Amsonia kearneyana (*Apocynaceae*) Kearney's Blue Star:

New Insights to Inform Recovery

by

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ABSTRACT

Amsonia kearneyana is an endangered herbaceous plant endemic to a small area of the Baboquivari Mountains in southern Arizona. It exists in two distinct habitat types: 1) along the banks of a lower elevation ephemeral stream in a xeroriparian community, and 2) a higher elevation Madrean oak woodland on steep mountain slopes. Half of the largest known montane population (Upper Brown Canyon) was burned in a large fire in 2009 raising questions of the species capacity to recover after fire. This research sought to understand how the effects of fire will impact *A. kearneyana's* ability to recruit and survive in the burned versus unburned areas and in the montane versus xeroriparian habitat.

I compared population size, abiotic habitat characteristics, leaf traits, plant size, and reproductive output for plants in each habitat area for three years. Plants in the more shaded unburned montane area, the most populated population, presented with the most clonal establishment but produced the least amount of seeds per plant. The unshaded burned area produced more seeds per plant than in the unburned area. Lower Brown Canyon, the xeroriparian area, had the fewest plants, but produced the most seeds per plant while experiencing higher soil temperature, soil moisture, photosynthetically active radiation, and canopy cover than the montane plants. This could indicate conditions in Lower Brown Canyon are more favorable for seed production.

Despite ample seed production, recruitment is rare in wild plants. This study establishes germination requirements testing soil type, seed burial depth, temperature regimes, and shade treatments. Trials indicate that *A. kearneyana* can germinate and grow

in varied light levels, and that soil type and seed burial depth are better predictors of growth than the degree of shade.

Finally, this study examined the law, regulation, policy, and physiological risks and benefits of a new management strategy and suggests that "conservation by dissemination" is appropriate for *A. kearneyana*. Conservation by dissemination is the idea that a protected plant species can be conserved by allowing and promoting the propagation and sale of plants in the commercial market with contingent collection of data on the fate of the sold individuals.

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CHAPTER 1

HOW AMSONIA KEARNEYANA RELATES TO OTHER AMSONIAS IN THE SOUTHWESTERN UNITED STATES, IN TERMS OF RARITY AND MANAGEMENT.

Plant species listed as Endangered all have a unique story. The understanding of that story can be informative in explaining why they are rare and if human intervention can assist them in recovery. The goal of this chapter is to provide history of human interactions with *Amsonia kearneyana*, describe how we came to know its status, and compare its status to others in the genus.

History:

The location of the type specimen of *Amsonia kearneyana* (collected 1926 and 1928 by F. Thackery) is South Canyon on the western side of the Baboquivari Mountains in Arizona (Phillips & Brian 1982). It was described by Robert E. Woodson, Jr. in 1928 and named after T.H. Kearney who brought it to the attention of the author and furnished him much of the current knowledge of the genus in in Arizona (Phillips & Brian 1982). Originally thought to be a sterile hybrid of the subgenera *Sphinctosiphon* and *Articulata* (possibly a cross between *A. palmerii* and *A. tomentosa*), Woodson considered *A. kearneyana* the most recently evolved *Amsonia* (McLaughlin 1982). However, due mainly to its assumed sterility, there was some debate surrounding *A. kearneyana's* status as a distinct species (Phillips & Brian 1982). Seeds from this only known population were subsequently tested by Steve McLaughlin who observed a 66% germination rate in the greenhouse (Phillips & Brian 1982). It was hypothesized that the original seeds collected were sterile due to damage inflicted by the stinkbug (*Chlorochroa ligata)*, common in the area, and known to predate on another Arizona *Amsonia*, *A. grandiflora* (Phillips & Brian 1982). In his 1982 "Revision of the Southwestern Species of Amsonia", McLaughlin retains *A. kearneyana*'s status as a distinct species based on its distinct morphological characteristics (McLaughlin 1982).

Amsonia kearneyana's extremely low population size prompted the species candidacy for federal listing under the *Endangered Species Act of 1973*. As such, in April 1982, Barbara Phillips and Nancy Brian were retained to survey the known habitat area and search for more plants. Phillips and Brian searched a 4.8ha area surrounding the one population in South Canyon and found only 25 individuals including one seedling and 24 adult plants (at a density of 25 plants per 12,000 square meters) (Phillips & Brian 1982). All plants except the seedling were flowering or fruiting with up to 50 stems per plant. The plants did not appear to be browsed despite the presence of cattle in the area and evidence of degraded conditions from grazing (Phillips & Brian 1982). Based on its extremely low population size, low replacement rate, and narrow range, Phillips and Brian advised the United States Fish and Wildlife Service (USFWS) to list the species as endangered (Phillips & Brian 1982). They also stated that the "species would be an excellent candidate for propagation and re-establishment in other favorable habitats" (Phillips & Brian 1982). *A. kearneyana* was listed as endangered by USFWS on January 19, 1989 (USFWS 1986).

This small population had declined to eight plants by the late 1980s, attributed to disruption of the hydrological cycle by overgrazing (Reichenbacher et al. 1994). Transplant efforts were undertaken to augment population size of *A. kearneyana*. In

1987, Howell searched many of the drainages in the east slope of the Baboquivari for potential reintroduction sites and for additional populations (Reichenbacher et al. 1994). (At that time, *A. kearneyana* had not been discovered in the higher elevations of the Baboquivaris, so high elevation areas were most likely not searched.) Though no new populations were found, Lower Brown Canyon, currently within the Buenos Aires National Wildlife Refuge, was identified as the best site for transplantation efforts. Lower Brown Canyon was private land at the time of the transplants and retired from grazing, with land owners sympathetic to the transplanting effort. Since its transfer to the Buenos Aires National Wildlife Refuge in 1993, this area has been protected from unsupervised public access and is managed for the conservation of local flora and fauna.

In 1988-1992, Reichenbacher and a team of researchers and volunteers transplanted a total of 245 two-year or four-year old plants of *A. kearneyana* to the riparian zone of ephemeral Brown Canyon, hereafter referred to as Lower Brown Canyon (Reichenbacher et al. 1994). Seeds used for his transplantations and study originated from the South Canyon population (Reichenbacher et al. 1994). Transplants were placed in two areas along the riparian area of Lower Brown Canyon that were separated by a 230m wide road which crossed the stream. In 1994, Reichenbacher et al. reported that only 64 of the original 245 plants were still alive. Many of these had been inundated (or scoured) by catastrophic floods in the area. Personal communication with Steve McLaughlin in 2013 indicated that as of the late 1990's, approximately 30 plants were surviving in this area. Unfortunately, records detailing the precise locations, plant heights, and number of surviving individuals recorded during this survey are no longer available.

South Canyon is currently managed exclusively by the Tohono O'Odham, and little is known of the current status of this or other possible populations by anyone outside of the tribal community. However, large patches of *A. kearneyana* likely exist in the upper elevations of Fresnal Canyon (a canyon on the tribal side of Baboquivari Mountain), where pools of water are more prevalent (personal communication with Dr. David Brown, 2014).

Most of the location information for *A. kearneyana's* in situ populations (including those in Upper Brown Canyon and Thomas Canyon) originated from Donovan's thorough searches of the Baboquivari peaks and drainages (Donovan 1998). Donovan found several small populations on the steep canyon slopes at higher elevations (1200-1800 m). The largest population was reported to be in Upper Brown Canyon and consisted of approximately 300 individual plants (Donovan 1998; USFWS 2013). Population size of the plants identified by Jim Donovan, Dan Austin, and others varied in abundance from 1998 to 2009, based on GIS data supplied by USFWS (Fig. 1). In June 2009, the Elkhorn Fire burned through parts of *A. kearneyana's* known habitat in the Baboquivari Mountains, creating further uncertainty around the status of its population. Surveys conducted by USFWS in 2012 and by ASU in 2013-2015 indicate this population remains intact. Donovan considered the Upper Brown Canyon population as one continuous population (Jim Donovan personal communication 2013). However, since the Elkhorn Fire burned through approximately half of this population, affecting the vegetation structure, I treat the two halves separately for the sake of analysis and hereafter refer to them as Upper Brown Canyon burned and Upper Brown Canyon unburned. (Fig. 2)

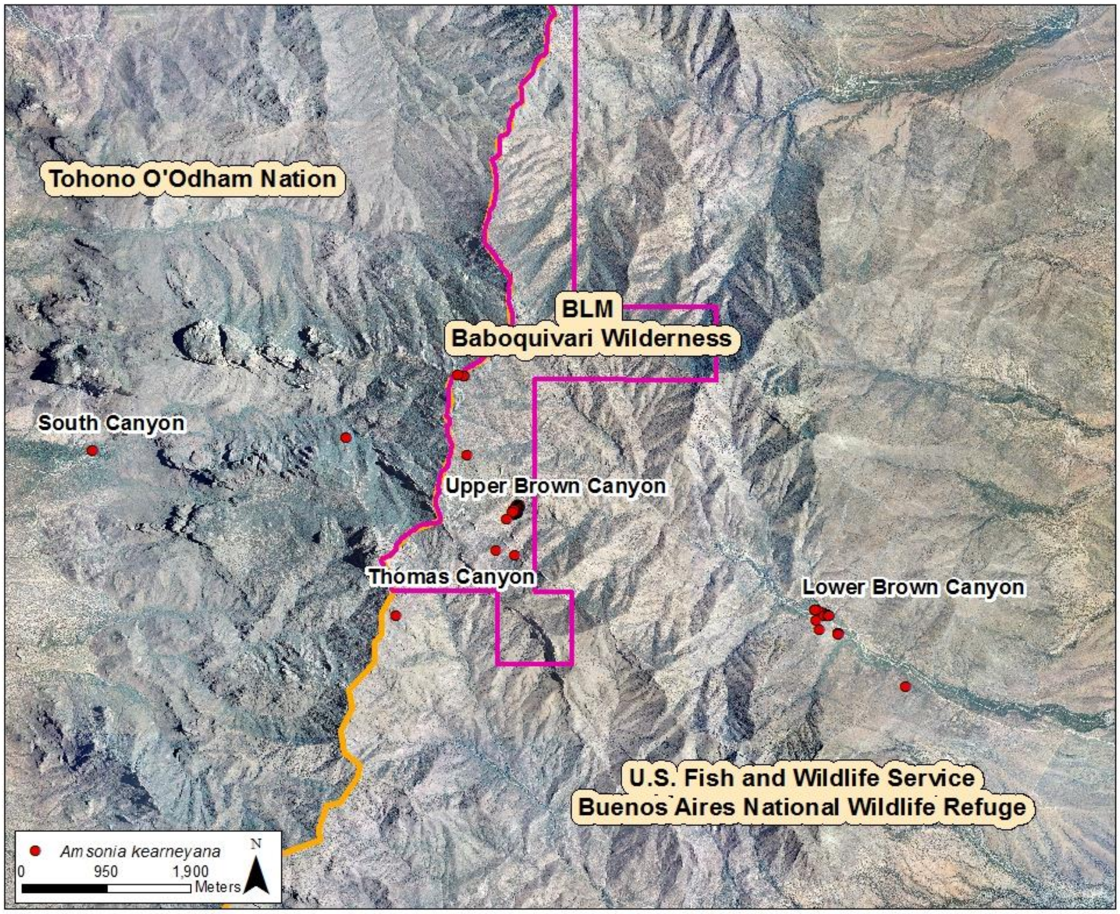


Figure 1: Map of known Amsonia kearneyana populations and administrative units. Baboquivari Mountains, Arizona.

Land Ownership:

Amsonia kearneyana exists entirely within the Baboquivari Mountains, which covers an area of 350 km^2. Extant populations occur on protected and non-use land managed by the Bureau of Land Management (Upper Brown Canyon) and the U.S. Fish and Wildlife Service (Buenos Aires National Wildlife Refuge; Lower Brown Canyon), as well as on the tribal lands of the Tohono O'Odham Nation. No known wild individuals are known to occur on private or State of Arizona lands. (Fig.1).

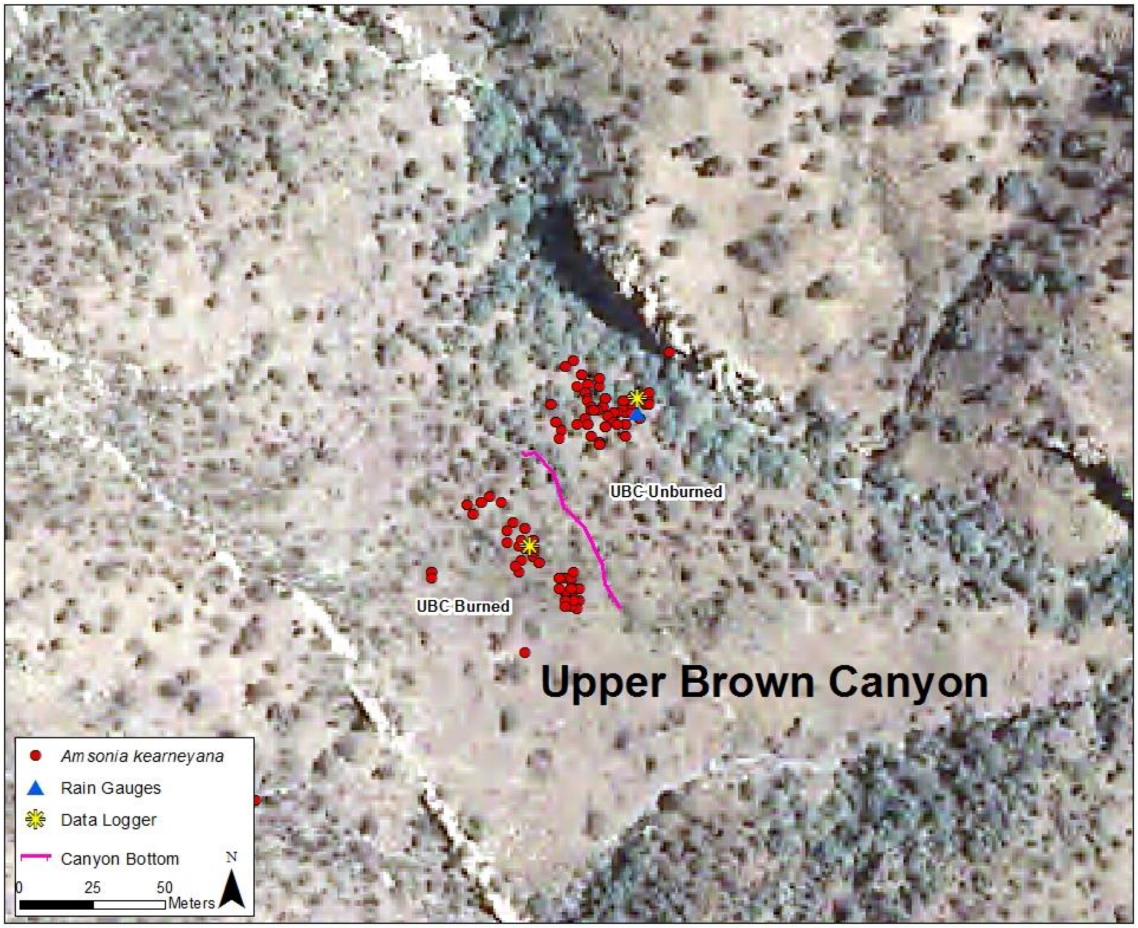


Figure 2: Upper Brown Canyon populations of *Amsonia kearneyana*. The areas burned in the Elkhorn Fire of 2009 still have spare vegetation (photo data, 2010).

