 C a	0.16 B	0.10 B	0.10 B	0.23 B a	0.22 A a	0.13 C a
2	2.06 A b	3.95 A a	3.12 B a	0.63 A	1.08 AB	0.77 A	0.54 A a	0.60 A a	0.30 B b
3	2.63 A b	5.52 A a	4.45 A ab	1.03 A	1.57 A	1.04 A	0.65 A a	0.66 B a	0.42 A a

Within tissues, means followed by different letters indicate a significant difference according to Duncan's Multiple Range Test ($P < 0.05$); uppercase within columns, lowercase within rows.

Table 18. Cl concentration (% of DW) of leaf, stem, and root tissues from *L. alyssoides*, *L. draba*, and *L. latifolium* subjected to saline irrigation treatments. Each value is presented as the mean of three replications, each comprised of three cells. For treatment composition and properties, see Table 1.

	Leaf			Stem			Root		
Treatment	*L. alyssoides*	*L. draba*	*L. latifolium*	*L. alyssoides*	*L. draba*	*L. latifolium*	*L. alyssoides*	*L. draba*	*L. latifolium*
1 (Control)	1.10 B c	1.63 B a	1.42 C b	0.11 C c	0.45 B b	0.66 B a	0.13 C c	0.23 B b	0.44 C a
2	3.85 A b	5.84 A a	3.56 B b	0.40 B c	1.94 A a	1.35 A b	0.43 B b	0.71 A a	0.57 B ab
3	4.52 A a	7.38 A a	4.99 A a	0.68 A b	2.83 A a	1.65 A ab	0.72 A a	0.96 A a	0.81 A a

Within tissues, means followed by different letters indicate a significant difference according to Duncan's Multiple Range Test ($P < 0.05$); uppercase within columns, lowercase within rows.

stem Cl concentrations increased more conspicuously than in roots, and reached as high as 2.8%. Conversely, *L. alyssoides* did not express such large increases in stem Cl as salinity was increased, neither in the present study (Table 18) nor in Objective 1 (Table 8). An important consequence for this noted species difference in stem Cl concentrations may be that, in addition to leaves (discussed previously), stems of *L. draba* and *L. latifolium* are also providing a "potent," recalcitrant (high-Cl) litter as they abscise and fall to the ground. That stem trait may further contribute to the invasibility of the latter two species and allow them to be "heavy-duty" ecosystem "engineers," more so than *L. alyssoides*.

As with Na, leaves of all species appeared to become the dominant Cl accumulation center in the plant with increasing salinity. For saline treatments 2 and 3 of the present study, the leaf Cl concentrations in *L. alyssoides* MQ (3.9 $\pm$ 0.5% and 4.5 $\pm$ 1.1%, respectively) were similar to those of Objective 1 (4.7 $\pm$ 1.2% and 5.0 $\pm$ 0.0%, respectively). Like Na, the leaf Cl concentrations reported in Table 18 (3.6–7.4%) greatly exceed the tolerance limits of most crop species, as discussed in Objective 1. The combined Na and Cl concentrations in leaves of these species reached halophytic proportions, that is, 7% for *L. alyssoides*, 10% for *L. latifolium*, and 13% for *L. draba*. Remarkably, these species failed to express leaf burn symptoms at any time during the study. For further implications, see Objective 1.

Objective 3: *Lepidium alyssoides, L. draba,* and *L. latifolium* Seed Germination Salinity Responses

Seed Germination

Others have reported on optimized temperature and light regimes for germination of seed from *L. latifolium* (Miller et al. 1986; Larson and Kiemnec, 2005) and *L. draba* (Kiemnec and Larson, 1991), but to our knowledge, we are the first to report on the germination of seed from *L. alyssoides*. More so, we are the first to conduct germination studies involving all three of these *Lepidium* spp. together. For these reasons, we needed to develop a temperature and light regime that would be suitable for the germination of all three of these species. In Objective 2, we achieved more than adequate germination of *L. alyssoides*, *L. draba*, and *L. latifolium* under the greenhouse temperature and light conditions that we observed during late January to early February. Therefore, the latter conditions served as the basis for the seed germination chamber diurnal temperature and photoperiod regime used in our seed germination studies (see Materials and Methods, Objective 3).

