

ECOLOGICAL ADAPTATION OF THE ENDEMIC *SHEPHERDIA ROTUNDIFOLIA* TO CONDITIONS IN ITS COLORADO PLATEAU RANGE

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ABSTRACT.—Due to limited water supplies, use of drought-tolerant species to conserve water in irrigated urban landscapes is increasingly important in the Intermountain West. The Colorado Plateau endemic shrub *Shepherdia rotundifolia* Parry is a potential candidate for use in sustainable urban low-water landscapes (LWLs) for its aesthetic and drought-tolerant qualities. However, the species is difficult to establish in urban landscapes of different fertility and water availability than found in regional native habitats. A better understanding of environmental and genetic constraints, as well as morphological adaptation in native habitats, may facilitate greater use of *S. rotundifolia* in LWLs. The goal of this study was to investigate variability in environmental conditions, morphology, and genetics among 6 populations of *S. rotundifolia* along an elevation gradient (range 1200–2500 m) in the species' native range. Aboveground environmental conditions were characterized from 30-year proximal weather station data, intra-annual weather collected on-site, and site relative light intensity (RLI) from hemispherical canopy images. Belowground, we analyzed site-specific soils for texture, pH, salinity, organic matter (OM), and macronutrients. We characterized plant morphology and genetics from leaf area and specific leaf area (SLA), scanning electron microscopic imaging of trichome structure and leaf thickness, and amplified fragment length polymorphism (AFLP) genetic variation among populations. Precipitation, air temperature, RLI, and soil properties varied widely among populations. Differences among leaf area, SLA, and leaf trichome structure suggest population-level adaptations consistent with environmental differences, particularly between high- and low-elevation populations. Similarly, distinct AFLP banding patterns among high- and low-elevation populations suggest differences due to isolation by distance. SLA was correlated with RLI, OM, and potassium (K). Relatively high native levels and positive correlation with SLA suggest that K may be a limiting factor in urban landscape soils. Selection of plants adapted to environmental conditions similar to those present in urban landscapes may enhance successful use of *S. rotundifolia*. Genetic variation also suggests potential for cultivar selection.

RESUMEN.—La conservación del agua en paisajes urbanos con riego, utilizando especies resistentes a la sequía, es cada vez más necesaria en la región Intermontañosa del Oeste debido al reducido suministro de agua. El arbusto endémico de Colorado Plateau, *Shepherdia rotundifolia* Parry, podría ser utilizado en los paisajes urbanos sustentables con bajo suministro de agua (LWL, por sus siglas en inglés) ya que aporta una imagen estética y es resistente a la sequía. Sin embargo, resulta difícil utilizarlo en los paisajes urbanos de suelo fértil y con disponibilidad de agua, en comparación con los hábitats regionales nativos. El comprender mejor las limitaciones ambientales y genéticas, y la adaptación morfológica en el hábitat nativo de *S. rotundifolia*, puede facilitar el aprovechamiento en LWL. El objetivo de este estudio es investigar la variabilidad en las condiciones ambientales, la morfología y la genética de seis poblaciones de *S. rotundifolia*, en un gradiente de elevación (de 1200 m a 2500 m). Describimos las condiciones ambientales sobre el nivel del suelo a partir de información de 30 años de estaciones meteorológicas cercanas, las condiciones climáticas del lugar a lo largo del mismo año, y la intensidad relativa de luz (RLI, por sus siglas en inglés) del lugar a través de imágenes hemisféricas del follaje. Por debajo del nivel del suelo, analizamos los tipos de suelo específicos del lugar para conocer la textura, el pH, la salinidad, la materia orgánica (MO) y los micronutrientes. Describimos la morfología y la genética de la planta analizando el área foliar y el área foliar específica (AFE), examinando imágenes de la estructura de tricomas y del grosor de la hoja con microscopio electrónico, y a través de la variación genética de los polimorfismos en la longitud de fragmentos amplificados (AFLP, por sus siglas en inglés) entre las poblaciones. Las precipitaciones, la temperatura del aire, la RLI y las propiedades del suelo mostraron grandes variaciones entre las poblaciones. Las diferencias entre el área foliar, el AFE y la estructura de tricomas de la hoja sugieren que se produjeron adaptaciones a nivel de población relacionadas con las diferencias ambientales, en particular, entre las poblaciones que se encuentran en elevaciones mayores y menores. Del mismo modo, los marcados patrones de bandas de AFE entre las poblaciones de mayor y menor elevación sugirieron que existían diferencias debido al aislamiento por distancia. El AFE estaba relacionado con la RLI, la MO y el potasio (K). Los niveles relativamente elevados en la población nativa y la correlación positiva con el AFE sugieren que el K puede ser un factor limitante en los suelos del paisaje urbano. El seleccionar plantas adaptadas a condiciones ambientales similares a las que se encuentran en los paisajes urbanos puede hacer que la utilización de *S. rotundifolia* sea más efectiva. Además, la variación genética sugiere que existe la posibilidad de seleccionar la variedad cultivada.

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Water conservation is critical for urban systems in the arid Intermountain West, USA (IMW). Low-water landscaping, specifically use of drought-tolerant native species, is an essential tool in urban water conservation (Kjelgren et al. 2009). Low-elevation, drought-tolerant IMW native species that require minimal supplemental water offer great potential for low-water landscaping. Native species provide a natural look to the urban landscape and support local native plant industries (McKinney 2002, Kjelgren et al. 2009). Exploiting drought-adapted IMW native species for low-water landscaping not only has potential to conserve water but also to increase biodiversity in urban environments.

Endemic plant species are key biodiversity elements in sustainable ecosystems. Endemic-species protection has typically focused on preserving natural habitats in biodiversity hot spots such as national parks, wildlife refuges, and national forests (Myers et al. 2000, Brooks et al. 2006). Urban landscapes, particularly low-water landscapes (LWLs) in the IMW, are a window of opportunity in promoting biodiversity and preserving endemic species (Alvey 2006). Endemic arid-adapted species used in biologically diverse urban landscapes become commercially viable assets, reduce water use and carbon footprint, support native pollinator species, and educate the public about natural systems (Mee et al. 2003).