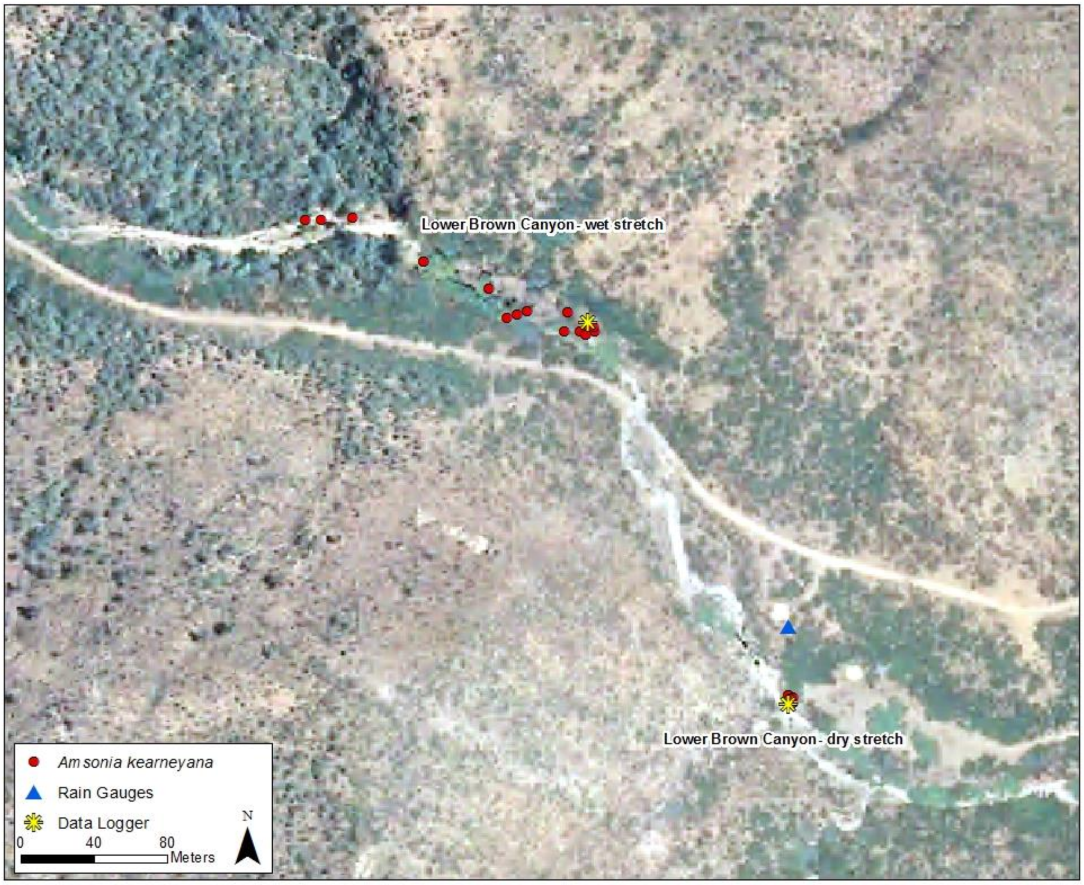


Figure 3: Map of Lower Brown Canyon populations of *Amsonia kearneyana*. Photo date 2010.

Similar Species:

Seven species of *Amsonia* are known to occur in Arizona. Several are considered rare. *Amsonia grandiflora*, a Forest Service sensitive species known from the Patagonia and Atascosa/Pajarito Mountains and northern Mexico, was considered but denied listing under the *Endangered Species Act* in 1993 due to lack of information (USFWS 1993c). It's sparse (11-25 individuals) clustered populations exist in only two mountain ranges (AGFD 1998; NatureServe 2015). This species occurs in similar habitat types as *A. kearneyana*, but seems to prefer canyon bottoms (AGFD 1998). Like *A. kearneyana*, *A.*

grandiflora also seems to have high reproductive potential, low recruitment, but stable populations due to low mortality (AGFD 1998).   Examination of *A. grandiflora* plants after a fire on private land suggests that fire does not pose a threat to established plants (NatureServe 2015). "The robust perennial rootstock allows for good regenerative abilities after burning" (NatureServe 2015) Unlike *A. kearneyana*, *A. grandiflora* seems most threatened by the effects of cattle grazing (trampling, habitat degradation) and habitat encroachment from humans (NatureServe 2015).

Amsonia peeblesii is an Arizona endemic found predominantly on the Navajo Nation in the Little Colorado River watershed. *A. peeblesii* grows in grasslands and Great Basin desert scrub communities mainly in alkaline soils. It is considered a Forest Service sensitive plant, a Bureau of Land Management (BLM) sensitive candidate, and vulnerable in Arizona, according to NatureServe (2015). Once also a candidate for listing by USFWS, *A. peeblesii* was denied because the species was determined more abundant and widespread than previously believed (USFWS 1990a).

Amsonia jonesii is a BLM sensitive plant located in northeastern Arizona, New Mexico, Utah, and Colorado. It is found in desert-steppe, rocky gorges and canyons in clay, sandy, or gravely soils (CSU 2012). It is said to be threatened mainly by off road vehicle use (CSU 2012).

Amsonia longiflora is more common in Texas and New Mexico, but is found in Arizona on the Coronado National Forest near Patagonia in canyon bottoms on a coarse, gravely substrate.

Amsonia tomentosa (also called *Amsonia brevifolia*), is common in northwestern Arizona, California and Utah at lower elevations than most other Amsonias in the American Southwest.

Considered to be *A. kearneyana's* closest relative (Topinka 2006), *Amsonia palmeri* is widespread across Arizona, New Mexico, and Texas. It, too, was once a candidate for listing as an endangered species until it was discovered to be more abundant than previously thought (Topinka 2006). *Amsonia palmeri* is found between 760 m and 1,370 m elevation in the open or among shrubs (NatureServe 2015). Like many *Amsonias*, *A. palmeri* often grows along streams and washes, in sandy soil (NatureServe 2015).

In 2009, USFWS found *Amsonia tharpii* warranted for listing. However, its listing was precluded by other species with higher recovery needs at the time (USFWS 2009c). It remains listed as endangered by the state of New Mexico and as a BLM sensitive species (Roth 2013). *A. tharpii* is threatened by human caused habit degradation and habitat encroachment, as well as by trampling and habitat destruction from grazing (USFWS 2009c). Their habitat is shortgrass grasslands or shrublands (USFWS 2009c), or Chihuahuan desert shrub communities (Roth 2013), in well drained sand, silt, or clay soils (USFWS 2009c). All populations are found in a substrate which contains gypsum (Roth 2013). A few small populations exist in New Mexico (50-100 plants) (Roth 2013) and two larger populations (a few thousand individuals) exist in Texas (USFWS 2009c).

CHAPTER 2

HOW HABITAT CHARACTERISTICS INFLUENCE GROWTH AND REPRODUCTION IN AMSONIA KEARNEYANA

Question: How does the population size and reproductive output vary among habitat types, specifically burned v. unburned and montane v. riparian?

Introduction

Wild *Amsonia kearneyana* plants are found in two distinct habitat types. The first is a lower elevation Interior Southwestern riparian deciduous forest and woodland community (Brown 1982). The second is a higher elevation Madrean oak woodland. It is not clear how plant performance of *A. kearneyana* varies among these montane and meso-riparian habitat types. Both habitat types experience periodic ecological disturbance, from fires and floods respectively. Though *A. kearneyana* does not seem to be habitat limited within its range, its narrow geographic range makes it more susceptible to the threats posed by environmental change or ecosystem disturbance compared to species with a wider geographic range (Martinez-Sanchez 2011). I sought to understand how each habitat type influenced the species by asking how the population size and reproductive output vary between montane and riparian habitats, and how fire influences the montane populations.

Methods

Study Sites

I selected study areas in two distinct habitat types. The first is the lower elevation (1145 m) (Phillips & Brian 1982) habitat of the first discovered South Canyon population characterized as an Interior Southwestern riparian deciduous forest and woodland community (Brown 1982). This relatively flat (0-5% slope) gravely, dry, rocky wash (ephemeral to intermittent flow) lies over a granite substrate and drains to the northeast from the base of Baboquivari Mountain (Phillips & Brian 1982). This area is within the Buenos Aires National Wildlife Refuge and has not been grazed since the Refuge was established in 1993. I subdivided this area into a drier and wetter section of the stream.

The second habitat type is a higher elevation (1200-1800 m) Madrean oak woodland montane area in the Baboquivari Mountains. Most plants are found near the tops of drainages on unconsolidated steep slopes of 20 to 30 degrees with sparse coverage by oak (*Quercus*) trees, though some are in the open (Donovan 1998). Dominant species include *Quercus oblongifolia*, *Quercus emoryi*, *Acacia greggi*, *Dasylirion wheeleri*, *Crossossoma bigelovii*, *Agave schottii*, and perennial grasses (Donovan 1998). The rugged terrain makes this area inaccessible to grazing and much human activity. The Bureau of Land Management (BLM) manages most of this habitat area on the eastern side of the Baboquivaris. The west side of the mountain is owned by the Tohono O'Odham nation. I subdivided the montane area into areas that were and were not burned by a 2009 fire (the Elkhorn fire).

Both habitat areas have a bimodal precipitation regime. However, the montane area may receive slightly more moisture due to its geographic relief. It presumably is colder, with frost possible during December and January (Donovan 1998).

Population Size

I obtained permits from the Buenos Aires National Wildlife Refuge, U.S. Bureau of Land Management, and U.S. Fish and Wildlife Service to assess the current population size of the wild populations of *A. kearneyana* in Brown Canyon. Field visits were made in November and December 2012 and February, June, August and September 2013 to several areas in Brown Canyon and associated canyon slopes (Fig. 1). During each trip, I used a Garmin Montana 650t GPS unit to record the latitude, longitude, and elevation of each plant that we could safely access. I recorded plant height and probable ramet growth for each plant as it was georeferenced. In addition, I noted the number of plants which could be seen from a distance but not accessed safely, to add to the approximate total population.

Abiotic Environment

In September 2013, I purchased two digital rain gauges with internal data loggers to better understand the local precipitation regime experienced by two *A. kearneyana* populations. One rain gauge was placed near the unburned montane population, Upper Brown Canyon B (UTM 12R 044873 351441 elevation 1492 m) and the other near the drier Lower Brown Canyon population (elevation 1206 m). Rain gauges were removed in July 2015, revealing a complete malfunction of the montane gauge.

I installed Hobo data loggers and photosynthetically active radiation (PAR) sensors and soil moisture and soil temperature sensors in the four habitat areas in July 2014 to compare the microclimatic conditions of the burned and unburned montane populations as well as the wetter (mesoriparian) and drier (xeroriparian) stretch of the Lower Brown Canyon riparian populations. The soil temperature and soil moisture sensors were buried approximately 8 cm below the soil surface, and the PAR sensors were exposed to sunlight, perpendicular to flat ground at 6 cm above the surface. Data loggers logged four data points per day (one point every 6 hours) throughout the experiment period (2014 to 2015). I collected these data loggers in July 2015.

Canopy Cover, Plant Density, and Associated Species

To determine average canopy cover and species density, I randomly selected two to three 2 m x 10 m plots within each habitat area using the random point generator function in ArcGIS. During September 2013, I recorded canopy cover at three points per plot with a densiometer and noted the number of *A. kearneyana* plants within each plot. At each of these sites, I also measured diameter of all woody plant stems, by species and sampled herbaceous cover, by species, in 6 1-m2 quadrants. At the same time, all plant species within each plot was recorded, noting dominant species, to determine species associated with *A. kearneyana* in each habitat type. For unknown taxa, a collection was made for later identification.

Leaf Traits

I collected leaves from 13 to 20 wild adult plants per habitat area (55 total) during July 2015. Leaves from the very top of the stem may not have been fully developed, and leaves from near the bottom of the stem would have been too self-shaded, so leaves were collected from mid-stem on an outside stem of the plant most likely to receive maximum sunlight. Due to recent monsoon rain activity, leaves were moist when collected. I stored them in individual sealed plastic bags until weight could be measured in the lab. I weighed leaves within 24 hours of field collection. Immediately after leaf wet weight was taken, I measured leaf area using a flatbed scanner and ImageJ software. Since *A. kearneyana* leaves are malleable and easily lay flat on a scanner, no further processing was necessary. Leaves were then dried for 72 hours in an oven at 90 degrees and then weighed again to obtain leaf dry weight in milligrams. I divided leaf area (mm) by leaf dry weight (mg) to obtain specific leaf area (CONICET-UNC 2014).

I also collected leaves from wild adult plants during July 2015 for stomatal density measurements. Stomatal density was measured on thirty leaves, one leaf per plant (10 leaves from the burned area of Upper Brown Canyon, 10 leaves from the unburned area, and 10 leaves from Lower Brown Canyon, three from dry stretch plants and seven from the wet stretch plants). Since only three plants were present in the dry stretch, I analyzed the wet and dry stretch as one population. I placed a 1sq mm cover on the largest area of the leaf which was not directly on top of midrib or secondary veins.

Stomatal counts were recorded only for the abaxial (lower) surface of leaves though some stomata were seen on adaxial side. I used a one way analysis of variance (ANOVA) to detect any differences in stomatal density between the three populations.

Soil Chemistry and Particle Size

I collected soil samples for analysis from the upper 10cm of soil, for each habitat area. Samples were collected in the burned area of Upper Brown Canyon and Lower Brown Canyon in November 2012, and in the unburned part of Upper Brown Canyon in June 2013. The soil was analyzed by Motzz Labs, Phoenix, Arizona, for pH, organic matter, and content of various minerals.

I analyzed soil for particle size in a lab at Arizona State University using a hydrometer method (Bouyoucos 1962), after first sieving the soil to remove particles >2mm. A control solution was first created by mixing 100ml of dispersing solution (5% $(NaPO_3)_6$) with 880ml room temperature deionized water. Soil samples were weighed and recorded to the nearest 0.01g, and mixed with 100ml of dispersing solution using an electric mixer. This mixture was then transferred to a 1000ml cylinder. Room temperature deionized water was added to the 1000ml line and then mixed well with a plunger to resuspend the solids. The temperature of the mixture and the control solution was recorded as well as the hydrometer reading for the control. After 40 seconds, the hydrometer reading of the mixture was recorded. Since the sand settles out of the mixture at this time, this reading reflects the amount of clay and silt remaining. Another hydrometer reading was recorded at 6 hours and 52 minutes. This represents the amount of clay still suspended as the silt had settled out. Percent sand, clay, and silt were calculated correcting for temperature and previous hydrometer calibration.