Germinability (G). There was a significant treatment x species interaction on germinability measured at 2-weeks ($P = 0.0029$). Germination percentage of *L. alyssoides* declined from treatment 2 to treatment 3,

while that of *L. draba* and *L. latifolium* did not (Table 19). Both main effects of treatment and species were significant ($P \leq 0.0132$). These effects were largely attributed to *L. alyssoides* with its inherently lower germination percentage irrespective of the treatment, and with its 19% germination decline when exposed to the treatment 3 solution. This indicates that seed germination of *L. alyssoides* may be more sensitive to high saline conditions than that of *L. draba* and *L. latifolium*. The lack of a high salinity effect on germination of *L. draba* seed matches similar findings by Kiemnec and Larson (1991). In contrast, the high germinability of *L. latifolium* (> 99%), irrespective of salinity (Table 19), was not consistent with findings by Larson and Kiemnec (2005), who reported that germination of a *L. latifolium* population from Oregon declined as salinity was increased (under constant light and 12-hr light/dark at 20°C). This inconsistency may be due to either a seed source (population) effect, or a conditional (temperature and light) effect, since temperature interacts with salinity in controlling seed germination (Ungar, 1995 and references therein). Further, it is worth noting that Larson and Kiemnec (2005) concluded that *L. latifolium* is "suited to" germinate in sodic conditions, yet non-sodic treatments (SAR of 2) were used in their study. We contend that their claim is unsubstantiated. While their study did show that *L. latifolium* is capable of germinating under saline conditions (EC up to 16 dS m^{-1}), our findings demonstrate that *L. latifolium* (and *L. draba*) can germinate unhindered in virtually infinitely high SAR conditions (deionized water + 24 and 48 mM NaCl). Therefore, we put forth quantitative data showing that *L. latifolium is* capable of germinating in both *saline* and *sodic* environments.

Table 19. Germinability (*G*) and mean germination time (*MT*) of *L. alyssoides*, *L. draba*, and *L. latifolium* under saline treatment solutions for two weeks.

	L. alyssoides		*L. draba*		*L. latifolium*	
Treatment	*G* (%)	*MT* (days)	*G* (%)	*MT* (days)	*G* (%)	*MT* (days)
1 (Control)	93.0 A b	5.1 A a	99.4 A a	4.3 C b	100.0 A a	3.6 B c
2	90.3 A b	4.7 A a	97.9 A a	4.8 B a	99.5 A a	4.0 A b
3	80.7 B b	5.4 A a	99.0 A a	5.4 A a	100.0 A a	4.2 A b

Means followed by different letters indicate a significant difference according to Duncan's Multiple Range Test ($P < 0.05$); uppercase within columns, lowercase within rows.

Mean Germination Time (MT). Treatment and species also interacted on MT ($P = 0.0210$). Across the three treatments, there was a lack of MT response by *L. alyssoides*, but as salinity was increased, so was MT of *L. draba* and *L. latifolium*, and the latter responses combined to produce the interaction as well as a treatment main effect ($P < 0.0001$) (Table 19). A significant species main effect ($P < .0001$) was associated with an overall shorter MT for *L. latifolium* as compared with longer MTs for *L. alyssoides* and *L draba*. If a delay in MT of a day or less for *L. draba* and *L. latifolium* would hold true in field

conditions, it would seem unlikely that such a delay would preclude infestations under the saline-sodic germination conditions of this study that, at least for those species, had no effect on germinability.