Dry habitats of the IMW harbor large numbers of endemic species (Mee et al. 2003, Meyer et al. 2009, Intermountain Native Plant Growers Association 2011) that have potential for use in LWLs. Roundleaf buffaloberry (*Shepherdia rotundifolia* Parry; Elaeagnaceae) is a promising LWL candidate: an evergreen shrub with distinct aesthetic qualities, apparent drought tolerance, and capacity for nitrogen fixation (Mee et al. 2003). Aesthetically, *S. rotundifolia* has a hemispherical canopy and silvery green evergreen foliage that would accent the LWL (Mee et al. 2003). The species is also important for wildlife habitat; the Utah Division of Wildlife Resources encourages use of *S. rotundifolia* in suitable landscapes because it provides food (fruit) and cover for quail and small mammals (Nordstrom 2001).

Shepherdia rotundifolia is endemic to the Colorado Plateau and is distributed from southern Utah into the Grand Canyon region of Arizona (Schmutz et al. 1967, United States

Department of Agriculture 2011). It occurs naturally on hillsides and cliff bases on well-drained rocky soils (Mee et al. 2003) from 1500 m up to 2400 m elevation (Kearney and Peebles 1960). Precipitation in the species' natural habitat is 170–480 mm annually (Brotherston et al. 1983).

Environmental variability within its native habitat may have created site-specific adaptations within *S. rotundifolia*. These adaptations may include leaf traits such as average area of a single leaf (LA) and specific leaf area (SLA = unit leaf area/unit weight). Specific leaf area is a signature adaptive adjustment to variations in environmental conditions and contributes to genotype discrimination within species (Rieger et al. 2003, Gomes et al. 2011, Jin et al. 2011). Furthermore, low SLA is an important strategy for plants living in harsh environmental conditions (Reich et al. 1998, Wilson et al. 1999, Ceriani et al. 2009, Liu et al. 2011). Variability in leaf traits as measures of environmental adaptation among populations of *S. rotundifolia* has not been described.

Shepherdia rotundifolia is, however, difficult to establish in irrigated urban landscapes (Mee et al. 2003). Anecdotally, *S. rotundifolia* fails to establish when planted in urban soils after being grown in containers. This failure automatically limits the species' adoption by nursery growers and the landscape industry. Better understanding of the link between genetic variability and leaf traits that characterize environmental tolerances may potentially be exploited to facilitate *S. rotundifolia* use in LWL. Understanding the tolerances of *S. rotundifolia* to light and soil conditions may improve landscape design and management options for increasing the odds of establishing the species in urban landscape soils. The goal of this study was to investigate variation in environmental conditions and the related morphological adaptations and genetic variation among populations of *S. rotundifolia* along an elevation gradient in its native habitats.

METHODS

Location

Shepherdia rotundifolia Parry is found only in extreme southern Utah and northern Arizona; we chose to collect from populations spanning southwestern to southeastern Utah. Six populations, representing a range of natural

TABLE 1. Environmental conditions among populations of *Shepherdia rotundifolia* in southern Utah, including elevation, 30-year means (1981–2010) of annual precipitation, maximum and minimum air temperature, and relative light intensity (RLI). Standard deviations are in parentheses.

Pop. ID	Location (latitude, longitude; elevation; nearest town)	Historical weather data, 30-year average ^a (1981–2010) ^b			
		Precipitation (mm · year ⁻¹)	Tmax (°C)	Tmin (°C)	Measured RLI ^{a,c} (%)
Tor-2500	38.13° N, 111.33° W; 2507 m; Torrey	285 (13.9) b	17 (0.2) d	3 (0.1) d	31 (4.3) b
Tor-2300	38.20° N, 111.35° W; 2295 m; Torrey	285 (13.9) b	17 (0.2) d	3 (0.1) d	98 (0.2) a
Tor-1600	38.19° N, 111.10° W; 1642 m; Torrey	210 (11.3) c	19 (0.1) b	6 (0.1) b	100 (0.0) a
Nat. Bridge	37.30° N, 109.54° W; 1342 m; Blanding	327 (14.5) b	17 (0.1) c	4 (0.1) cd	99 (0.1) a
Bluff	37.28° N, 109.53° W; 1342 m; Bluff	199 (11.5) c	17 (0.2) d	4 (0.2) c	94 (0.6) a
Springdale	37.19° N, 113.00° W; 1188 m, Springdale	409 (23.2) a	25 (0.2) a	9 (0.1) a	88 (5.5) a

^aValues within a column with different letters indicate statistical significance at $\alpha = 0.05$.
^bMean precipitation and maximum and minimum temperatures over a 30-year record at each site were obtained from an existing weather station closest to the site; the weather station Boulder (37.9° N, 111.4° W; elev. 2036 m.) was used for the populations Tor-2500 and Tor-2300; the weather station Capital Reef NP (38.3° N, 111.3° W; elev. 1676 m.) was used for the population Tor-1600; the weather station Natural Bridge NM (37.6° N, 110.0° W; elev. 1981 m.) was used for the population Nat. Bridge; the weather station Bluff (37.3° N, 109.6° W; elev. 1317 m.) was used for the population Bluff; and the weather station Zion NP (37.2° N, 113.0° W; elev. 1234 m.) was used for the population Springdale.
^c $n = 3$.

habitats, were selected along an elevation gradient to maximize the possibility of variation in morphological and genetic characteristics. The 6 populations included 3 at different elevations in the town of Torrey in central Utah (Tor-2500, Tor-2300, and Tor-1600), one in Bluff (Bluff) in far southeastern Utah, one near Natural Bridges Monument (Nat. Bridge) in southeastern Utah, and one in Springdale near Zion National Park (Springdale) in southwestern Utah (Table 1; Fig. 1).