Reproductive Output

I recorded GPS coordinates for up to 20 *A. kearneyana* plants in each habitat area (or as many as existed there) in July of 2013, 2014, and 2015. To determine reproductive potential and plant size, I gathered data on plant height and width (cm), number of stems, fruits per stem, rhizomatous growth (indicated by presence of ramets). Though impossible to verify ramet production without disturbing the roots, observations of probable ramet clones were noted. I searched for seedlings as an indicator of recruitment. Stems were defined if they extended completely to the plant base. Where stems did branch, they were included with their parent stem. I collected one or two fruits per plant in each year to determine number of seeds per fruit and thus per plant. Seeds per plant, number of stems, and plant height were compared between the four habitat areas, for 2013, 2014, and 2015, using two way analysis of variance and post-hoc comparisons using the Holm-Sidak method. Pearson product moment correlation was used to detect correlation between number of stems and plant height. To investigate seed predation, I collected any insects or eggs observed on plants or seeds and vouchered them for further identification at an insect lab at Arizona State University. Evidence of predation was documented qualitatively.

Results

Population size

Upper Brown Canyon: Jim Donovan (1998) identified the largest known population of *A. kearneyana*, 300 individuals, in Upper Brown Canyon. In revisiting the segment of this population which had burned in the 2009 Elkhorn Fire, in November 2012, I located and georeferenced 43 individual plants. Plants are easily detected in November, after their seasonal color change to a bright yellow so individuals were easily seen from a distance and delineated from the surrounding vegetation. This is an important advantage in finding new plants in this steep terrain (30-45% slope). While more plants could be seen but not reached due to the difficult terrain, and still others may have been missed, I estimate that the Upper Brown Canyon burned population contains approximately 68 individuals. No obvious ramet clones were identified in this area.

A visit in mid-June 2013 to the unburned portion of this previously identified population yielded especially encouraging results, with 128 individual plants georeferenced. Another 150 plants could be seen across the canyon in areas too steep to access safely. Adding these plants to the previous 68 found in the burned area, I approximate the total for the Upper Brown Canyon population to be 346 individuals, a gain of 46 plants from the time of Donovan's searches (Donovan 1998) (Table 1). This total may however overestimate individuals, as some plants observed may have been ramet clones. This was especially evident during the field visit in 2015 when at least half of the plants in this population had surrounding ramets. These plants presented with generally shorter stems and fewer fruit, and grew adjacent to another plant approximately

30 cm away. This pattern continued in a downhill direction, up to six times in a row. These cases did, however, occur in patches of many larger plants so individual ramet clones were difficult to delineate.

A thorough search of Lower Brown Canyon in June 2013 yielded 15 individuals, all less than 40.5cm in height and seemingly vigorous. Two individuals had ramet growth. In one of these cases, the surrounding soil had eroded to such a degree as to expose the underground stem connecting the parent plant to the ramet. This connecting underground stem was approximately 30cm in length. By November 2014 this ramet was noted to be torn nearly off of the parent plant near the root. Surrounding soil had been scoured from the root during a large flooding event in September 2014 further exposing the roots. In a later visit, that ramet clone was gone. It is also postulated that one of the plants in the dry stretch of Lower Brown Canyon is actually a ramet of the largest plant in that area

Table 1. Population numbers of *A. kearneyana* at each surveyed site.

Population	Elevation (m)	Number geo-referenced	Additional viewed	Approx. population total
Upper Brown Canyon A	1500	43	25	68
Upper Brown Canyon B	1494	128	150	278
Lower Brown Canyon	1159	15	0	15
Totals:		186	175	361

Vegetation

Canopy cover, plant density, and percent ground cover varied widely among populations. Lower Brown Canyon wet stretch had the highest canopy cover (62%)

followed by the unburned montane area (47%), and Lower Brown Canyon dry stretch (12%). The burned area of Upper Brown Canyon was devoid of canopy cover and had no live trees (Fig. 8). Both montane areas were rich in herbaceous species. No grasses were present in Lower Brown Canyon though they dominated in Upper Brown Canyon. The burned area had more shrubs than the unburned area. In Lower Brown Canyon, the wet stretch was dominated by mostly small diameter trees and herbs hugging the water line (which is where *A. kearneyana* plants occur). The dry stretch was more shrubby with fewer large diameter trees. Plant density of *A. kearneyana* was higher in the unburned montane area (17.5 per 20 m^2) than the burned area (2.7 per 20 m^2). No *A. kearneyana* plants were found within the random plots in Lower Brown Canyon reflecting its low species density.

The plots in the burned montane area were dominated by *Garrya wrightii*, *Gutierezia sarothrae, Mimosa aculaeticarpa* var. *biuncifera, Bidens leptocephala*, *Muhlenbergia emersleyi*, and *Mirabalis linearis* (Table 5). Burned remains of dead *Quercus* trees were evident. The unburned plots were more diverse. Dominant in these plots were *Quercus turbinella, Quercus oblongifolia*, *Quercus emoryi*, *Amsonia kearneyana*, *Bidens leptocephala*, *Gossypium sp*., and many grasses such as *Bothriochloa barbinodis*, *Bouteloua curtipendula*, *Garrya wrightii*, *Leptochloa dubia*, and *Panicum bulbosum*.

In the wet stretch of Lower Brown Canyon, *Platanus wrightii*, and *Artemisia ludoviciana,* were dominant. The drier stretch supported these same species as well as *Baccharis salicifolia, Celtis reticulata,* and *Juglans major,* and the forbs *Bidens leptocephala* and *Boerhavia coccinea,* *Abiotic Environment*

In the Upper Brown Canyon, PAR was higher in the burned area, with mean (SD) of 178 uE (333). The 47% average canopy cover of the unburned area was reflected in its reduced PAR 104 uE (206) (Fig. 1). Lower Brown Canyon experienced higher PAR than both montane areas with 211 uE (332) for the wet stretch (Fig. 4). Two large flooding events corroded the batteries in those two data loggers causing them to stop collecting data early. Data that was collected indicates PAR was similar to the burned montane area until early October and then much higher than the montane areas through the winter months likely owing to the prevalence of deciduous trees along the stream and evergreen trees on the mountain.

For the period of 01 January 2014 to 01 January 2015, Lower Brown Canyon experienced 402 mm of accumulated precipitation with the most rain occurring in July and August. The closest lower elevation weather station (in Ajo, Arizona) at a distance of 142 kilometers and an elevation of 533 m, averages 174 mm annually with the same temporal precipitation patterns. Precipitation was last measured in Lower Brown Canyon during the time *A. kearneyana* plants were being transplanted there (1987-1993). The average precipitation measured for that time period was 525 mm (Reichenbacher et al. 1994). Upper Brown Canyon likely receives slightly more precipitation. The nearest higher elevation weather station is 23 kilometers away (at Kitt Peak, Arizona) at an elevation of 2070 meters and averaged 597 mm precipitation annually from the period of 1960 to 2005 (WRCC 2005). The mean temperature in Lower Brown Canyon in 2014 was 22 °C with a range from 5 °C in December to 36 °C in July.

General temperature and precipitation patterns during the three years of the study, drawing from the Kitt Peak station show that 2013 was the wettest at 540 mm, followed by 434 mm in 2014, and 371 mm in 2012. All of these years are much drier than the 597 mm average. Although data for 2015 for this location was not yet available, Arizona generally has received unusually high amounts of rainfall in 2015. 2013 was also the warmest with a mean temperature of 18 °C compared to 11 °C in 2014 (NOAA 2015).

Soil temperature was highest in Lower Brown Canyon with a mean (SD) of 17.7°C (7.1) (Fig. 5). The burned 16.1°C (8.3) and unburned 16.3°C (6.4) montane areas were virtually equivalent to one another though the soil temperature in the unburned area was less variable. Soil moisture also was highest in Lower Brown Canyon with a mean (SD) of 0.24 m^3/m^3 (0.07) (Fig. 5). For the montane populations, soil moisture was higher in the unburned area (0.18 m^3/m^3 (0.06) than in the burned area 0.14 m^3/m^3 (0.09).

Soil Chemistry and Particle Size

Soil conditions differed sharply between the mountain slope and the riparian populations. The riparian soil was considerably sandier than the mountain soil (Table 2), and had lower content of most macro- and micro-nutrients (Table 3). Based on large-scale mapping, this soil is classified as primarily type 37: Keysto extremely gravely sandy loam with 2-8 percent slope (USDA 1961). The mountain soil is a dense sandy clay loam, and is distributed among abundant rock cover. Some of the mountain soil has high content of metals including copper and iron, reflecting underlying geology of schist and/or granite and gneiss. Most of the known mountain populations occur within two classified soil type areas. The first is type 20: Cortaro-Rock outcrop-Faraway complex with 15 to 45 percent slopes. The second is type 28: Far-Spudrock-Rock outcrop complex with 35 to 85 percent slopes. Both are well drained with 5-20 inches to lithic bedrock (USDA 1961). It is not known how deeply *A. kearneyana* can root, but based on preliminary observations of greenhouse plants, we believe 30-40 cm is a conservative range.

Table 2. Soil particle size analysis for three populations of *A. kearneyana*.

	% Clay	% Silt	% Sand
Upper Brown Canyon burned	25	14	61
Upper Brown Canyon unburned	28	10	62
Lower Brown Canyon	3	7	91

Table 3. Soil chemical properties of three populations of *Amsonia kearneyana*.

	Upper Brown Canyon burned	**Upper Brown Canyon unburned**	**Lower Brown Canyon**
pH (SU)	7.5	6.9	8.5
Electrical Conductivity, EC (dS/m)	0.35	0.3	0.17
Calcium, Ca (ppm)	3300	1900	680
Magnesium, Mg (ppm)	190	150	96
Sodium, Na (ppm)	20	14	35
Potassium, K (ppm)	310	240	43
Zinc, Zn (ppm)	4.3	6.1	0.58
Iron, Fe (ppm)	16	25	3.1
Manganese, Mn (ppm)	36	63	2.5
Copper, Cu (ppm)	1.5	1	0.32
Nickel, Ni (ppm)	0.32	0.33	0.096
Nitrate-N, NO3-N (ppm)	9.2	1.8	2.7
Phosphate-P, PO4-P (ppm)	52	24	17
Sulfate-S, SO4-S (ppm)	9.6	2.3	4
Boron, B (ppm)	0.66	0.35	0.57
Free Lime, FL ()	None	None	Low
ESP (%)	0.5	0.5	3.4
CEC (meq/100g)	19	11.4	4.5
Organic Matter (WB) (%)	4.7	4.3	0.19

Leaf Traits

Overall, mean (SD) specific leaf area of *A. kearneyana* was 4.1 mm/mg^{-1} (0.6) which was lower than expected for a herbaceous plant (Kattge et al 2011). The plants in the unburned montane population area had significantly higher SLA than those in the burned area (p=0.007) with means (SD) of 4.4 (0.6) and 3.9 (0.08) respectively. SLA was negatively correlated with the number of seeds produced per plant (p=0.03, correlation coefficient= -0.29; n=55).

The one way ANOVA did not reveal a statistically significant difference in mean stomatal density among the three treatment groups (p = 0.12). However, leaves from the burned area, with values of 46.6 (10.9) did have a higher mean stomatal density than the other two groups (37.9 for unburned montane and 40.2 for riparian). Overall, for the 30 samples the mean (SD) stomatal density was 41.6/mm (9.9) (Fig. 9)

Plant Size

As expected, plant height and number of stems were strongly correlated (p< 0.001. correlation coefficient of 0.32). Plant height was significantly different between populations over all years (p <0.001) with plants in the wet stretch of Lower Brown Canyon being the tallest (Fig. 6). Population location was also a significant factor predicting number of stems produced per plant, although patterns varied among years (p=0.017). The unburned montane population produced the most stems in 2014 and 2015 whereas in 2013 the Lower Brown Canyon plants produced the most stems (Table 7).

Reproductive Output

Plants in the wet stretch of Lower Brown Canyon produced significantly more seeds per plant than the unburned montane population during years 2013 and 2014. These were both wet years for the area compared to very dry conditions seen in 2012. However, in 2015, though local precipitation cannot yet be established, the number of seeds produced per plant was nearly identical across populations. Both montane populations produced more seeds per plant in 2013 than in 2014. Statistically, habitat type, represented by location, was significant (p=0.028). Specifically, the Lower Brown Canyon wet stretch population produced more seeds per plant than the unburned Upper Brown Canyon population regardless of year (p=0.043) (Fig. 10, Table 7). Number of stems was significantly positively correlated with seeds produced per plant, as measured in 2015 (p<0.001, correlation coefficient= 0.54).

Seed and Fruit Predation

Predation was evident on several fruits on all populations in all years, though observed more frequently in 2013. One large true bug, identified as *Chlorochroa ligata,* was seen feeding on the fruit of *A. kearneyana* in Upper Brown Canyon B. Many very small insects, identified as nymphs of *C. ligata,* were collected from inside the predated fruits. Insect eggs were present on *A. kearneyana* leaves and are consistent in appearance with eggs of this species.

Fruit predation was most frequent at Lower Brown Canyon. Nymphs of *C. ligata* were found inside and on the surface of fruits that were predated upon. Many fruits, with and without these nymphs appearing inside, seemed to be consumed from the inside out. Reichenbacher notes this same type of damage in 1988 on this same population, but he could not identify the insect predator at the time. Reichenbacher (1994) also saw *C. ligata* in the area of *A. kearneyana* but none were observed on the plants by botanists who contributed to the search efforts.

One plant, which had *C. ligata* nymphs on its fruit in June, was found to have evidence of seed predation in August when seeds had dispersed and dropped to the ground. Ten of the at least 100 seeds surrounding the plant contained small bore holes in the side of the seed coat. This is the type of damage typically associated with *C. ligata* (Reichenbacher et al. 1994). One of these seeds was still trapped in the base of the fruit, attached to the plant, indicating this damage had occurred while the seeds were within the follicle and not after dispersal. Though several like this were also seen around other plants, the vast majority of *A. kearneyana* seeds appeared robust and unpredated. Seeds still visible around plants in September 2013 showed no evidence of insect predation.