Seed Vigor

Seed vigor, as inferred by radicle extension after 7-days, was also affected by the treatment x species interaction ($P = 0.0083$). The final radicle length of *L. draba* decreased linearly with increasing salinity, whereas that of *L. alyssoides* and *L. latifolium* was unaffected by the increasing salinity (Fig. 9). The treatment and species main effects were significant ($P \leq 0.0122$). The treatment effect was limited to the reductions in radicle length of *L. draba* as noted previously, and the species effect to the long radicles of *L. draba* in treatments 1 and 2. *Lepidium draba* had an inherently larger seed mass (grams per 100 seed) than *L. alyssoides* and *L. latifolium* (unpublished data from our laboratory). This may explain the longer radical length of *L. draba* under non-saline and moderately saline solutions, but there may be an upper limit to radical extension under sufficiently high salinity, as shown here.

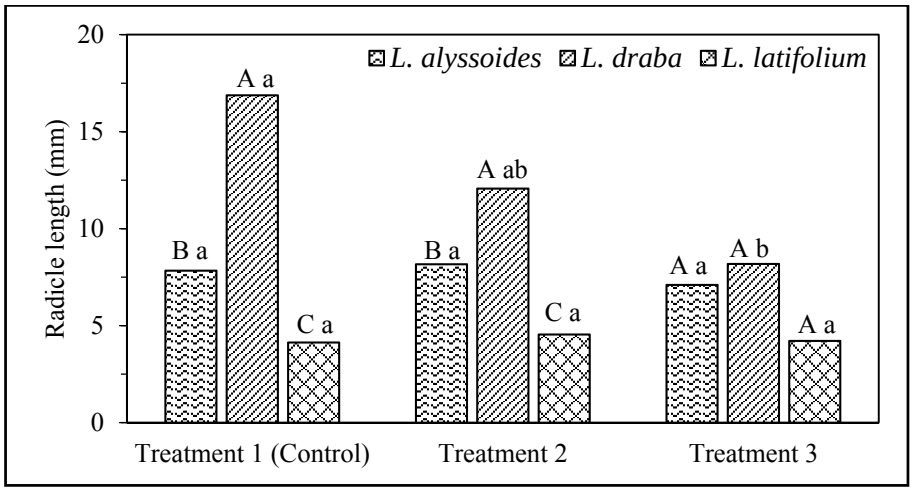

Figure 9. Radicle length (mm) of *L. alyssoides*, *L. draba*, and *L. latifolium* after seven days under saline treatment solutions. Different letters indicate a significant difference according to Duncan's Multiple Range Test ($P < 0.05$); uppercase within treatments, lowercase within species.

CONCLUSIONS AND RECOMMENDATIONS

This final chapter will integrate our findings to provide useful and practical information on salinity and invasive plants to aid in the management of land and water in the semiarid U.S. Along the way, we will be discussing the biology of a fascinating plant taxon, and for good measure, we will pose some interesting research questions emanating from the current study. The salinity responses of *Lepidium draba*, *L. latifolium*, and *L. alyssoides* have been carefully evaluated in our NM WRRI-funded research. Two of the listed species, *L. draba* and *L. latifolium*, have earned their reputations as alien aggressive invaders throughout much of the western U.S. Comparatively little information has been published on the third species, *L. alyssoides*, and this report is the first that we are aware to explain why this indigenous plant should be considered as invasive under saline conditions, behaving much like its two relatives. These *Lepidium* spp. are by no means the only invasive plants in our region. Thus, the final chapter will also propose for further testing to: 1) replicate the present study for additional weedy species, 2) develop predictive and intervention management tools supported by quantitative data, 3) overcome weaknesses in the vegetation science literature, and 4) simply become more educated on the role of salinity in jeopardizing our semiarid lands and soil water supply.