Environment

Elevation, precipitation, relative light intensity (RLI), temperature, evapotranspiration (ET_o), and soil properties were recorded for populations of *S. rotundifolia* in their native habitats. Relative light intensity, the ratio of incoming solar radiation at the top of the *S. rotundifolia* canopy to total unobstructed incoming solar radiation, was estimated from 3 canopy images taken with a CI-110 Plant Canopy Digital Imager (CID Inc., Camas, WA) at each collection site. The canopy images were analyzed with HemiView Canopy Analysis Software 2.1 (Delta-T Devices Ltd., Burwell, Cambridge). Air temperature and precipitation data for a 30-year time span (1981–2010) were collected from existing weather stations that were paired with each site on the basis of proximity and similarity in elevation (data obtained in 2012 from <http://climate.usu.edu/>; Table 1). One weather station was applied to both Tor-2500 and Tor-2300 because it was similar in elevation and distance to both sites. The environmental variables among populations were

subjected to analysis of variance (ANOVA) using PROC GLM in SAS software (SAS Institute, Cary, NC). The 3 populations located in the town of Torrey (Fig. 1) represented variation in seasonal environmental conditions due to differences in elevation and canopy closure. In situ weather, including air temperature and solar radiation, was monitored at each site from July to December 2009 using 2 sensors connected to a data logger (HOBO-U30, Onset Computer Corporation, Pocasset, MA). Maximum and minimum air temperatures at each site were used to calculate local reference evapotranspiration (ET_o) according to methods of Hargreaves and Allen (2003). Finally, 2 soil samples taken from the soil surface to a 30-cm depth were collected for each of the 6 populations. Soil samples were analyzed for electrical conductivity (salinity), pH, phosphorus (P), potassium (K), and organic matter (OM) at Utah State University (USU) Analytical Labs (Logan, UT).

Morphology

Leaf traits that may be representative of environmental tolerance were measured. Average leaf area (LA), specific leaf area (SLA), leaf thickness, and leaf pubescence were collected from the 6 populations of *S. rotundifolia*. Due to the small number of plants (approximately 5–10 individuals) at several of the sites, 5 plants were used to represent each population. Approximately 15–20 mature leaves located at the top of the canopy (i.e., sun leaves) were randomly subsampled from each individual plant in summer 2009. Specific leaf

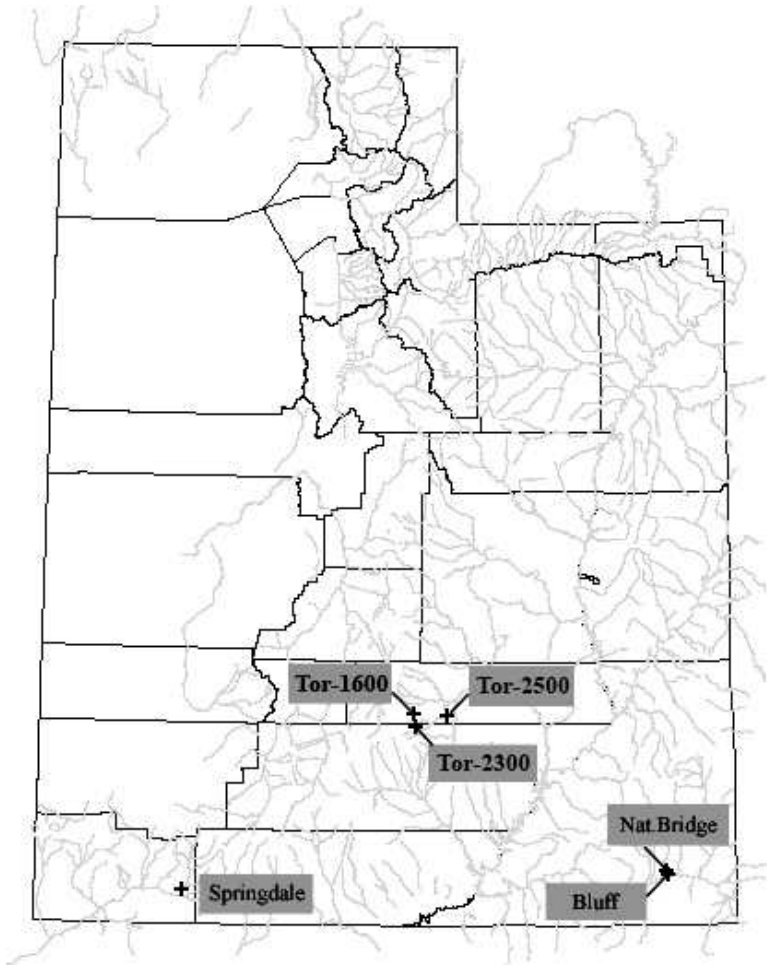


Fig. 1. Locations of *Shepherdia rotundifolia* populations sampled in southern Utah.

area was calculated as single-sided leaf area, determined using a LI-3100 leaf area meter (Li-Cor, Lincoln, NE), divided by the leaf weight after drying at 65 °C for 24 h. Leaf thickness and leaf pubescence were measured on leaf samples collected from the Natural Bridges population and the 3 populations located in Torrey, Utah. From these samples, leaf punches were collected at each site and fixed in formalin-aceto-alcohol (FAA) solution in the field. The fixed leaf tissues were critical-point dried using Samdri-PVT-3D (Tousimis, Rockville, MD). The fixed leaf tissues were used to observe leaf trichomes on the adaxial and abaxial surfaces, as well as leaf thickness on the cross-sectional surface, via a scanning electron microscope (Hitachi S4000, Pleasanton, CA).

Leaf area, specific leaf area, and trichome thickness among populations were subjected to ANOVA using PROC GLM in SAS software (SAS Institute, Cary, NC). In addition, morphological variables were correlated with environmental variables at the 6 sites (Pearson's correlation coefficient in PROC GLM). The environmental variables included elevation, precipitation, air temperature, relative light intensity, and soil properties.

Genetics

Leaf samples were collected (2–3 leaves per plant) from each population (5 plants per population) and dried in 28–200 mesh silica gel (Fisher Scientific, Pittsburgh, PA). DNA was extracted with the DNeasy 96 Plant Kit (QIAGEN, Valencia, CA). Amplified fragment length