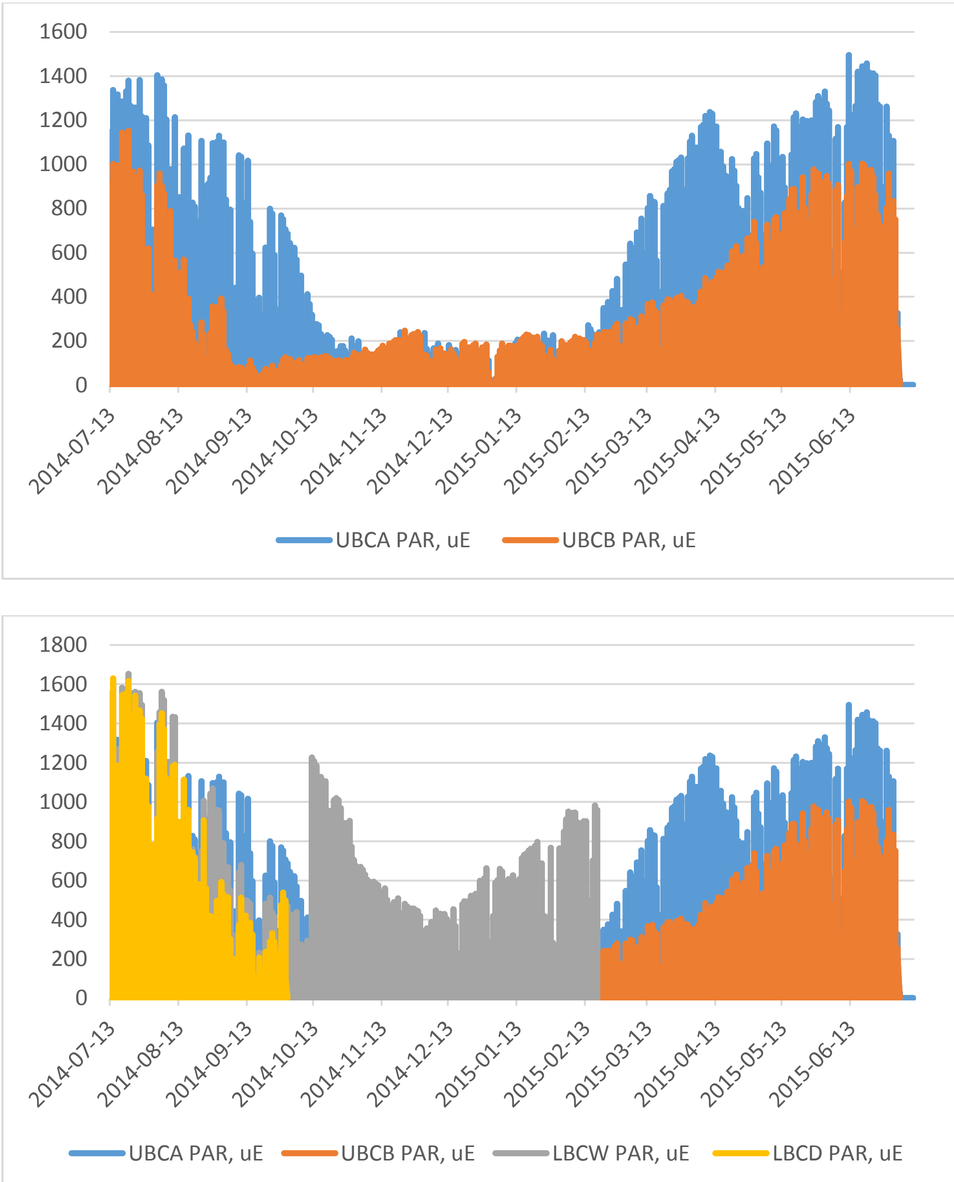


Figure 4: Photosynthetically Active Radiation (PAR) measured in microEinsteins for one year. Top panel shows two montane population areas, Burned (UBCA) and Unburned (UBCB). Bottom panel shows wet and dry stretches of Lower Brown Canyon (LBCW/LBCD)

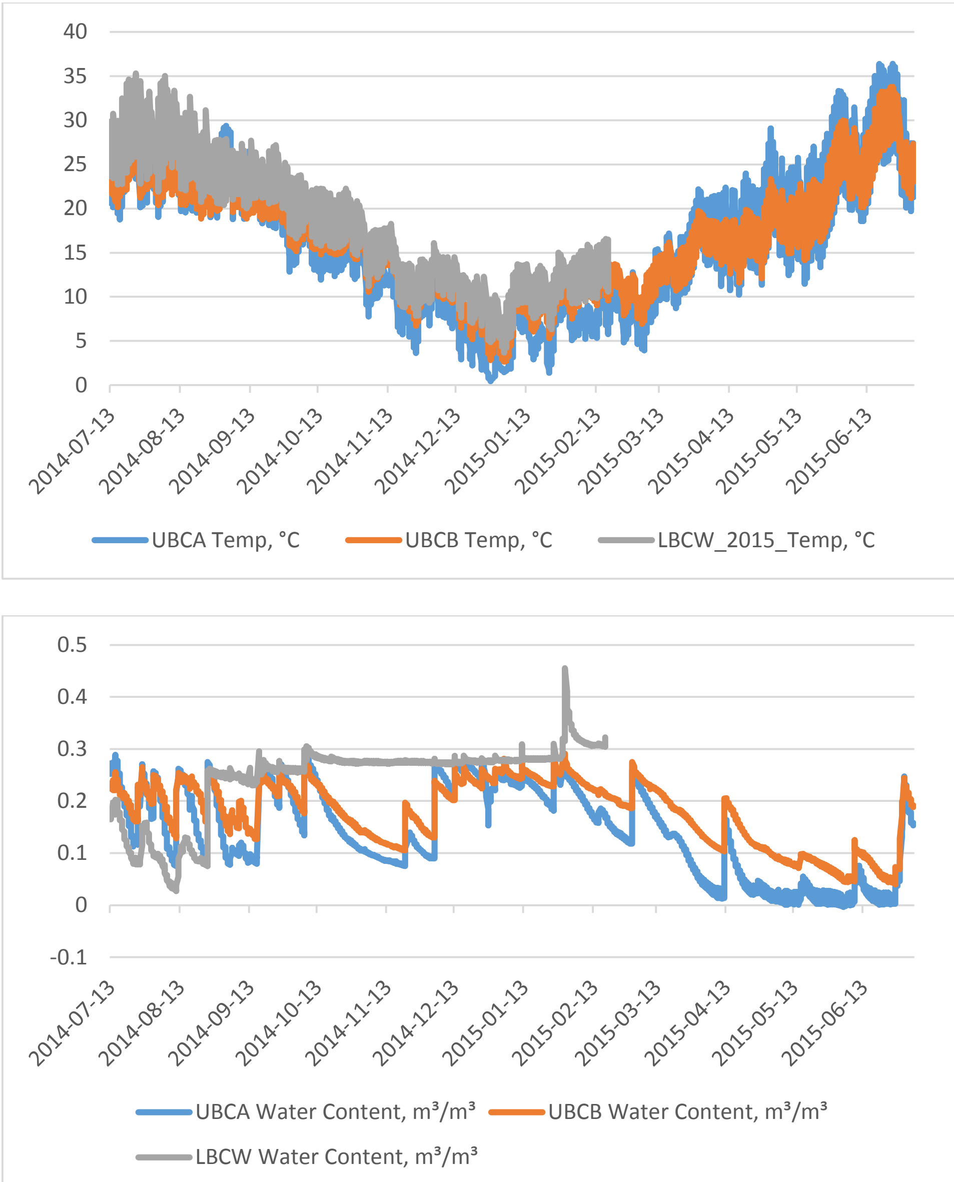


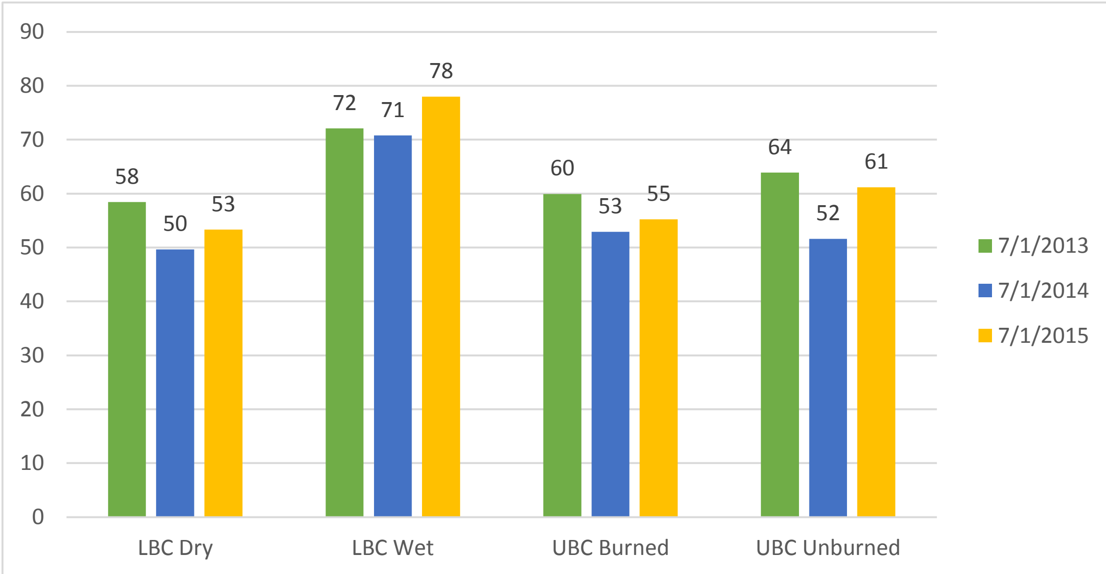
Figure 5: Soil temperature (top panel) and soil moisture (bottom panel) for one year for three population areas (Burned, UBCA; Unburned, UBCB; and the wet stretch of Lower Brown Canyon, LBCW).

Table 4. Abiotic environment.

	Upper Brown Canyon- burned	Upper Brown Canyon- unburned	Lower Brown Canyon	South Canyon (Phillips & Brian 1982)
Elevation	1492 m	1492 m	1145 m	1145 m
Slope	15-45%	15-45%	2-8%	0-5%
Aspect	Northwest	northwest	All	All
Soil Type (USDA 1961)	20: Cortaro Rock Outcrop Faraway Complex	20: Cortaro Rock Outcrop Faraway Complex	37: Keysto extremely gravely fine sandy loam	
Parent material (USDA 1961)	granite, gneiss, schist	granite, gneiss, schist	mixed alluvium	granite
Soil Texture (USDA 1961)	extremely gravely sandy loam	extremely gravely sandy loam	extremely gravely fine sandy loam	gravely wash
Dominant Soil Texture near plants	sandy clay/loam	sandy clay/loam	sand	unknown
Mean annual precipitation (USDA 1961)	40-51 cm	40-51 cm	30-40 cm	30.53 cm (Sells, AZ)
Frost free period (USDA 1961)	160-080 days	160-080 days	180-230 days	272 days (Sells, AZ)
Canopy Cover	0%	47%	62%	unknown
SLA($mm^2 mg^{-1}$)	3.859	4.403	4.055	unknown
Biotic community	Madrean oak woodland	Madrean oak woodland	riparian woodland association (Reichenbacher 1994)	Interior southwestern riparian deciduous forest and woodland

Table 5: Dominant Associated Species near five population areas of *A. kearneyana*.

Upper Brown Canyon- burned	Upper Brown Canyon- unburned	Lower Brown Canyon- wet	Lower Brown Canyon- dry	South Canyon (Phillips & Brian 1982)
Bidens leptocephala, Garrya wrightii, Gutierezia sarothrae, Mimosa aculaeticarpa var. biuncifera, Mirabalis linearis Muhlenbergia emersleyi,	*Amsonia kearneyana, Bidens leptocephala, Bothriochloa barbinodis, Bouteloua curtipendula, Garrya wrightii, Gossypium sp. Leptochloa dubia, Panicum bulbosum Quercus turbinella, Quercus oblongifolia, Quercus emoryi,*	*Platanus wrightii, Artemisia ludoviciana*	*Baccharis salicifolia, Celtis reticulata, Juglans major*, and the forbs *Bidens leptocephala Boerhavia coccinea*	*Acacia greggii, Anisacanthus thurberi Celtis reticulata, Dasylirion wheeleri, Juglans major, Ptelea trifoliata Quercus oblongifolia,*



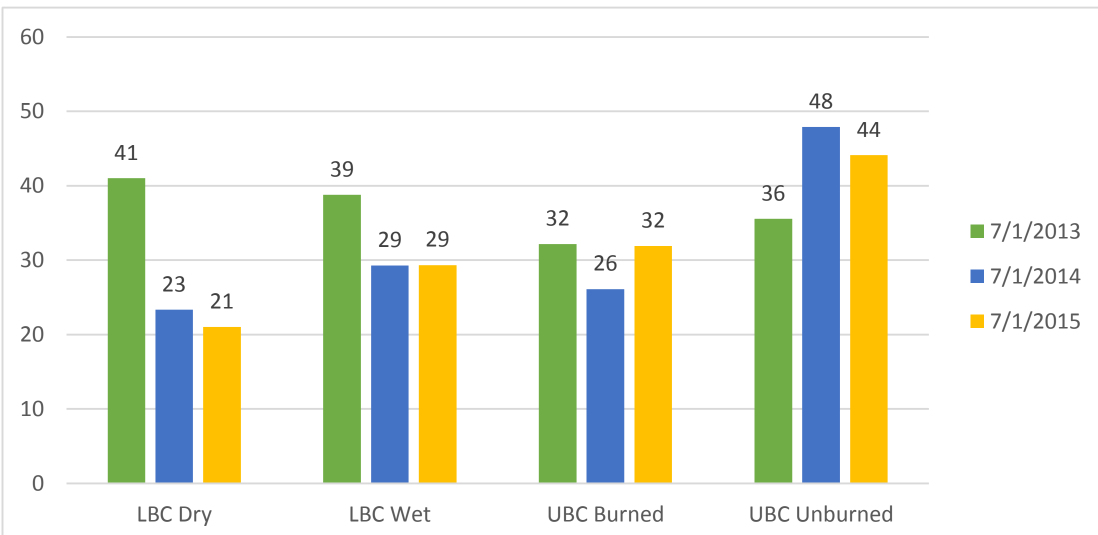


Figure 6: Average plant height (cm) (top figure) and average stems produced per plant (bottom figure) for three years in four habitat types.

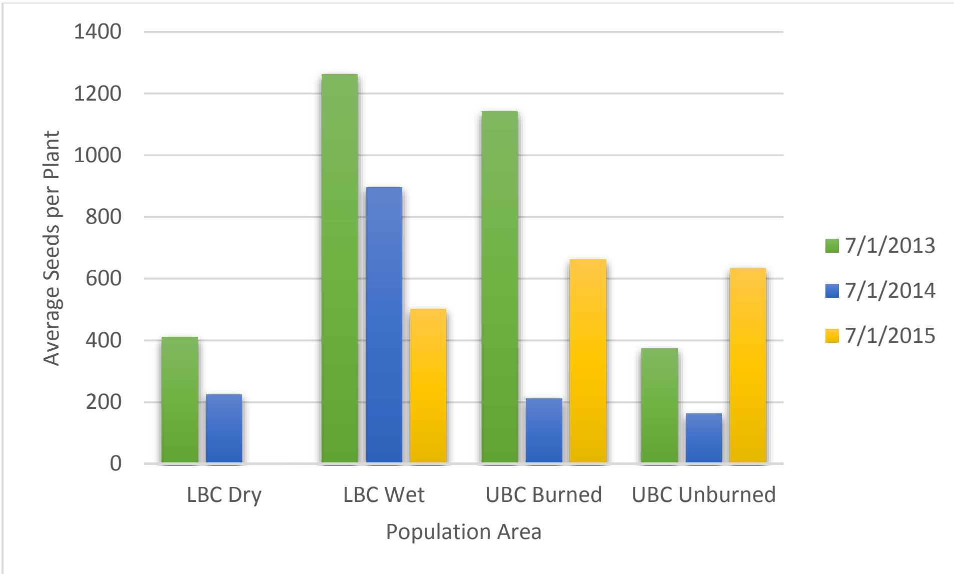


Fig. 7: Average seeds produced per *A. kearneyana* plant for three years in four habitat types.