"Non-resource" factors, such as soil salinity, may be important determinants of plant species pools on semiarid lands (Cox et al. 2006), and the makeup of the pools may rest upon their most tolerant members (Grace, 2001). When we first proposed the study, evidence supported our hypothesis that *Lepidium* spp. may be able to occupy a vacant, high-Na niche that is either unusable or lethal to other plant species. As we close the study, we now have additional evidence to state that high-Cl soils should also be considered in addition to high-Na soils. The *Lepidium* spp. under study tolerate categorically high leaf Na and Cl concentrations in saline conditions and there is every reason to believe that this tolerance enables them to monopolize salinized landscapes. In addition, the seed germination stage is important in determining a species ability to establish in saline conditions (Ungar, 1995). High seed germinability and vigor of *L. alyssoides*, *L. draba*, and *L. latifolium* in saline-sodic solutions indicate that these species are capable of establishing in similar saline-sodic environments, particularly in the arid southwest, such as roadways, irrigation ditches, disturbed landscapes, and brackish water land application sites. Seed propagule pressure of *L. latifolium* has attracted significant attention (Leininger and Foin, 2009; Francis and Warwick, 2007) and through our own field observations, we also know that *L. alyssoides* and *L. draba* are prolific seed producers (Appendix A). In addition, we observed aggressive vegetative budding through creeping rhizomes in all three of these *Lepidium* spp. Overall, it seems these species express tremendous resilience and propagule pressure exerted by both seed and vegetative reproductive centers,

and that disturbed, saline-sodic sites of the arid southwest may be particularly susceptible to new infestations, provided that the seed banks are available.

Despite the observed growth suppressions, our findings have demonstrated that the growth and water use characteristics of three different *L. alyssoides* populations are largely independent of various isosmotic saline stress solutions. The isosmotic solutions in Objective 1 were included to address questions on *L. alyssoides*, like the following: Would there be a growth stimulation in response to high-Na waters as was suggested by findings from the earlier and somewhat crude field study (Picchioni et al. 2012b)? Would Na serve as a beneficial element as in other species (Subbarao et al. 2003)? Would either high-Na waters or high-Cl waters impose specific ion toxicity? Would the growth response be indifferent to the ionic composition of irrigation waters and instead, would the osmotic effect predominate? Such finely tuned questions about plant salinity stress underpin critical knowledge gaps that are blatantly obvious in the vegetation science literature, and these insufficiencies have hindered the understanding of factors regulating weed invasions upon natural terrestrial systems.

The use of isosmotic salts to separate osmotic and specific ion effects on salt-stressed plants is not a simple and clear-cut process (Munns and Tester, 2008) even though in our case, it served an important purpose. Bernstein (1975) stated that the consequences of successfully delineating osmotic and specific ion effects on plants are not at all trivial, and in his review, he went on to write the following: 1) if salt effects are driven by specific ion concentrations, then soil salinity assessment requires chemical determination of the specific ions in question, and 2) if osmotic effects are driving the growth response (as is suggested in our study on *L. alyssoides*), then only the total soil salinity (i.e., electrical conductivity of the soil saturation extract, "EC_e") needs to be assessed—a much simpler task.

We acknowledge the need for more data on isosmotic salt effects on *L. draba* and *L. latifolium*, but nonetheless, assessing the risk of *Lepidium* spp. invasions should unquestionably involve measuring the electrical conductivity of the soil saturation extract (EC_e), and this quick and reliable tool is precisely what is needed for water and land managers. Based on our close comparisons of dry matter productivity between the *Lepidium* spp. and salt-tolerant cotton under similar experimental conditions, we now know that the three species in question—*L. alyssoides, L. draba,* and *L. latifolium*—are truly salt tolerant. Therefore, soils of high salinity are at-risk to invasions by these species and wherever the seed banks are available, high salinity would draw the line through the species pool and set the stage for invasions, much like what we observed under relatively crude field conditions near Las Cruces (Picchioni et al. 2012a, 2012b, 2014).

Most of our data are on *L. alyssoides* and even for that species, it is not as simple as to rely solely upon the EC_e to predict its encroachment and invasiveness. This species became invasive on a saline−sodic wastewater land application site and its invasiveness was coincident with substantial

increases in shallow-depth soil saturation extract sodicity (SAR) and pH, without an attendant rise in EC_e (Picchioni et al. 2012a, b). While more data are needed for *L. draba* and *L. latifolium*, soil saturation extract SAR and alkalinity would be useful assessment tools for the prediction of *L. alyssoides* invasions.