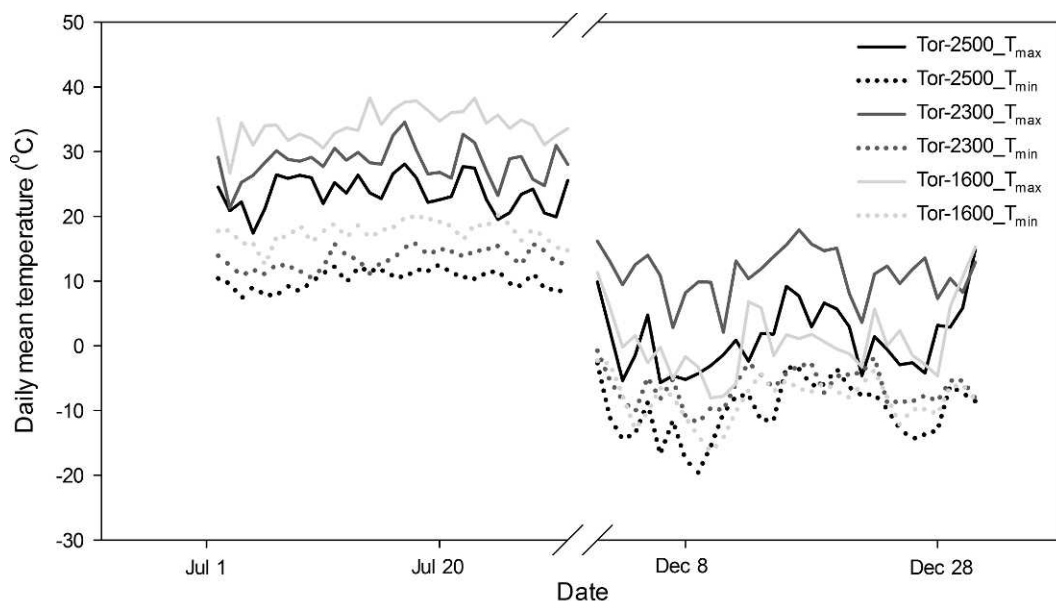


Fig. 2. Daily mean maximum and minimum air temperatures recorded from July 2009 to December 2009 at 3 *Shepherdia rotundifolia* populations in Torrey, Utah: Tor-2500, Tor-2300, and Tor-1600.

polymorphisms (AFLP) were assayed as described by Vos et al. (1995) with described modifications. The DNA samples were preamplified with EcoRI +1 / MseI +1 using A/C-selective nucleotides. Selective amplification primers consisted of 5 EcoRI +3 / MseI +3 primer combinations using AAC/CAA-, AAG/CAG-, ACC/CAT-, ACG/CTA-, AGG/CTA-, and AGA/CCC-selective nucleotides. The EcoRI-selective amplification primers included a fluorescent 6-FAM (6-carboxy fluorescein) label on 5' nucleotides. Selective amplification products were combined with GS600 LIZ internal lane size standard and fractionated using an ABI 3730 instrument with 50-cm capillaries and sized between 50 and 600 bp with Genescan software (Applied Biosystems, Foster City, CA). Although DNA molecules vary in length by increments of 1 bp, the relative mobility of bands is also affected by sequence composition. Thus, nonhomologous bands of the same length may not have the same relative mobility. Genescan trace files for each individual were visually analyzed using Genographer software (<http://hordeum.oscs.montana.edu/genographer>) for the presence or absence of DNA bands in bins that were at least 0.3 bp or more apart. Bayesian clustering (Structure v2.1) of individual plants without a priori assignment of

individuals to hierarchical groups was used to determine genetic structure, which might otherwise confound phylogenetic analysis (Pritchard et al. 2000). Three analyses were used of each model with either 100,000 iterations and 10,000 burn-in or 200,000 iterations and 20,000 burn-in with the dominant-allele admixture model of Structure v2.2 (Pritchard et al. 2000, Falush et al. 2007).

RESULTS

Environment

Environmental conditions of the 6 *Shepherdia rotundifolia* Parry populations varied widely. All but one of the sites were found within open areas of the pinyon-juniper community, indicating a general preference for full sun. The exception was the high-elevation population Tor-2500, which was found beneath the canopy of a ponderosa pine forest, resulting in the lowest relative light intensity of all sites (Table 1). On the basis of a 30-year record (1981–2010), mean annual precipitation, mean maximum temperature, and mean minimum temperature also varied among populations. The population at Springdale, located adjacent to Zion National Park, had the greatest mean annual precipitation due to late summer

TABLE 2. Soil properties, including texture, salinity (EC), pH, phosphorus (P), potassium (K), and organic matter (OM) at the 6 *Shepherdia rotundifolia* sites in Utah. Each value is the mean of 2 soil samples, with standard deviation in parentheses.

Pop ID	Texture	EC (dS · m ⁻¹)	pH	P (mg · kg ⁻¹)	K (mg · kg ⁻¹)	OM (%)
Tor-2500	Silt loam	0.7 (0.2)	6.5 (0.1)	27.1 (7.9)	373.5 (105.5)	8.7 (2.1)
Tor-2300	Sandy loam	1.2 (0.5)	7.5 (0.2)	5.2 (1.2)	148.5 (48.0)	1.8 (0.8)
Tor-1600	Sandy clay	0.8 (0.1)	7.7 (0.1)	8.5 (5.5)	193.5 (3.5)	0.7 (0.2)
Nat. Bridge	Sandy loam	1.0 (0.1)	7.6 (0.0)	1.6 (0.0)	243.0 (15.0)	2.0 (0.4)
Bluff	Sandy loam	1.1 (0.4)	7.4 (0.0)	3.3 (0.5)	190.0 (103.0)	3.9 (2.8)
Springdale	Sandy loam	0.8 (0.1)	7.9 (0.2)	8.3 (1.2)	360.0 (73.0)	6.3 (3.3)

monsoonal flow from the Gulf of California. The Springdale precipitation was approximately 2 times greater than that at the Tor-1600 and Bluff sites. Mean minimum and maximum temperatures from the weather station that represented the high-elevation populations (Tor-2300 and Tor-2500) were generally lower than temperatures at the low-elevation population sites.

In situ weather data recorded in the field in 2009 at the 3 sites near Torrey, Utah, showed daily high temperature to be highest in July (40 °C at Tor-1600) and lowest in December (–20 °C at Tor-2500; Fig. 2). In July, mean maximum air temperatures at Tor-1600 were higher than those at Tor-2300 and Tor-2500, a relative pattern repeated with mean minimum air temperature. Maximum and minimum air temperatures at the 3 sites dropped at least 10 °C in December compared to temperatures in July. December maximum and minimum temperatures at Tor-1600 dropped to levels similar to the high-elevation sites during the same period. Interestingly, Tor-2300 had the highest winter maximum and minimum temperature, whereas winter temperatures at Tor-1600 were sometimes lower than those at 2500 m. The Tor-1600 site was exposed rock in a desert region to the west of Capital Reef National Park with no buffering from vegetation, unlike the Tor-2300 site.