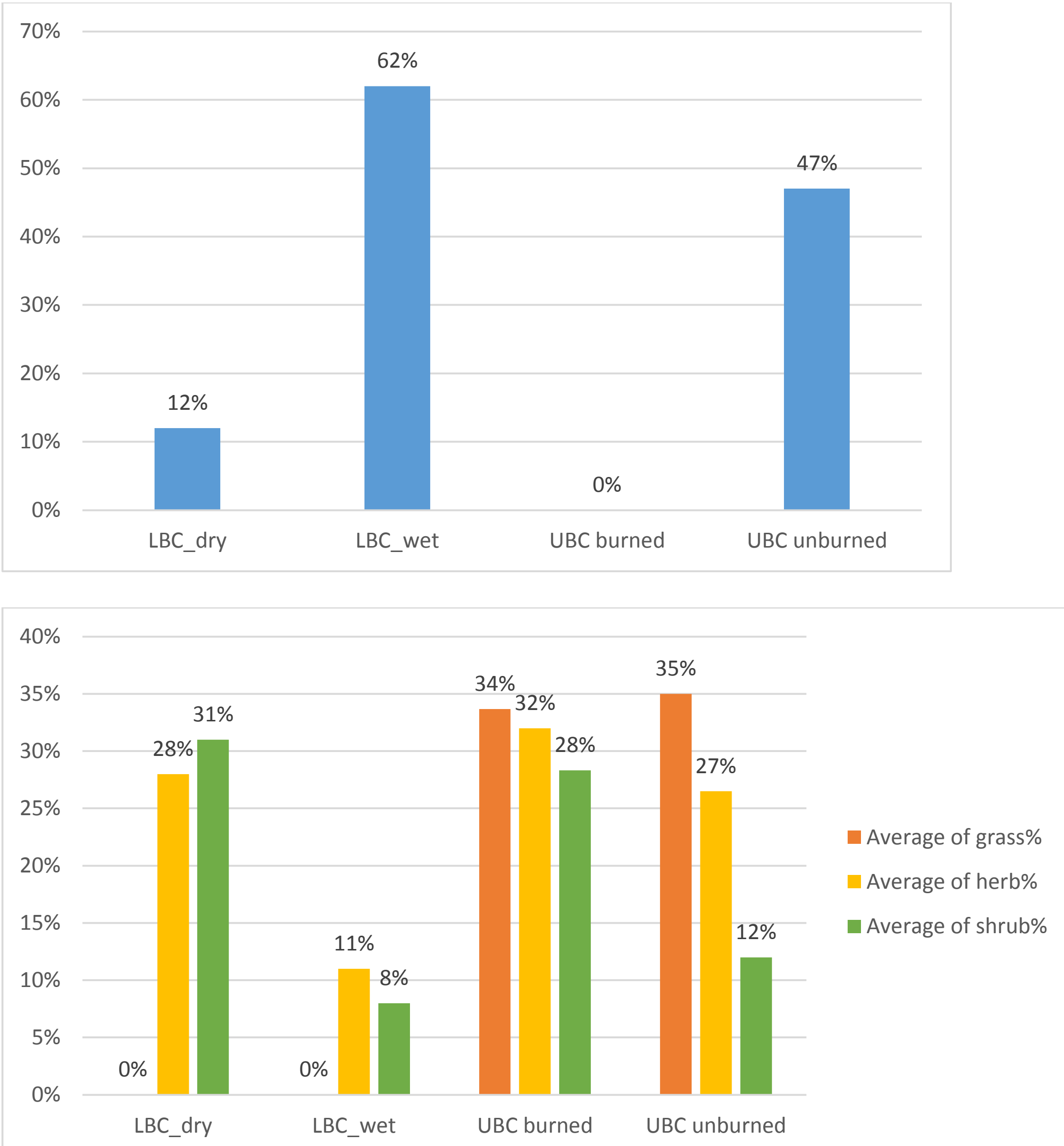


Figure 8: Canopy cover (top figure) and percent ground cover (bottom figure) for four *A. kearneyana* population areas. Data are from the late summer growing season of 2014.

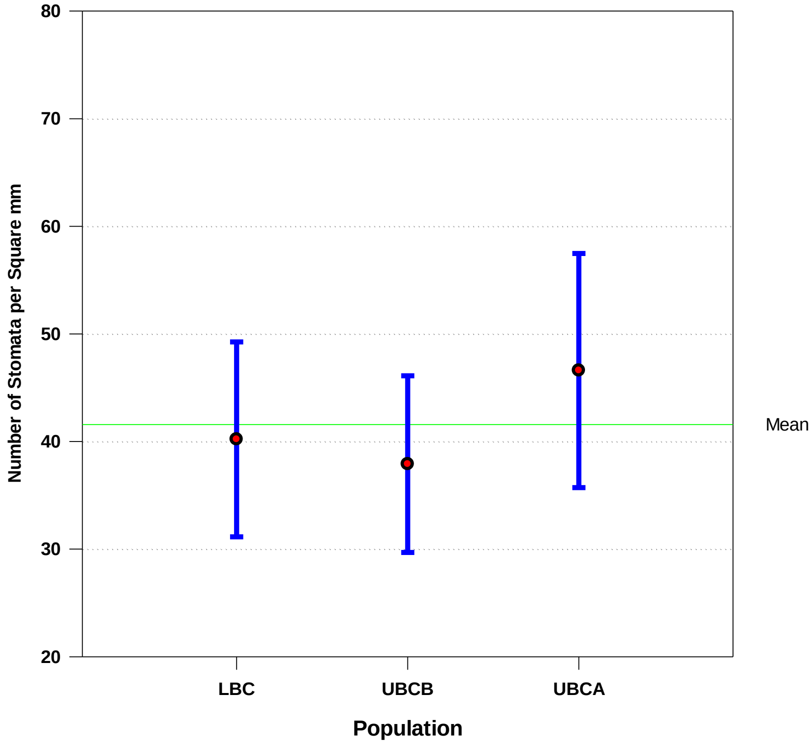
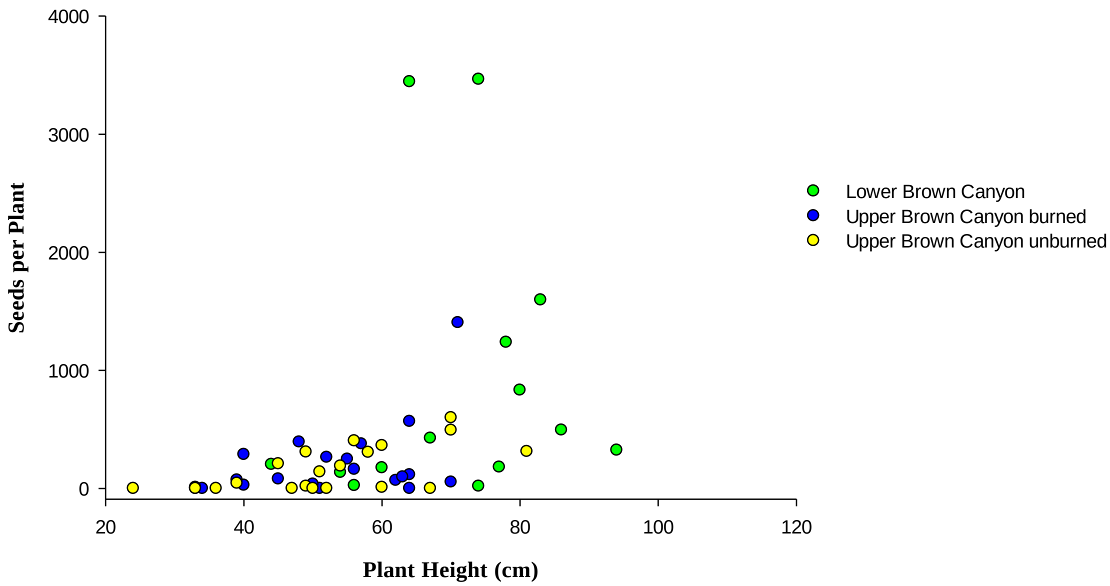


Figure 9: Stomatal density for three *A. kearneyana* population areas (Lower Brown Canyon, LBC, Upper Brown Canyon unburned, UBCB, and Upper Brown Canyon burned, UBCA) Mean and standard errors, n=30.



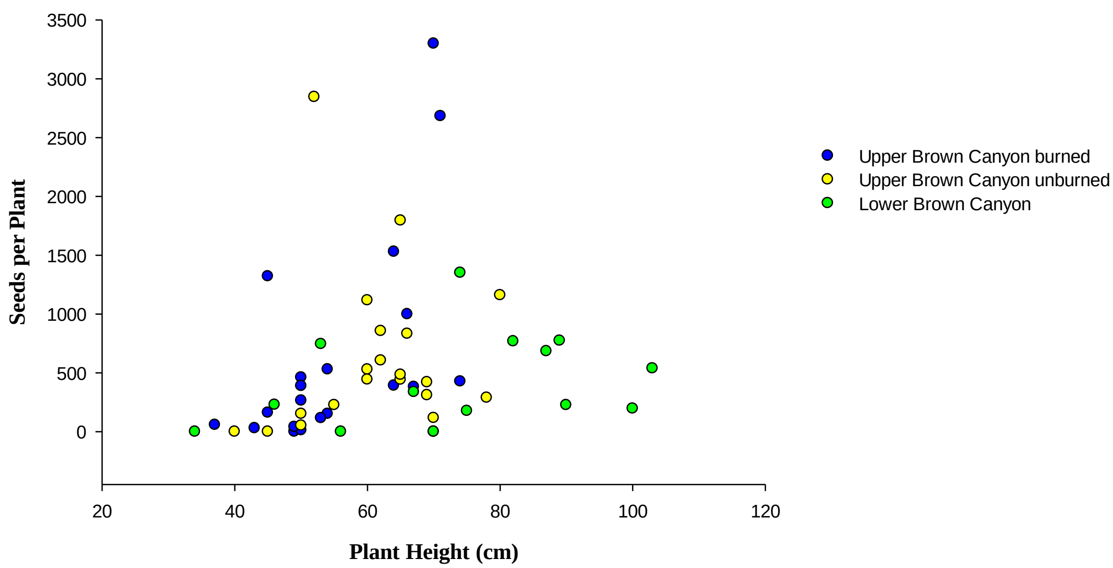


Figure 10: Seeds per plant increases with plant height in 2014 (upper) and 2015 (lower) for three population areas (Lower Brown Canyon, Upper Brown Canyon unburned, and Upper Brown Canyon burned)

Table 6: Summary of results indicating the population area of *A. kearneyana* with the greatest performance for the given parameter:

Parameter:	Results:
Precipitation	LBC = 402 mm (total for 2014)
Soil moisture	LBC > UBC unburned > UBC burned
Soil temperature	LBC > UBC burned = UBC unburned
PAR	UBC burned > LBC > UBC unburned
Canopy cover	UBC unburned > LBC > UBC burned
SLA	UBC unburned > LBC wet > UBC burned > LBC dry
Stomatal density	UBC burned > LBC > UBC unburned

Table 7: Summary of results indicating the population area of *A. kearneyana* with the greatest performance for the given parameter for each year surveyed:

	2013	2014	2015
Plant height	LBC wet	LBC wet	LBC wet
Number of stems	LBC	UBC unburned	UBC unburned
Seeds produced per plant	UBC unburned	UBC unburned	All equivalent
	(wettest year)	(driest year)	

Discussion:

This study showed the Lower Brown Canyon population produced more seeds and experienced higher soil temperature, soil moisture, PAR, and canopy cover than the montane plants particularly in the unburned area. This could indicate conditions in Lower Brown Canyon are more favorable for seed production in mature plants. However, the lack of recruitment here (and elsewhere) is concerning and should be examined further before any more reintroduction projects are considered for the species. It is possible that dispersed seeds become covered by sand and leaf litter too quickly, before they can germinate. Alternatively, emerging seedlings may be washed away by high flows so close to the water's edge. Flow regime should be established in Lower Brown Canyon and compared to flow conditions in the originally discovered South

Canyon site to determine what distance up the bank would provide less disturbance for seedling establishment.

Frugivory and granivory can contribute significantly to the available seed pool that enables expansion of existing populations. The observation of a large decline in the type locality *A. kearneyana* population in the late 1980's included speculation of post-dispersal seed granivory by Bruchid beetles (Reichenbacher et al.1994). Additionally, it has been postulated that *A. kearneyana* may also fall victim to seed granivory by *C.ligata* (McLaughlin 1982, Reichenbacher et al.1994). This study did find evidence of frugivory and granivory on *A. kearneyana*, but in insufficient abundance in any surveyed year to suggest it as a predominant cause of the species' lack of recruitment.

The reduced PAR evident in the montane areas from early November to late February is most likely due to a combination of increased mountain shading with a higher angle of sunlight during winter months and snow cover at high elevations. Lower Brown Canyon is lower in elevation, flatter terrain, and not exposed to snow. Light would also be expected to reflect off water during times the stream is flowing. This would explain the higher PAR levels, in the wet stretch though canopy cover in this area was also higher than other population areas. In Lower Brown Canyon, there was also less abundance of surrounding vegetation, allowing light to permeate intermittently throughout the day. As air temperatures are generally warmer at lower elevations, soil temperature was also highest in Lower Brown Canyon. Due to their close proximity and elevations, air temperature would be consistent among the montane areas as reflected in their similar soil temperature. The lower variability in the unburned area may be explained by its greater vegetative cover, insulating the soil.

Higher soil moisture in Lower Brown Canyon was not surprising as the sandy soil is inundated during short term flooding events from heavy monsoon rains and mountain snowmelt in the spring. *A. kearneyana* is senescent from late October through early spring, but free water availability during this time would be advantageous, as they invest in below ground structures before investing in above ground growth in March (USFWS 2013). While free water is available in Lower Brown Canyon, in Upper Brown Canyon, water is frozen during winter months and unavailable to plants. Greater canopy cover and greater vegetative ground cover in the unburned montane area moderates evaporation keeping soils moister than in the burned area. The dominant species shift in the burned area from *Quercus oblongifolia*, *Quercus emoryi*, *Acacia greggi*, *Dasylirion wheeleri*, *Crossossoma bigelovii*, and *Agave schottii* pre-fire in 1998 (Donovan 1998) to current dominants of *Garrya wrightii*, *Gutierezia sarothrae*, and *Mimosa aculaeticarpa var. biuncifera* also indicates the ongoing drier conditions here (USFWS 2012). Complete lack of recovery of *Quercus* six years after the Elkhorn fire may indicate a community composition shift is occurring. If it is, recruitment in *A. kearneyana* here may become even more rare if drier conditions preclude it.