All wastewater land application sites in New Mexico are under the jurisdiction of the New Mexico Environment Department (NMED). Among their numerous compliance mandates, NMED has adopted a voluntary secondary federal drinking water guideline into its enforcement policy, specifically pertaining to Cl emissions by wastewater generators (companies, processors, farms, etc.). The NMED strictly enforces wastewater discharge Cl concentration to be kept at or below 250 mg L^{-1} (Picchioni et al. 2014). Sparing the manifold details here (see Picchioni et al. 2014, specifically pages 8–11, 22–25, and 37), a four-year scenario that we experienced was a hard and painful lesson for water and land managers who lack a preventative and proactive soil management plan to predict and control weed infestations before they become a problem. If the knowledge of *L. alyssoides* tolerance to sodicity had been in place, and if the Cl discharge restriction had not existed, the *L. alyssoides* invasion may not have occurred. Further discussion and recommendations pertaining to the Cl discharge regulation and water reuse practices in New Mexico may be found on page 37 in Picchioni and others (2014).

For assessing the invasive risk of any of these three *Lepidium* spp., we must also consider their high potential to colonize aggressively sites affected by human activity, and irrespective of soil salinity or sodicity. In addition to the salinity-related factors discussed previously, land-use practices such as those associated with the plant populations of our study—construction, grading, surface and storm water diversion, excavation, and farming—must be accounted for in assessing the invasive risk of these *Lepidium* spp. In our study (Objective 2), the leaf Na, and particularly the leaf Cl concentrations of *L. draba* and *L. latifolium* exposed to the non-saline control treatment provide a good case in point. These two species appeared to accumulate preferentially Na in leaves, from 0.5–0.7% of leaf dry weight, and at a low external Na concentration (2.8 meq L^{-1} in the tap water). Those leaf Na concentrations should be considered as high for such a low-Na water source. Perhaps more impressive were the high leaf Cl concentrations of these two species (1.4–1.6% of leaf dry weight) when exposed to the non-saline control solution that contained Cl at but a meager 0.5 meq L^{-1} in the tap water. It is relevant to note that at the *L. draba* and *L. latifolium* seed collection sites, the soils were non-saline (EC_e of 2.1–2.4 dS m^{-1}) although Cl was certainly available at 5–7 meq L^{-1}, 10 times or more the Cl concentration of our tap water in the greenhouse study. Questions thus arise as to whether these two species could consume the soil water supply on dry, non-saline sites under the water-limited conditions of our prolonged drought, and in those sites, whether they could "set the rules" for plant species pools by depositing Na and Cl-containing leaf litter resulting in a cumulative salt buildup over time. Can the species "mine" Na and Cl at low external concentrations for "cheap" osmotica, allowing them to extract the little water we have left? More

research is needed to address quantitatively these intriguing possibilities under field conditions, because the findings could be relevant to the countless number of landscapes affected by human disturbance, and irrespective of salinity and sodicity.

Greenhouse conditions, as used in this project, do not impose the high evaporative demand constraints that are characteristic of an outdoor semiarid climate, especially when considering our salt treatment durations of 1.5 to 3 months. Compared to the greenhouse conditions used for our experiments, Chihuahuan Desert outdoor growing seasons last much longer with intrinsically higher temperatures, lower relative humidity, higher light intensities, and the hot, dry, and dusty winds. None of these outdoor climatic elements are imposed in the shaded, protected, and evaporatively cooled greenhouse environment. Ironically, we selected a greenhouse environment to turn-the-tide on the many shortcomings of the outdoor field study cited many times previously in this report (Picchioni et al. 2012a, b). Because of the limitations on our resources, the field study included the following drawbacks: mixed vegetation analysis; high spatial variability in plants and soils; fluctuation in the volume and chemical composition of treated, saline wastewater; confounding effects of water, plant nutrients, and saline ions on plant productivity; absence of true replications; lack of root analyses; and the inability to confirm, with certainty, a cause and effect relationship between soil sodicity and growth stimulation of *L. alyssoides*, or between the sodicity and mortality and disappearance of the other species. A controlled greenhouse environment was clearly necessary to overcome the problems associated with the field study.