During a representative period (mid-August to mid-September 2009), the Tor-2500 site was cloudier and had lower solar radiation intensity compared to Tor-1600, likely due to the site’s elevation causing greater cloud formation (Fig. 3A–C). Higher temperatures and greater solar radiation were integrated into greater mean reference evapotranspiration (ET₀) at the Tor-1600 site (781 mm · year⁻¹; data not shown). Conversely, cooler temperatures, less solar radiation, and likely greater rainfall resulted in only 564 mm · year⁻¹ ET₀

at the higher-elevation sites. Though the Tor-2300 site was only 200 m lower than the highest-elevation site, the surrounding pinyon-juniper tells the story of a much drier environment consistent with 705 mm · year⁻¹ ET₀.

Soil properties varied among populations (Table 2). Phosphorus (P) and pH were different among populations; soil pH level was lowest at the Tor-2500 site where soil OM, mostly from ponderosa pine leaves, was greatest due to a cooler and moister environment. The site Tor-2500 also had the highest level of P in its soil beneath the ponderosa pine canopy. Soil OM was lowest at Tor-1600 due to the site’s widely scattered perennial herbaceous forbs rooted in open, hot, dry ground adjacent to Capitol Reef National Park.

Morphology

Shepherdia rotundifolia showed morphological traits that were adaptive to the environmental gradient. Although LA was not significantly different among sites, plants growing beneath the canopy of ponderosa pine at the higher elevation Torrey sites trended toward larger individual LA than plants at the other sites (Table 3). Similarly, SLA was not significantly different among sites; however, plants at Tor-2500 invested less in leaf thickness than plants at the other sites, as SLA (area per mass) was greater than that of plants at the other sites, suggesting a stronger response to lower light intensity (Table 1).

At the open, hot and dry habitat at Tor-1600, leaf size was relatively small compared to that of plants at the other sites, with a greater carbon investment, as the SLA of plants at this site was among the lowest. Leaf thickness as derived from SEM images was significantly different among the subset of sampled populations (Fig. 4). Leaf thickness was largely accounted for by the leaf trichome layer (Table 3; Figs. 4A–C, 5A–B). Leaf thickness and trichome

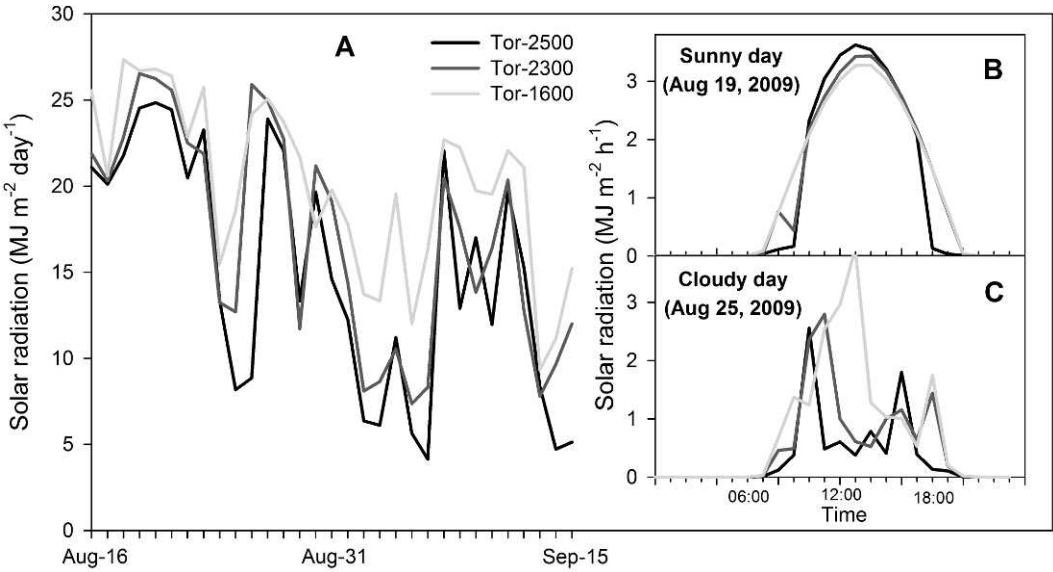


Fig. 3. Solar radiation intensity recorded at the *Shepherdia rotundifolia* sites Tor-2500, Tor-2300, and Tor-1600: **A**, daily solar radiation from 16 August 2009 to 16 September 2009; **B**, hourly solar radiation on a sunny day (19 Aug 2009); **C**, hourly solar radiation on a cloudy day (25 Aug 2009).

TABLE 3. Leaf morphological characteristics of *Shepherdia rotundifolia* ($n = 5$) from the 6 populations in Utah, including leaf area, specific leaf area (SLA), and mesophyll, leaf-trichome, and leaf thickness. Each value is the mean, with standard deviation in parentheses. Values within a column with different letters indicate statistical significance at $\alpha = 0.05$.

Pop. ID	Leaf area (cm ²)	SLA (cm ² · g ⁻¹)	Mesophyll thickness (mm)	Trichome thickness (mm)	Leaf thickness (mm)
Tor-2500	2.3 (0.5) a	48.6 (5.5) a	0.18 b	0.83 b	1.01 b
Tor-2300	2.2 (0.3) a	37.0 (1.2) a	0.20 b	0.76 b	0.96 b
Tor-1600	1.3 (0.2) a	37.8 (1.5) a	0.15 b	1.16 a	1.31 a
Nat. Bridge	1.8 (0.2) a	40.0 (1.8) a	0.25 a	0.87 b	1.12 ab
Bluff	1.5 (0.1) a	40.4 (3.0) a	—	—	—
Springdale	1.6 (0.2) a	41.7 (2.2) a	—	—	—

thickness of *S. rotundifolia* at Tor-1600 were substantially greater than at the other sites, consistent with a hot and dry environment. SLA of plants at the 6 sites was negatively correlated with RLI (Pearson’s $r = 0.886$, $P = 0.019$) and positively correlated with K (Pearson’s $r = 0.832$, $P = 0.040$) and OM (Pearson’s $r = 0.854$, $P = 0.030$; Fig. 6A–C).

Genetics

In the Bayesian cluster analysis, AFLP banding patterns of plants from the high-elevation populations, Tor-2500 and Tor-2300, were distinct from those of plants at the low-elevation populations (Fig. 7A–D). The distinction between the 2 groups occurred in the test of a 3-population model ($K = 3$) using the related

species *S. argentea* as an outgroup. Genetic variation among plants of the low-elevation populations appeared to be greater than genetic variation among the high-elevation populations in the test of a 3-population model in the structure analysis, and the variation remained separated when the number of test populations in the model was increased to 7.