Specific leaf area (SLA) is one of several leaf functional traits which can be predictive of a plants ability to respond to the environmental factors influencing its habitat at several levels. For example, it has been found to be positively correlated to net photosynthetic rate at the level of the individual leaf and to the plant's relative growth rate at the whole plant level (Violle et al 2007). Specific above ground net primary productivity is also positively correlated with SLA at the plant community level (Violle et al 2007). It has also been found to explain much of plant functional trait variance across plant species (Kattge et al 2011). This study measured SLA at only one point in time and can therefore make no comparisons to relative growth rate in *A. kearneyana*. However, further experiments could provide further evidence of the potential range of environmental conditions for which it can adapt.

Many mesic adapted species (deep root systems, large leafed, with low SLA) require nurse plants as opposed to more xeric adapted species (Butterfield & Callaway 2013). Nurse plants can buffer effects of periodic resource limitations, disturbances, or other unfavorable conditions such as high ambient temperature and low soil moisture. Whereas available nurse plants, defined as any plant taller than a small *A. kearneyana* plant, were rare in the burned montane area, nurse plants were plentiful in the unburned area and in Lower Brown Canyon. It is in Lower Brown Canyon where *A. kearneyana* presented with more stems and greater height. *A. kearneyana* was found to have a surprisingly low mean SLA compared to other herbaceous species which average about $10mm^2mg^{-1}$, and even other shrubs which average about $6mm^2mg^{-1}$ (Kattge et al 2011). These factors are all consistent with mesic adapted plants. They also have deep tap roots and broad leaves. Deep roots can be found in species of all biomes (Canadell 1996). Roots deep enough to reach the water table have been shown to be much more efficient in absorbing water than roots in drier soil (Reicosky et al. 1964). Though maximum rooting depth has not been established for *A. kearneyana*, plants appear robust through the hottest summer months indicating they are able to access water where the surrounding dry vegetation cannot.

Areas which tend to favor low SLA also tend to favor species with a high total mass of seeds (Butterfield & Callaway 2013). In 2015, *A. kearneyana* produced an average of 580 seeds per plant in 2015 over all surveyed population areas. Considering the rarity of recruitment in the wild, this would seem to qualify as ample seed mass. Low SLA species tend to also be excluded from areas with a brief growing season or in "windows of opportunity, as can occur after physical disturbance and brief precipitation pulses in arid ecosystems." (Butterfield & Callaway 2013)

Amsonias are known to have long tap roots which would allow them to access water deep below the surface. Greenhouse seedlings revealed a consistent trend where tap roots were at least three times the length of above ground growth. Field observations showed *A. kearneyana* plants green and robust even as surrounding vegetation was dry and dormant. If water is therefore not limiting, the higher stomatal density of the plants in the burned area, where shade is not present, may be conducive to cooling the leaves by inducing transpiration, though further study investigating the physiological processes of *A. kearneyana* would be necessary to confirm this theory.

As no recruitment has been documented in Lower Brown Canyon, plants in that population are presumed to be the same individuals transplanted there in 1988-1992, making them 23-27 years old. Plant ages for the montane plants are unknown though the populations found there in 1998 appeared well established. Therefore, it is reasonable to assume plants of similar reproductive maturity were represented in my montane samples. Differences in height and stem abundance were most likely due to differences in environmental conditions favoring those in Lower Brown Canyon, and to a lesser extent, the unburned area of Upper Brown Canyon.

A. kearneyana can reproduce clonally, via ramets or by seed. It was concluded that many of the plants in unburned montane population had ramet growth. No obvious seedlings were noted during any visit in any population. However, it is possible that some of the assumed ramet clones were actually young individual plants which germinated from seed and happened to grow in the immediate vicinity of other plants.

Factors which influence or are required for ramet production in *A.* kearneyana are unknown. In general, production of ramets can adapt a plant to a frequently disturbed environment (Huber 2005) such as the unstable substrates of the montane areas and the flood scour of the riparian zone. However, the virtual lack of ramet growth in the population that produces significantly more seeds (Lower Brown Canyon) and the plentiful production of ramets in the population that produces far less seeds (unburned Upper Brown Canyon) may indicate a biological trade off within the species in response to some environmental factor facilitating seed production versus vegetative reproduction.

Topinka (2006) suggested that the transplanted population located in Lower Brown Canyon has a lower seed set potential than the larger natural population in Upper Brown Canyon but no supporting data was available for this statement (Topinka 2006). Surprisingly, although there was evident variability between years, despite weather differences, seed production per plant was not statistically significant over the time of this study.

CHAPTER 3

GERMINATION REQUIREMENTS OF AMSONIA KEARNEYANA

Question: What are the germination requirements of *A. kearneyana*, as to water, temperature, soil type, burial depth, and shade? Also, how does fire influence germination?

Introduction

Seedling recruitment of *Amsonia kearneyana* is rare in the wild. The one seedling documented in the wild was growing in the sandy wash in South Canyon (Phillips and Brian 1982). There are no reports of seedlings in the montane areas. Rugged terrain and their remote location is likely a factor in this lack of observations.

Many abiotic factors can influence seed germination, including water, temperature, soil particle size, and light availability (Finch-Savage & Leubner-Metzger 2006). Given that the soil, shade and other physical conditions differ between the two main habitat types of *A. kearneyana* (montane and riparian), these factors must be considered when establishing the germination requirements of the species.

Seed morphology also influences germination (Finch-Savage & Leubner-Metzger 2006). *A. kearneyana* has a hard and thick seed coat (McLaughlin 1982) and such traits can indicate adaptation to fire (Khurana and Singh 2001). A hard thick seed coat may also afford protection from predators and microbial decay and, as such, has been associated with greater seed longevity (Long et al. 2015). Many arid adapted plants rely on fire, (heat or charate), to stimulate germination, either to break seed dormancy or to physically break the seed coat (Rieks & Goulier 2013, Keeley 1987). *Amsonia* *kearneyana* grows in areas influenced by fire and co-occurs with fire-adapted species such as *Garrya wrightii* and *Mimosa aculaeticarpa* var. *biuncifera* (USFWS 2012). Other species require temperature diurnallity or moist heat to break seed dormancy of a hard thick seed coat (Rieks & Goulier 2013, Mousavi et al. 2011). *A. kearneyana* is in the *Apocynaceae* family which has been identified to have a nondeep physiological seed dormancy (Finch-Savage & Leubner-Metzger 2006). This class is defined as species first requiring light, and then requiring gibberellin treatment (a plant hormone involved in growth) or scarification, after-ripening in dry storage, or temperature stratification to induce germination (Finch-Savage & Leubner-Metzger 2006). Additionally, as the species specific temperature requirements are reached, seeds will become even more sensitive to light and hormonal cues (Finch-Savage & Leubner-Metzger 2006).

Adult *A. kearneyana* plants have been found in areas of full sun and partial shade (Phillips and Brian 1982, Donovan 1998). However, the conditions tolerated or required by seeds and seedlings typically differ from those tolerated by mature plants (Long at al. 2015). It has not been determined if fire has the potential to benefit *A. kearneyana* by opening areas for seedling growth or providing soil nutrients, or if it hinders the plants by removing shade (USFWS 2013). It is also unknown if shade, or lack thereof, is a factor limiting germination or seedling survival.

Species vary in their capacity to germinate from depth (Stromberg et al. 2011), with larger seeds tending to emerge from deeper depths than do smaller seeds. *A. kearneyana* has large seeds (30.5 mg), and thus it may have capacity to germinate from depth. However, information about how fire, seed burial depth, or shade affects *A. kearneyana* (and other species of *Amsonia*) is lacking in the literature.

Methods:

Germination and survivorship trials were conducted in the greenhouse at Arizona State University to determine germination requirements and to determine how seed germination, seedling growth, and survivorship are influenced by factors which differ between the habitat types of wild growing *Amsonia kearneyana* populations. Specifically, I conducted trails to determine how different soil types, burial depth, fire, and shade affect *A. kearneyana's* ability to germinate under varying conditions in the wild.

Assessing germination requirements:

Initial germination trials were conducted in 2013 using seeds from two sources: those collected from wild plants in 1986 and held in accessions at the Desert Botanical Garden in Phoenix, Arizona. and seeds harvested in 1999 from cultivated Desert Botanical Garden plants. Though not specified in accession records, the only wild populations identified as of 1996 were in South Canyon and Sycamore Canyon, both on tribal lands with similar habitats to Lower Brown Canyon, so these plants can be assumed to be the seed source. For all additional trials, seeds were collected from parent plants in July 2014 and allowed to air dry at room temperature until planting in September 2014.

Seeds were weighed on a laboratory scale to obtain average seed mass. To confirm seed viability of the older seeds and to determine germination rate and days-to-germination, 18 of these seeds were planted in the greenhouse in seed starter containers and in standard potting soil. To determine how watering frequency influences germnination, 45 seeds were planted 1 cm beneath the soil surface in a temperature controlled growth chamber, simulating spring/cool summer conditions (maximum 25 C) and using soil collected in the field near the montane populations. I used five watering interval treatments (watering every 3,6,9,12, or 15 days) with three seeds per treatment group.

To initially investigate the effects that different temperature regimes would have on soil type and seed burial depth experiments, I planted seeds in two temperature controlled growth chambers; one under spring/ cool summer conditions (maximum of 25 degrees Celsius), and the other at a cooler temperature (maximum 20 degrees Celsius) with a shorter day length, with abundant water. I had three soil types: the indigenous clay/loam from near the montane populations, standard potting soil, and the sandy soil gathered from near the population transplanted in the riparian area of Lower Brown Canyon. Seeds were positioned at three burial depths: 4cm, 0.5cm, and on the soil surface. All seeds were soaked for 20 minutes in deionized water before planting.

To examine soil type, I collected soil from near the wild populations (clay/loam from the montane area and sandy soil from the meso-riparian area). and used standard potting soil as a control (Table 3). To examine how using soil affected by fire influenced germination rates, I treated montane soil with the ash of Oak (*Quercus*) trees. Oak wood was burned to ash. Four tablespoons of ash was added to each 20 ml of indigenous clay/loam soil used in the clay plus ash group. For burial depth, seeds were plants on the surface and at a depth of 0.5 cm. To investigate the effects of shade, four treatments that differed in PAR (microeinsteins per second per square meter; $\mu E\ m^{-2}\ s^{-1}$). were created by loosely hanging an appropriate number of layers of canvas strips over the seed trays to block out some of the ambient light from all sides while allowing adequate air flow to reach the plants. Immediately before planting, all seeds were soaked in deionized water for at least five minutes or until visibly water logged. For each group of 5 seeds used for each treatment, 3 seeds were from montane parent plants and 2 were from the meso-riparian parent plants.

At the end of the germination trial period (23 weeks), data for shade treatments, soil type, and burial depth were analyzed using a 3 way analysis of variance (ANOVA) with Germination and maximum stem length as dependent variables. Pairwise comparisons were made using the Holm-Sidak Test.

To track seedling growth and survival as a function of shade, surviving seedlings were transplanted to larger pots (2 liters) on February 21, 2015 using the same soil type that they were germinated in. The transplanted plants were returned to their respective shade treatments to track the seedlings' survival. Sample size varied among shade treatments because of varying rates of mortality at the seed germination stage.

To examine how the greenhouse experimental conditions compared to *A. kearneyana* field conditions, HOBO data loggers were installed in the greenhouse to measure soil moisture, soil temperature, and PAR in one of the experimental replicates in each shade treatment. A replicate with either clay/loam or clay/loam plus ash soil was chosen to house the soil moisture and soil temperature sensors to allow the best comparisons to the montane field conditions. Results from the greenhouse data loggers were compared to the data logger results from the field.

Table 8: Greenhouse germination trial treatments for *A. kearneyana*. Unless otherwise indicated, there were 5 replicates per treatment

Germination study				
Shade Treatment	Clay/loam	Clay/loam + Ash	Sand	Potting Soil
Full sun	soil surface	soil surface	soil surface	soil surface
	buried 0.5cm	buried 0.5cm	buried 0.5cm	buried 0.5cm
33% shade	soil surface	5 replicates- soil surface	soil surface	soil surface
	buried 0.5cm	5 replicates- buried 0.5cm	buried 0.5cm	buried 0.5cm
66% shade	soil surface	5 replicates- soil surface	soil surface	soil surface
	buried 0.5cm	buried 0.5cm	buried 0.5cm	buried 0.5cm
100% shade	soil surface	soil surface	soil surface	soil surface
	5 replicates- buried 0.5cm	5 replicates- buried 0.5cm	5 replicates- buried 0.5cm	5 replicates- buried 0.5cm

Table 9: Seedling survival trial treatments

Seedling survival				
Shade Treatment	Clay	Clay + Ash	Sand	Potting Soil
Full sun	2 replicates	2 replicates	2 replicates	2 replicates
33% shade	2 replicates	2 replicates	1 replicate	3 replicates
66% shade	1 replicates	0 replicates	4 replicates	6 replicates
100% shade	0 replicates	1 replicate	3 replicates	5 replicates
Totals:	5	5	10	16

Results: Moisture and Temperature

A. kearneyana seeds yielded an average weight of 30.5mg per seed. Of the 18 17-year old seeds, 11 germinated (at 9-12 days past planting) and 9 of the seedlings survived. In the trial to determine watering frequency, only one plant germinated. This showed the ability of *A. kearneyana* to germinate from beneath the soil surface and the ability of the aged seeds to germinate. However, the most frequent watering interval (3 days) was required to stimulate germination in the clay/loam soil.

More frequent and faster germination was observed in warmer conditions. In the warmer growth chamber, seeds sown on the surface of all soil types germinated in as little as 7 days with a total germination of 44% (12 of 27 seeds),. The radicle emerges from the flatter end of the conical seed and first orients to point upward away from the soil. It should be mentioned that *Amsonia* seeds, though large, become buoyant when wet. When seeds were soaked in a clear beaker, they initially sunk to the bottom, but then floated to the water's surface within a few minutes. Most seeds planted at 0.5cm below the soil surface also floated to the soil surface with very little water added. This phenomenon occurred in the sandy soil and potting soil, but not in the clay/loam. The two seeds originally planted at 0.5cm which did germinate were of those which had floated to the surface before germinating. Two seeds from the 4cm burial depth germinated in potting soil. Only one of the seeds of the clay/loam soil lot germinated (sown on the surface). This plant survived to the seedling stage but grew much slower than the others.A total of seven survivved to the seedling stage,

In the cooler growth chamber, only 4 of the 27 seeds (15%) germinated. The first germination was observed 15 days from planting, for two seeds that were from the surface-sown group (one in the potting soil group and one in sand). Three days later, two more seeds germinated, both from potting soil, one from a 0.5cm burial depth and another from the surface. No seeds germinated from a 4cm burial depth, and none germinated from the clay/loam soil.