Under the greenhouse conditions, we were able to detect a significant growth suppression in cotton at a leachate EC of 8.1 dS m^{-1} corresponding to a soil saturation extract EC of about 6.2 dS m^{-1} that was surprisingly lower than the published, field-based soil saturation extract salinity threshold of 7.7 dS m^{-1} for this salt-tolerant crop species (Mass and Hoffman, 1977). Given the known cotton salt tolerance standard, and given the similar experimental conditions wherein the three *Lepidium* spp. performed as well or better than cotton, we conclude that the three *Lepidium* spp. under study would indeed be classified as salt tolerant. That conclusion represents a significant contribution to the literature that has not provided sufficient quantitative data to substantiate the claims of *Lepidium* spp. salt tolerance.

The homogeneous coarse sand medium along with the small plant growing cell served as an efficient experimental system in controlling the leaching fraction, maintaining a steady-state salt balance, providing high reproducibility, and keeping systematic error to a minimum (Tables 3 and 10; Figs. 4 and 6). In light of the good control over our experimental units, we can make meaningful estimates of the equivalent depth of ET over a 3-month experimental duration. From the final total volumetric ET estimates of *L. alyssoides*, *L. draba*, and *L latifolium* (Table 11), and using the average canopy diameter measurements in the greenhouse conditions during Objective 2, the consumptive use per projected canopy area and on a depth basis can be calculated. Such calculations are minimal estimates on semiarid outdoor

(real world) standards because of the climatic and growing season duration discrepancies noted previously, but also because of the low leaf area index of our young test material that would eventually increase to much higher values in field infestations. To construct the minimal estimates of the potential impact that these *Lepidium* spp. could have on the soil water supply of our semiarid lands, we will apply the following realistic assumptions and conditions: full canopy coverage of the ground, young seedlings as in Objective 2 conditions, regular soil moisture, 20 cm annual rainfall primarily during summer months, a northern Chihuahuan Desert location (i.e., Las Cruces, NM), the average canopy diameter of the *Lepidium* spp. plants of Objective 2 midway through the 89-day experimental period (June 4, 2014; grand average of nine total seedlings per treatment and per species), and the final cumulative ET per three seedlings after 89 days treatment taken from Table 11. "Regular" soil moisture is an important assumption that was not overlooked in our study, since in Objective 2, irrigation was applied once or twice a day with less than a 50% depletion in total water storage, whereas in Objective 1, we scheduled irrigations at 50% of total water storage depletion. That adjustment (Objective 2) was reflected in higher ET values for *L alyssoides* in Objective 2 conditions compared to Objective 1 conditions, suggesting that if high soil moisture is available continuously, the consumptive use of that species would increase over and above that of an intermittent water depletion to 50%. Further study is necessary to confirm this possibility for all of these *Lepidium* spp.

In our completed Objective 2 and across the three different *Lepidium* spp., the average canopy diameter (data not shown) and the final cumulative ET (Table 11) varied by less than 15% within the non-saline control treatment, and within the high saline treatment 3 (NaCl at -0.2 MPa). For discussion purposes, we will use the average canopy diameter and the average final (total) ET across the three species, i.e., *L. alyssoides*, *L. draba*, and *L. latifolium.* For treatment 1 (non-saline control), the overall canopy diameter average midway through the study was 24 cm per single plant and the total water use per three plant cells by the end of the study was 10.0 L. For treatment 3 (NaCl at -0.2 MPa), the overall canopy diameter average midway through the study was 21 cm per single plant and the total water use per three plant cells by the end of the study was 7.3 L. Under the non-saline control condition, 10.0 L water per projected canopy area of three plants (1357 cm^2) equates to 7 cm equivalent depth of water use. For the analogous calculation on saline solution treatment 3 (1039 cm^2 canopy area, 7.3 L water use by three plants), the equivalent depth of water use is 7 cm, which is identical to the non-saline condition. In either of the non-saline or saline scenarios, the consumptive use of 7 cm represents over one-third of the annual rainfall at the expense of other species. That is a "soft" description for the reality of *Lepidium* spp. water use and how these species could consume the water supply, under either saline or non-saline conditions.