DISCUSSION

Variation in the native habitats of *S. rotundifolia* suggests that the species is tolerant to a range of rainfall, light, and temperature. The tolerance ranges are relevant to the species’ potential use in LWL. The population at Springdale, located in southwestern Utah (Fig. 1),

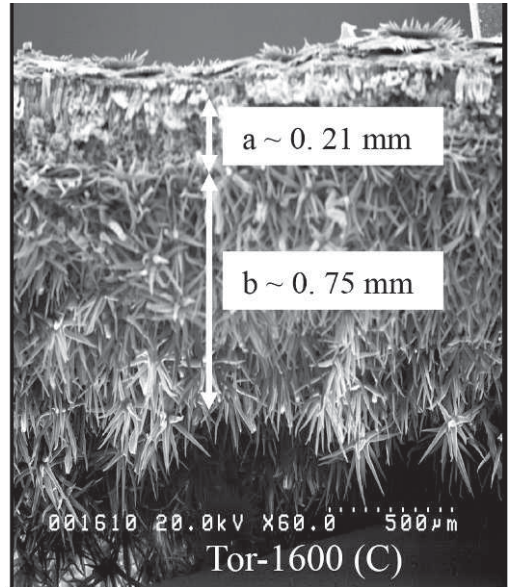
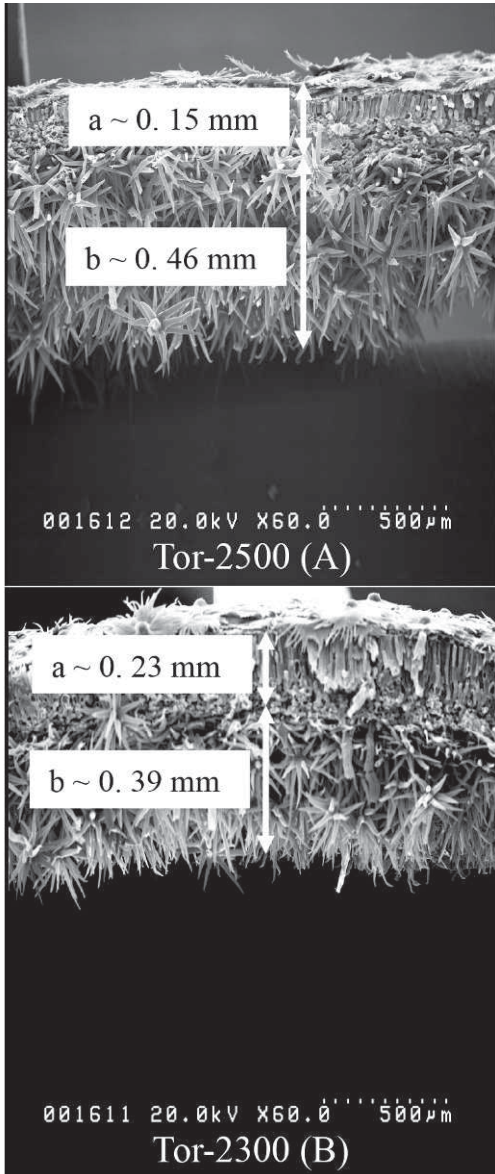


Fig. 4. Scanning electron micrographs of leaf cross sections of *Shepherdia rotundifolia* plants from 3 populations in Torrey, Utah: A, Tor-2500; B, Tor-2300; C, Tor-1600.

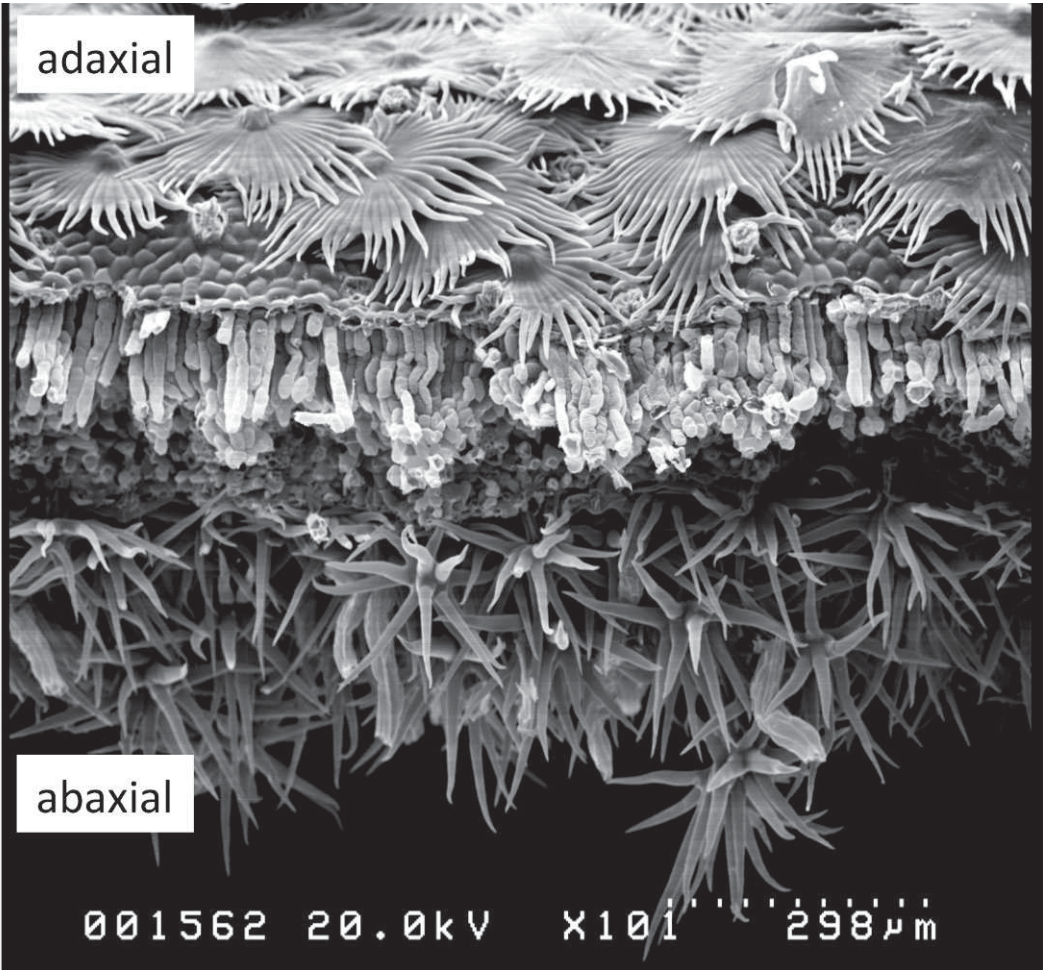
receives the greatest mean annual precipitation and greatest mean maximum and minimum temperatures compared to the other populations (Table 1) due to the effects of the southwest monsoonal subtropical ridge from the Gulf of California. The mean annual precipitation at the Springdale site was approximately 2 times greater than mean annual precipitation at Tor-1600 and Bluff, with most of the increased rainfall occurring in summer, suggesting a wide range of soil moisture tolerance.