Results: Germination and seedling growth response to burial depth, soil type, and shade parameters:

Overall, the experiments using freshly collected seeds produced a 36% germination rate. However, only 24% of the seeds planted survived to 20 weeks. Seeds sown on the soil surface had a significantly higher germination rate than those buried 0.5 cm beneath the surface over all treatments (p=0.113) (Fig. 11). Of the total of 140 seeds used in in the experiment, 57 seeds germinated. Of these 57, 48 germinated on the surface.

Soil type also was significant factor in predicting germination (p=0.004), although the differences were between the control (potting soil) and the other soil types. Three times as many seeds germinated in potting soil as in the clay/loam montane soil. Twice as many seeds germinated in the sandy soil than in either the clay/loam or clay/loam plus ash (Fig. 12). Trends were similar for stem length of the seedlings, with potting soil producing significantly more stem length than any other soil type (Fig. 12). Of the wild soil types, sandy soil from Lower Brown Canyon produced the most growth.

There was a statistically significant effect of soil type on stem length based on a two way ANOVA in which the maximum stem(s) length in millimeters of the plants after transplantation was the dependent variable, and four soil types and the four shade treatments were independent variables (p=0.037). However, multiple comparison tests using the Holm-Sidak method did not detect significant differences between individual soil types. The power of the performed test with alpha = 0.0500 for soil type was 0.616.

Shade treatment affected seed germination at p=0.113 (Fig. 13). The two way ANOVA did not indicate a statistically significant effect of shade treatment on stem length. (*p*=0.708) The power of the performed test with alpha = 0.0500 for shade treatment was 0.0509. Although the mean stem length was similar among all shade treatments, it was more variable in the 66% and full shade plants (Fig. 14).

How greenhouse conditions relate to field conditions

Soil moisture was much higher in the greenhouse experiment than in montane field conditions (Fig. 15). However, Lower Brown Canyon had higher soil moisture conditions during flood pulses. During those events, soil moisture values would be consistent with greenhouse soil moisture conditions. The full sun greenhouse shade treatment most resembled the burned montane area, and the 33% shade treatment mimicked the unburned area. The shade level in Brown Canyon was closest to the 66% experimental treatment.

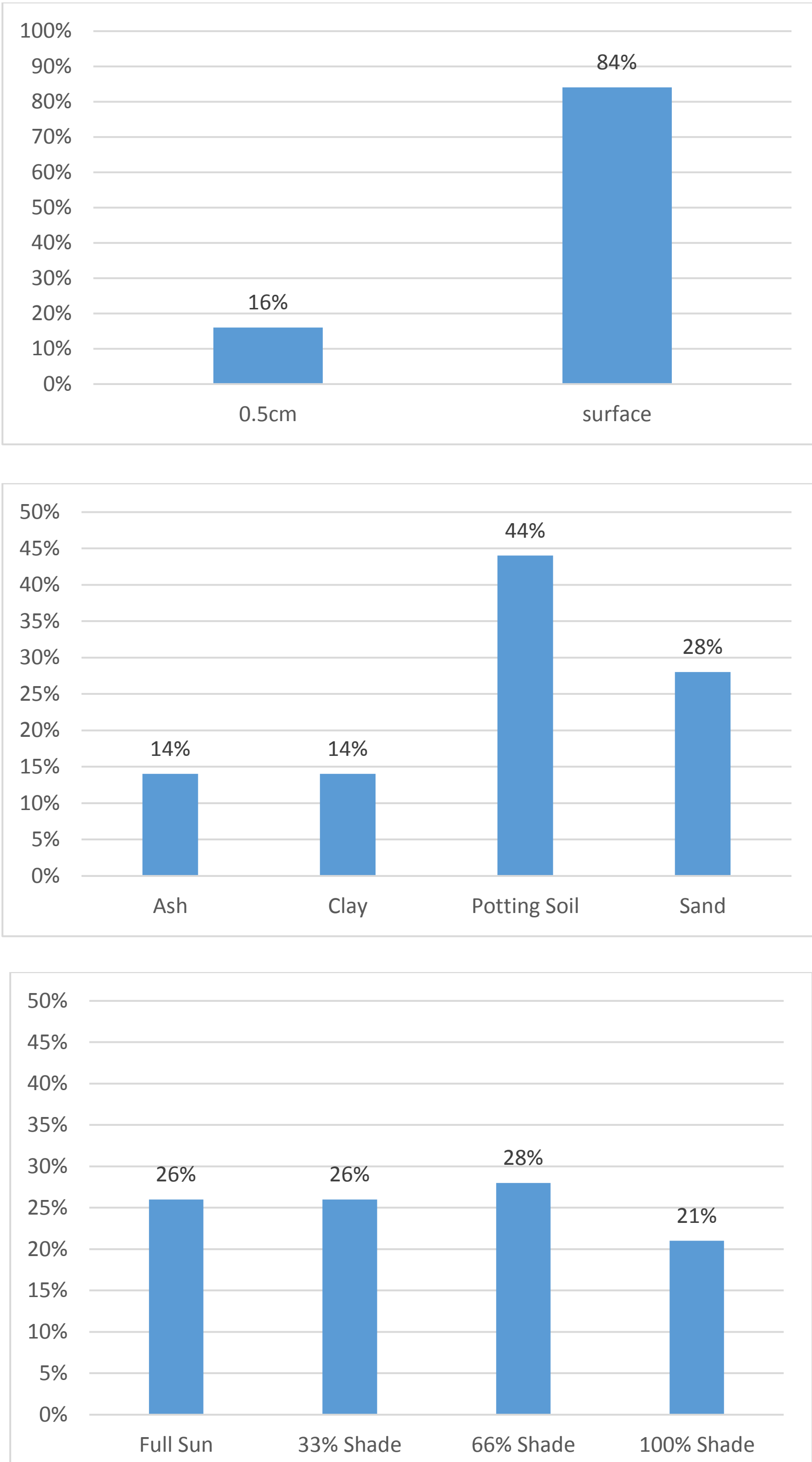


Figure 11: Percent of *A. kearneyana* seeds germinated, by burial depth (0.5cm and soil surface) soil type, and shade treatment, n=57.

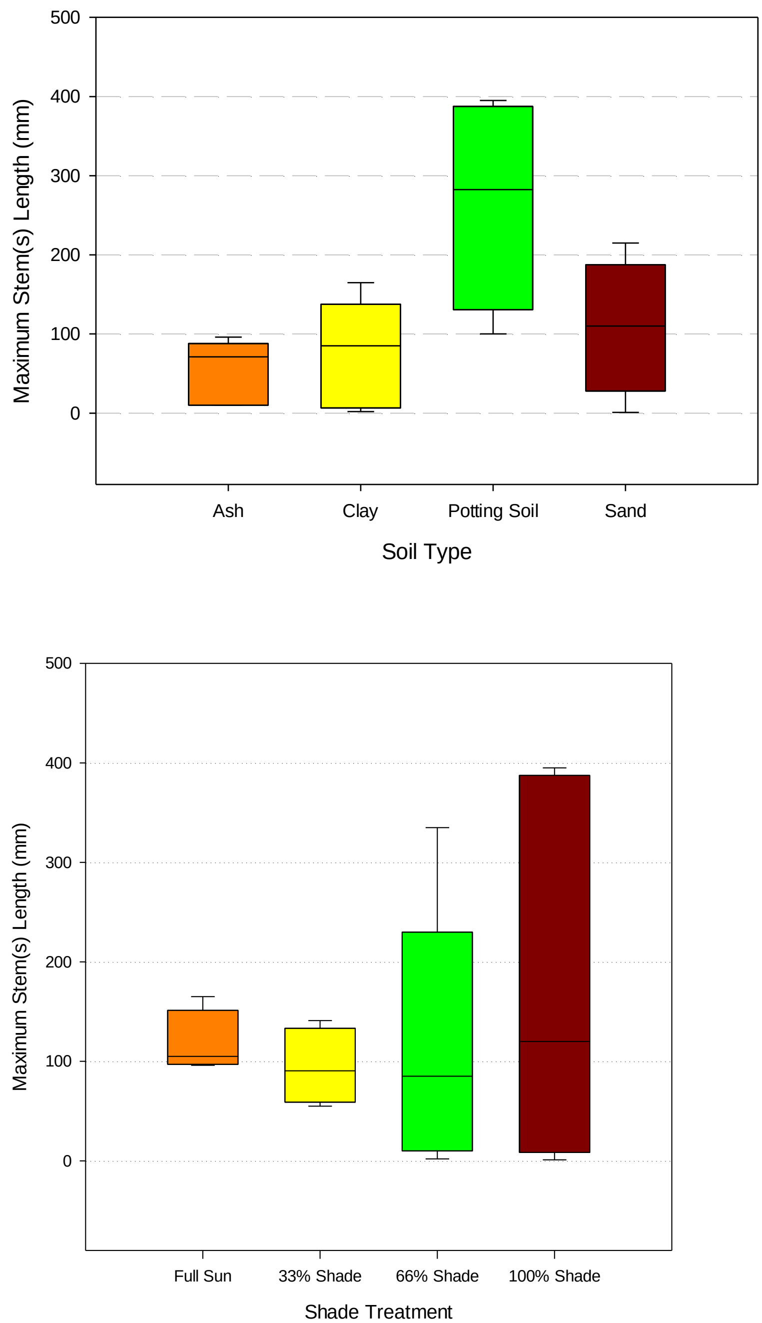


Figure 12: Maximum stem length for *A. kearneyana* in four soil types (top) and four shade treatments (bottom).

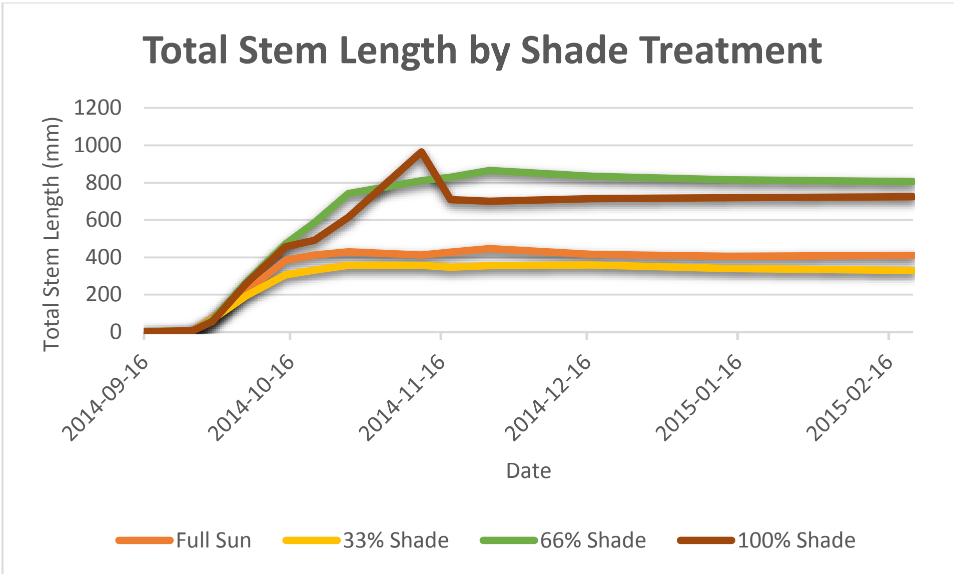


Figure 13: Total stem length for greenhouse-grown *A. kearneyana* in four shade treatments.

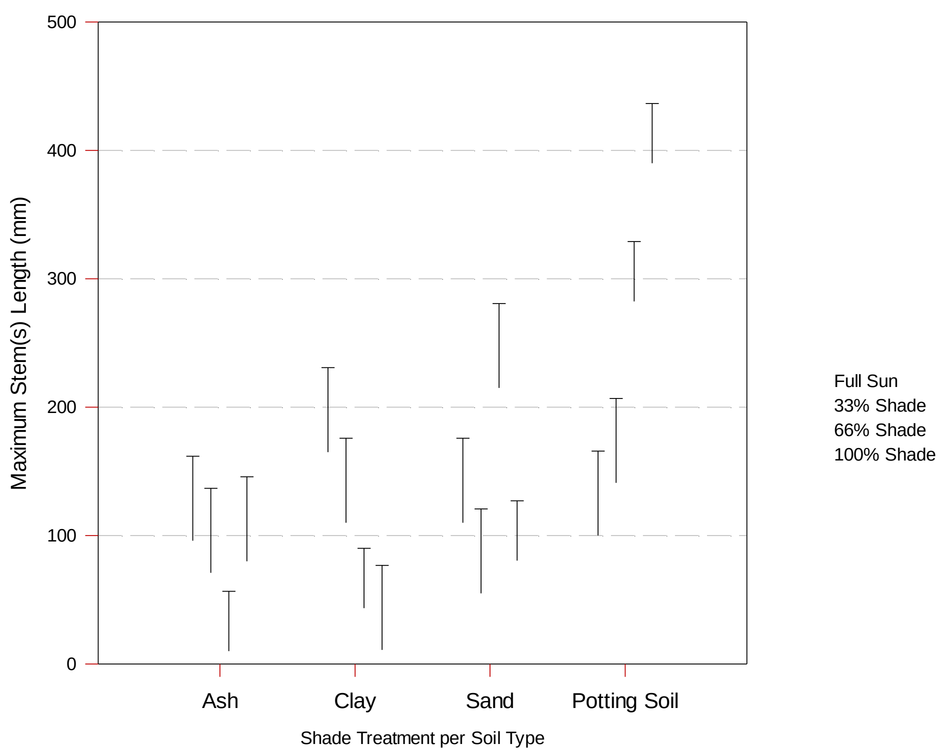


Figure 14: Effects of soil type and shade treatment on maximum stem(s) growth in millimeters on surviving *A. kearneyana* plants.

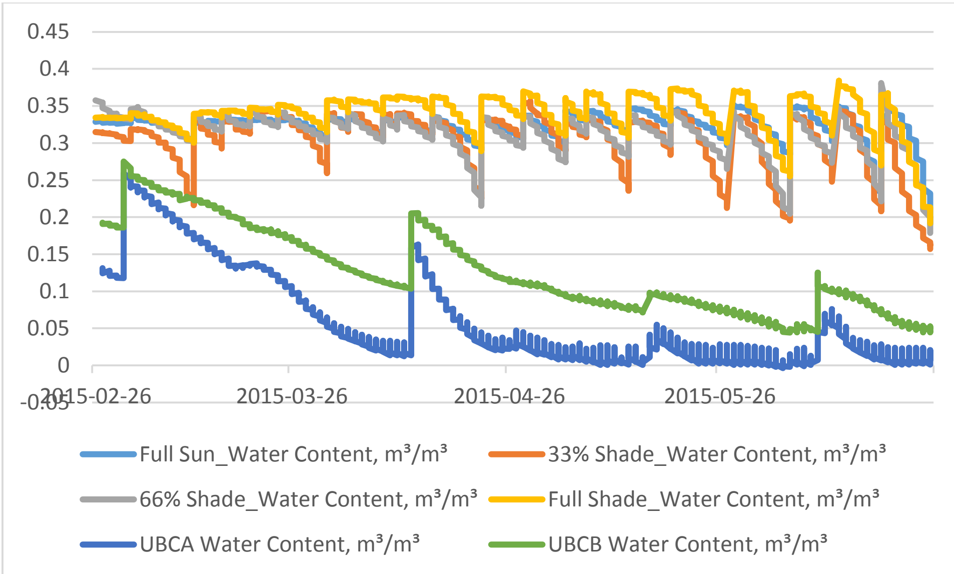


Figure 15: Soil moisture values of Upper Brown Canyon burned (UBCA), Upper Brown Canyon unburned (UBCB), and the four experimental treatments in the greenhouse seedling survival experiment.