Our findings showed step-wise salinity-induced reductions in the volumetric ET of *Lepidium* spp. on a per-plant basis (i.e., 3-cell experimental units). However, the expression of ET on the basis of

canopy coverage and water depth presents a different picture. It should not be surprising as to how these species can aggressively occupy saline sites. What *is* somewhat surprising is the lack of quantitative data in the literature to support the claims of "salt tolerance." These species produce dense stands that literally cover a salt-affected landscape (Picchioni et al. 2012b and 2014; Francis and Warwick, 2007). To our knowledge, this report is the first to provide a biological and quantitative basis for the invasions. Namely, we have shown how these species may be able to maintain water use under saline conditions by exploiting the otherwise toxic Na and Cl in leaves in ways that other plant species cannot. Next, they drop their high Na and Cl litter to the ground to increase salinity of the soil water supply and "engineer" the soil to suit their own existence, and further intensify the invasive cycle at the expense of other plant species.

Taken all together, we have reported data pertaining to edaphic factors that may regulate invasiveness of *Lepidium* spp., which addresses the very essence of research need discussed in detail and supported by the voluminous documentation. It is a fitting punctuation that we reiterate a basic and aligned research need for *L. latifolium* that has invaded much of North America and apply the need to any other plant that becomes invasive under saline conditions, including a few "household" names. For *L. latifolium*, land managers are eager for an improved understanding of the habitat requirements in order to identify landscapes that are vulnerable to future invasions (Andrew and Ustin, 2009). That statement resonates throughout our literature review regarding a bigger picture for New Mexico, that of considering other invasive plants, such as Kochia, Russian thistle, and Palmer amaranth. It is noteworthy that the latter three species require Na as a micronutrient. Thus, do they have a competitive advantage on high-Na soils and are they less dominant on low-Na soils? The ultimate value of this research is neither site nor species-specific and should be applied to the larger picture of integrated weed management on diverse salt-affected lands. Application of the present research to the greater diversity of invasive plant species would strongly aid in the understanding of plant and soil traits that govern invasions to protect the supply and quality of soil water on semiarid lands. Our study on *Lepidium*, a novel and fascinating taxon, represents a positive step in the right direction to strengthen and expand the research effort.

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APPENDIX A – SEED POPULATION SITE PHOTOGRAPHS

L. alyssoides West Mesa (WM)
Las Cruces, NM
June 2012

L. alyssoides Mesquite (MQ)
Mesquite, NM
June 2012

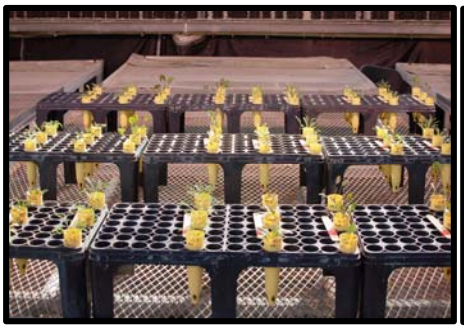
L. alyssoides East Mesa (EM)
Las Cruces, NM
June 2012

L. draba
Los Lunas, NM
July 2013

L. latifolium
Los Lunas, NM
July 2013

APPENDIX B – REPRESENTATIVE PHOTOGRAPHS OF THE EXPERIMENTS


Objective 1. Three populations of *Lepidium alyssoides* (WM, MQ, and EM). Initial (left) and final (right) days.




Objective 2. Three species of *Lepidium (L. alyssoides, L. draba, and L. latifolium)*. Initial (left) and final (right) days.


Objective 2. Bean. Initial (left) and final (right) days.


Objective 2. Cotton. Initial (left) and final (right) days.


Objective 3. Seed germination (left) and seed vigor (right).