However, conditions at Tor-1600 were the most extreme of the 6 sites. The Tor-1600 site

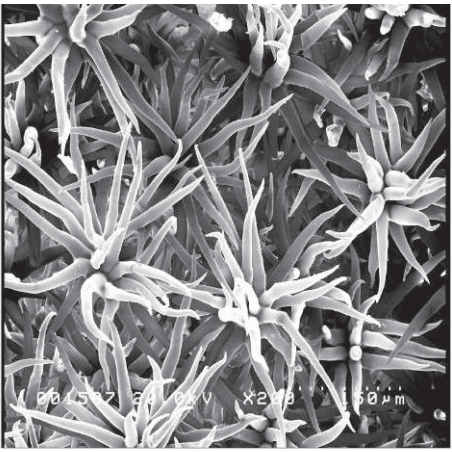
was the driest habitat (Fig. 2), with high summer maximum and minimum temperatures and negligible summer rainfall. Though average rainfall was similar to that at Bluff, the southwest monsoon is much weaker in central Utah, so Tor-1600 receives less growing season rainfall. Tor-1600 consists of a very sparse perennial herbaceous species canopy, resulting in greater temperature extremes at this site during both summer and winter than at the Bluff site, which is situated in a narrow canyon. To tolerate environmental variation in its native habitats, *S. rotundifolia* appears to have adaptive morphological characteristics.

Plants at Tor-1600 have adapted to a hot, dry habitat by having a relatively small LA and a relatively low SLA, although LA and SLA were not significantly different among populations. The small leaf size of plants at Tor-1600 helps to reduce water loss by reducing the transpiration surface area and thus protecting against high ambient temperature and ET_o . Leaf morphological properties also appear to be important in moderating temperature extremes and water loss.

Leaf trichomes, peltate and stellate (Cooper 1932), were present on adaxial and abaxial surfaces, respectively (Fig. 5A–B). Abaxial trichome density was approximately 5 times greater than



(A)



(B)

Fig. 5. *Shepherdia rotundifolia* leaf trichome morphological characteristics observed under a scanning electron microscope: A, adaxial trichome (upper side of leaf); B, abaxial trichome (lower side of leaf).

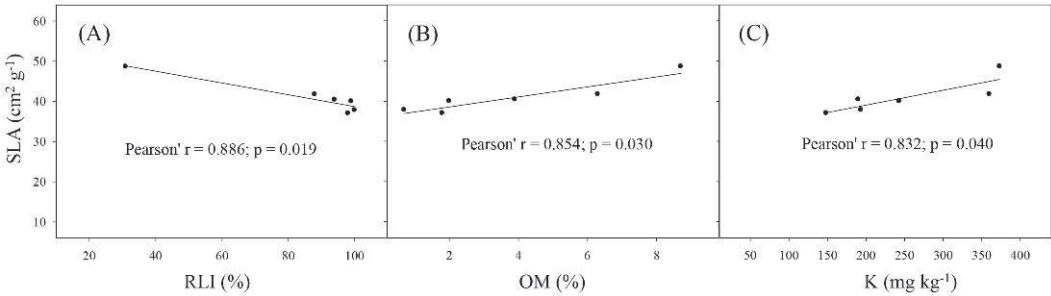


Fig. 6. Pearson's correlation of *Shepherdia rotundifolia* specific leaf area (SLA) with 3 variables: **A**, relative light intensity (RLI); **B**, soil organic matter (OM); **C**, soil potassium (K).

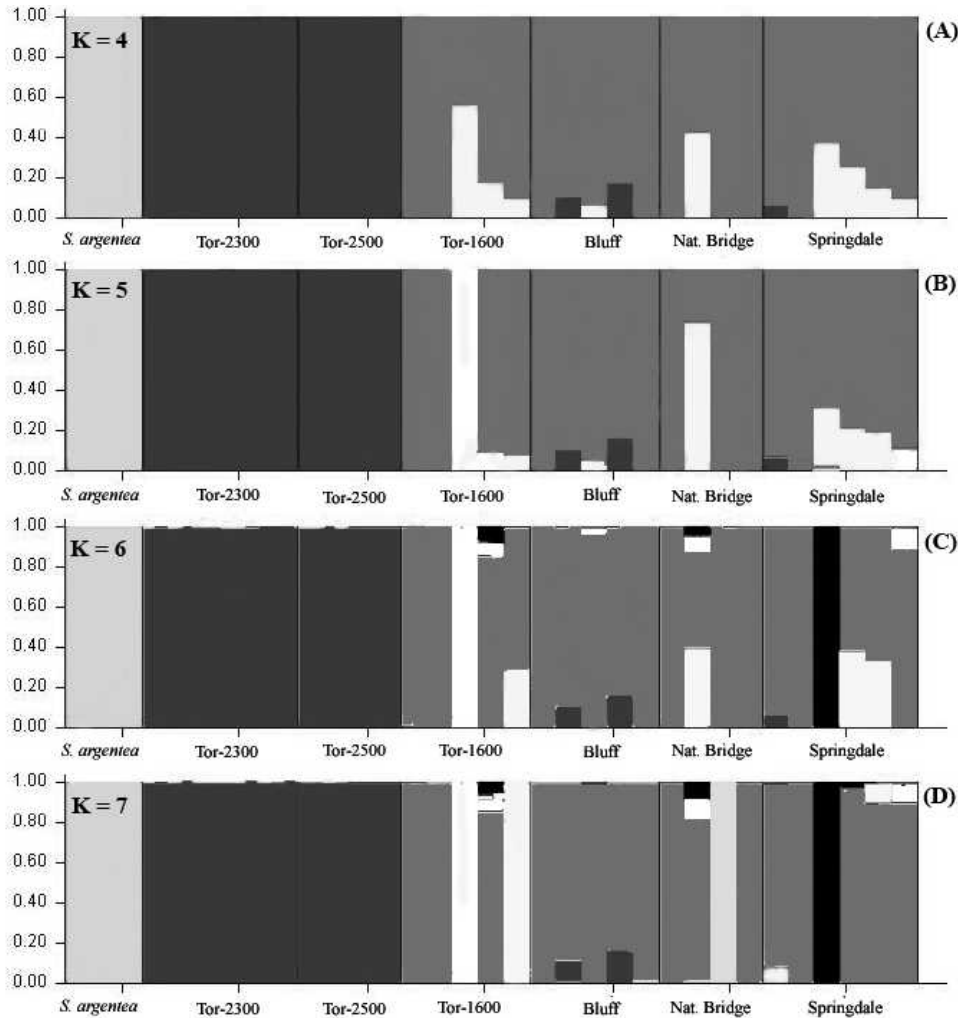


Fig. 7. Inferred population structure of *Shepherdia rotundifolia* AFLP genotypes from 6 populations in the field: **A**, testing a 4-population model (K = 4); **B**, testing a 5-population model (K = 5); **C**, testing a 6-population model (K = 6); **D**, testing a 7-population model (K = 7). A thin vertical line represents each individual, and black lines separate individuals of different populations.

adaxial density (Fig. 4A–C), similar to findings of Bissett et al. (2009) on *Elaeagnus umbellata*, also in the family Elaeagnaceae. The peltate trichomes on the upper surface reflect excess radiation to protect the underlying tissues against ultraviolet-B radiation damage (Karabourniotis et al. 1993). The thicker underside layer of stellate trichomes helps with insulation and provides a moisture trap on the leaf surface to protect against heat and water loss. It also increases the leaf boundary layer, thus reducing transpiration and the impact of wind on the plant energy budget (Press 1999). The relatively thick stellate trichomes on plant leaves at Tor-1600 suggests that regulation of transpiration is more critical for plants at this site than at Tor-2500.

The 2 high-elevation sites demonstrate the range of low-temperature and low-light tolerance of *S. rotundifolia*. The Tor-2300 site was probably the most moderate of the 6 in terms of rainfall and temperature, with the greatest winter maximum and minimum temperatures likely due to surrounding dense pinyon-juniper canopy. Just 200 m higher, the environment and plant responses were quite different. Measured winter low temperatures approaching -20°C suggest that *S. rotundifolia* can tolerate the colder temperatures that would be found in most IMW urban areas.

The significant correlation between SLA and relative light intensity (RLI; Fig. 6A) suggests adaptive traits to shady environments. An increase in leaf area increases surface area for greater absorption of light for photosynthetic carbon assimilation under low-light conditions (Schumacher et al. 2008). Tor-2500 was not the only site where we observed *S. rotundifolia* growing under tree canopies. Though the studied plants at Natural Bridges were in the open, many other plants grew directly under pinyon, probably as a result of bird dispersal. Similarly, we observed a number of *S. rotundifolia* growing under pinyon at the Springdale location. *Shepherdia rotundifolia* at Tor-2500 trends toward having a degree of shade tolerance that would make it well suited to the variable light environments in urban landscapes.

Responses of *S. rotundifolia* to variation in soil properties among sites may be most limiting to its use in LWL. SLA was positively correlated with OM and K (Fig. 6B–C). Those correlation results and *S. rotundifolia*'s evergreen leaf habit were consistent with work

reviewed by Poorter and de Jong (1999), which showed that low SLA indicates efficient conservation of nutrients. The correlation between SLA and K supports the importance of transpiration regulation in allowing *S. rotundifolia* to tolerate its hot, dry native habitats. Potassium is involved in many physiological processes, including plant water relations (Pettigrew 2008) through control over turgor (Amtmann and Armengaud 2007). The high level of K in soils in habitats of sampled populations (Table 2) may improve efficiency of plant water use (Egilla et al. 2005, Sangakkara et al. 2011).

The genetic distinction between the high-elevation populations and the low-elevation populations (Fig. 7) may suggest genetic isolation by distance of the 2 population groups. The high-elevation population groups, including Tor-2300 and Tor-2500, had relatively greater leaf area than the low-elevation population groups (Table 3). This difference may further suggest underlying morphological characteristics of *S. rotundifolia* that are adaptive to different environmental conditions in the species' native habitats. Hence, genetic variation could be exploited in cultivar selection of *S. rotundifolia* for use in LWLs.

Shepherdia rotundifolia appears able to adapt morphologically to light, temperature, and drought—environmental conditions that are very relevant to low-water landscaping. Morphological traits that allow tolerance to hot, dry climates would be advantageous in extensively paved or mulched urban landscapes that also generate high heat loads (Kjelgren and Clark 1993) or are minimally irrigated. Further extending the parallels, *S. rotundifolia*'s adaptation, including evergreen leaf habit, to dry, infertile desert soils suggests that the species should tolerate commonly infertile urban soils (Lorenz and Kandeler 2005). Similarly, low-light response traits in the high-elevation population would be an advantage in shaded conditions under larger trees and next to buildings or under tree canopies that typify urban landscapes (Kjelgren and Clark 1992, Chen et al. 2005).

However, even with its adaptations to a variable environment, *S. rotundifolia* is difficult to establish in urban landscapes. Anecdotally, *S. rotundifolia* plants growing in urban soil conditions often show a yellowing in old leaves that progressively spreads to new growth and ultimately kills the whole plant.

This response may be consistent with K deficiency symptoms (Amtmann and Armengaud 2007). Further work is needed to determine whether K deficiency indeed leads to mortality of *S. rotundifolia* in urban landscapes. The use of plants adapted to environmental conditions similar to a given landscape environment, as well as K fertilization in managed settings, may facilitate use of *S. rotundifolia* to increase biodiversity in urban low-water landscapes.

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