Discussion:

Though originally thought to be a sterile hybrid (Woodson 1928), *A. kearneyana* has been found to have a 66% germination rate in greenhouse conditions (McLaughlin 1982). No information is known about the soil medium or burial depth used by McLaughlin, but *A. kearneyana* at the Desert Botanical Garden (DBG) in Phoenix and the Arizona-Sonora Desert Museum (ASDM) in Tucson are currently grown in standard potting soil and have similar germination rates (personal communication from Steve Blackwell, DBG and George Montgomery, ASDM). These values are similar to those in my experiment.

Overall, germination can be expected in as little as 7 days from planting. Though warm temperatures encouraged a faster growth rate and a higher germination rate, cool temperatures did not appear to be a limiting factor. These findings coincide with Finch-

Savage & Leubner-Metzger (2006) predictions that once species specific temperature requirements are achieved, germination would progress at a higher rate. Though the specific temperature could not be precisely determined by this trial, it seems to be around 25 degrees C. Gibberellin levels could be measured in future germination studies to evaluate their role for this species. In a non-experimental trial, one *A. kearneyana* seed was planted in potting soil within 24 hours of collecting it directly from its fruit in the field. This seed germinated in 9 days from planting showing temperature stratification is not necessary for germination. Physical scarification by scratching the seed coat was also not necessary, though soaking seeds in water before planting did seem to progress germination. Other than this one seed trial, all seeds used in germination trials were held in dry storage for varying times, ranging from weeks (for personally collected seeds) to years (for seeds acquired from Desert Botanical Garden). This after-ripening in dry storage may have inadvertently aided in breaking seed physiological dormancy in germination trials making them more sensitive to light and hormonal cues (Finch-Savage & Leubner-Metzger 2006).

Achieving a 61% germination rate from 17 year old seeds is encouraging. While seed granivory and frugrivory was observed on some wild plants by a common true bug, *Chlorochroa ligata*, it is doubtful this would destroy all seeds produced. Given the excess of seeds produced by wild plants, a viable seed bank may be present in wild habitats far beyond the bounds of current year's seed production. However, burial depth results suggest that, even though wild *A. kearneyana* plants produce copious seeds, those seeds may rarely germinate if natural disturbances such as soil erosion, strong winds, or heavy rain events bury them prior to germination. With the steep terrain and loose, rocky substrate of Upper Brown Canyon, this type of erosion seems eminent. Lower Brown Canyon is fairly flat, but experiences flooding events which can easily cover seeds with sandy soil and debris. While germination was achieved in two seeds from as deep as 4 cm beneath the soil surface, this only occurred in non-compacted potting soil. This substrate most likely not dense enough to mitigate much light, air flow, or other factor which may have otherwise inhibited below surface germination.

Soil type (potting soil or sand) and burial depth (surface of the soil) have strong influence on germination and growth; fire had no discernable effects. The sandy soil from the Lower Brown Canyon area is more facilitative to germination and stem length than the montane soil. Root elongation may be met with less resistance in sand than in the denser clay/loam, allowing faster growth, especially in drier soil conditions. The low but equal germination rates and stem length rates in the montane clay/loam soil and the ash treated montane soil suggests that the effects of fire on soil are negligible on germination rates. In other words, the presence of ash (effects of fire on the soil) does not preclude or stimulate germination.

Shade treatment trials indicate both that A. kearneyana can germinate and grow in a full spectrum of light levels, but that shade and soil type interact. Interestingly, 66% shade was most conducive to seedling survival in sandy soil. Other than the potting soil plants, these plants also grew faster and appeared healthiest with broader leaves and higher turgor. These are the closest to field conditions of the wet stretch of Lower Brown Canyon. Though fewer plants survived in the clay/loam soil from Upper Brown Canyon, the highest stem length of these plants was seen in full sun and 33% shade conditions which is closer to the field conditions of the unburned Upper Brown Canyon area.

CHAPTER 4

CONSERVATION BY DISSEMINATION OF AMSONIA KEARNEYANA

Question: Would *Amsonia kearneyana* be a good candidate species for a conservation by dissemination approach? If so, what would be involved in amending its recovery plan?

Introduction:

Underlying all endangered species policy is a common assumption. If a species is in such a vulnerable position as to warrant federal protection, then each individual will have a weightier influence on the survival of the species as a whole. In other words, every individual matters when a species is so few. The ultimate goal is the preservation of the species in the wild. However, sometimes, recovery is predicated on captive breeding and controlled propagation programs which make reintroductions and population augmentation possible. I argue that, for plants in the United States, an additional conservation technique, conservation by dissemination, should be used in certain circumstances for threatened and endangered plants to support conservation goals. I examine here if *Amsonia kearneyana* would be a good candidate species for a conservation by dissemination approach, and if so, what, if any, amendments to its recovery plan would be needed.

Conservation by dissemination

"Conservation by dissemination" is not a coined term in the literature. It refers to the idea that a protected plant species can be better conserved by allowing and promoting the propagation and sale of plants in the commercial market, with contingent collection of data on the fate of the sold individuals. The individuals introduced in commerce would be propagated from private collections or botanic gardens and not from wild collected plants. These individuals would not be intended as a source for reintroduction into wild habitats, but rather as an additional source of scientific information on the species, utilizing a quasi-citizen science approach. Commercial propagation, per the United States Fish and Wildlife Service (USFWS), refers to the growing and selling of threatened or endangered plants specifically for the commercial market. It is virtually unregulated and seen more as an inherent problem of global trade markets than as a potential conservation tool. There is no implication that commercial propagation will be used to support the survival of wild plants except where it may indirectly reduce the threat of over collection of wild plants. Conservation by dissemination, as I am coining it here, differs from commercial propagation in that its intent is to support the survival of wild plants indirectly by augmenting scientific knowledge of the species for use by USFWS in managing wild populations.

Methods:

To determine if *A. kearneyana* would be a good candidate for conservation by dissemination, I identified all relevant laws, regulation, and policy at an international, national, and state level that govern commercial trade of endangered plants. Knowledgeable persons from USFWS, Arizona Department of Agriculture, botanic gardens, and academia were identified and consulted. I also identified the biological, ecological, and pragmatic ethnobotanical characteristics of *A. kearneyana* to evaluate the potential risks and benefits of applying conservation by dissemination to this species.

Law, regulation, and policy

There are several laws pertaining to commerce of listed plants. The Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) restricts international trade, requiring permits to be obtained from both the importing and exporting country for plants and wildlife placed on its list. My focus is domestic trade, so this will not be explored further.

Under the *Lacey Act of 1900*, as amended in 1981,

> *"it is unlawful to import, export, sell, acquire, or purchase fish, wildlife or plants that are taken, possessed, transported, or sold: 1) in violation of U.S. or Indian law, or 2) in interstate or foreign commerce involving any fish, wildlife, or plants taken possessed or sold in violation of State or foreign law."*

This was the first US law to assert federal authority over interstate commerce involving wildlife, primarily addressing problems incurred from overhunting, profiteering, and poaching (Doremus 2010)(USFWS 1990b).

The *Endangered Species Act of 1973* (ESA), as amended in 1982, prohibits removal of listed plants from federal lands and prohibits their sale in interstate commerce (USFWS 1990b). Under the assumption that "what nature needs most is for people to leave it alone", it follows the traditional thoughts of John Muir and Aldo Leopold and seeks to remove the economic incentives of overharvesting (Doremus 2010). The USFWS, the federal agency charged with administering the Act as applied to terrestrial and fresh-water species, has the authority to allow, with permits, actions such as interstate commerce and plant re..moval from federal lands if it deems these actions in the best interests of the species (USFWS 1990b). Under the ESA, “taking” (killing, removing, harassing, etc.) of endangered species is expressly prohibited anywhere in the United States when applied to vertebrates. However, it is only illegal to “take” plants on federal land. This discrepancy makes more sense in the light of the historical context of common law (Rolston 1990). Whereas wild animals may just as easily be on a one piece of property as another, plants do not move and are seen as a piece of one’s land. American common law has its roots in Old English law. In Old English laws, vegetation, especially large trees, belonged to the King, no matter who owned the land (Rolston 1990). American common law sees all vegetation included in the land and therefore belonging to the land owner (Rolston 1990). However, land use and property rights laws are generally promulgated by the state, and therefore any endangered plants on state lands are under the state’s authority (Rolston 1990). Endangered plants on state or private land may be killed, removed, bought or sold legally under the ESA as long as this is not prohibited by state law. The ESA does not prohibit intrastate commerce or commerce of plants from private collections.

State laws vary widely. Most allow endangered plants to be removed or destroyed with a permit from the state (Rolston 1990). Some, like Hawaii, encourage endangered species sales in the commercial market within the state, only requiring a four cent state permit tag be placed on each plant sold indicating it comes from cultivated stock (HAR 1997). However, no penalties are in place for ignoring this law, and large batches of these tags can be obtained at one time so it is likely hard to enforce. Some states completely prohibit the destruction of endangered plants, and Congress has backed them by supporting federal penalties when these state laws are violated (Rolston 1990).

In the state of Arizona, all federally listed plants native to Arizona are also protected as Highly Safeguarded Species under Arizona's Native Plant Law. This listing is not exclusive to federally listed plants, and includes many other more common species valued highly by the state including the Saguaro Cactus and most native cacti. Under this law, it is illegal to kill, damage, or remove Arizona listed plants within the state. Any land owner may destroy or remove for sale listed plants from their property, but must notify the Arizona Department of Agriculture 60 days prior to obtain a permit. It is unknown what level of compliance occurs. If the plant will be transferred to another property that they own, a permit is not required (personal communication with Zeke Austin, Arizona Department of Agriculture 2014). Where an Arizona permit can be obtained, protected plants can be sold within the state. However, selling or moving the plants to another state is interstate commerce and would invoke the federal law, the *Endangered Species Act*, and involve permission from the United States Fish and Wildlife Service, but only if the plants are obtained from federal land or invoke a federal nexus. In the case of *A. kearneyana*, all known wild growing individuals (except whatever may grow on tribal lands), exist on federal land and would have such federal nexus.

The USFWS and its sister agency the National Marine Fisheries Service (NMFS)(collectively referred to as the Services) promulgated a *Policy Regarding Controlled Propagation of Species Listed Under the Endangered Species Act* (USFWS 2000) in September 2000 clarifying its official position on the issue of controlled propagation of the animals and plants under its direction. Controlled propagation is not the same as commercial propagation. Controlled propagation refers to the process of growing plants with the intent of using them for species reintroduction into the wild or to hold as living material for refuge populations or for scientific studies. Since these plants are intended to augment wild populations in some way, the focus of this technique is to preserve a genetically representative sample of the wild populations. To accomplish this, USFWS defers to guidance and protocols established by the Center for Plant Conservation. (USFWS 2000, CPC 1991).

Commercial sales of listed species may be permitted by the Services under *50 CFR §17.22*. USFWS issued a final rule in 1977 promulgating regulations relating to commercial propagation of threatened and endangered plants. These regulations specify under what conditions permits could be issued for this purpose (USFWS 1977). The stance of the agency with these regulations is quite liberal, and requirements to obtain a permit are lax. Regarding the commercial propagation of private seed stock and cultivated plants, the Service only commits to monitor these activities and not interfere as long as the commercial propagation does not represent a threat to the species, thus fulfilling the requirements of the ESA (USFWS). This being said, USFWS does not have any policy regarding commercial propagation of threatened or endangered plants (personal communication with USFWS 2015).

While the *Policy Regarding Controlled Propagation of Species Listed Under the Endangered Species Act* (2000) does not specifically refer to commercial market sales, all responses from USFWS to my inquiries for current agency policy on commercial propagation directed me to this document. It describes controlled propagation as having a supportive role in recovery and consistent with the ESA. With this direction, I will attempt to extrapolate the Services' general characterization of *ex situ* recovery. Additionally, if the commercial sales of species are authorized, funded, or carried out by the Services and implemented by controlled propagation for the recovery of the species, this policy would apply (USFWS 2000). Conservation by dissemination is designed to make use of the best available scientific and commercial information in a way that commercial propagation cannot. This policy states:

> *"Though the Act (Endangered Species Act) emphasizes the restoration of listed species in natural habitats, section 3(3) of the Act recognizes propagation as a tool available to us to achieve this end. The controlled propagation of animals and plants in certain situations is an essential tool for the conservation and recovery of listed species. …To support the goal of restoring endangered and threatened animals and plants, we are obligated to develop sound policies based on the best available scientific and commercial information."*(USFWS 2000)

As with any ex situ conservation strategy, the associated benefits and risks would need to be considered on a case-by-case basis by the Services and alternatives requiring less intervention would need to be objectively evaluated (USFWS 2000). There are times when this method has been authorized, specifically to authorize commercial sales as a recovery strategy by lessening the collection pressure on wild plants.

Commercial propagation examples:

The ESA requires a recovery plan be developed for most species. Among other things, it serves as the central guiding outline of the recovery strategies best suited for the species. One requirement of the *Policy Regarding Controlled Propagation of Species Listed Under the Endangered Species Act* (USFWS 2000) is that controlled propagation be explicitly called for in the species recovery plan or be included in an amended recovery plan (USFWS 2000). Though this requirement has never been enforced by USFWS (personal communication with USFWS 2015), I will explore some examples of species recovery plans where controlled propagation and commercial propagation were advised.

For several endangered plants, the ESA Recovery Plans already call for commercial propagation as a method to recover the species by alleviating collection pressure. *Ancistrocactus tobuschii* (Tobusch Fishhook Cactus), *Coryphantha minima* (Nellie Cory Cactus), *Coryphantha ramillosa* (Bunched cory cactus), *Echinocactus horizonthalonius* var. *nicholii* (Nichol's Turk's-head Cactus), *Echinocereus chisoensis* var. *chisoensis* (Chisos Mountain Hedgehog Cactus), *Echinocereus reichenbachii* var. *albertii* (Black Lace Cactus), and *Echinocereus triglochidiatus var. arizonicus (*Arizona Hedgehog Cactus) are all examples of this (see Appendix A for examples of language used in recovery documents). Another example is *Betula uber* (Virginia round leaf birch tree), which was threatened mainly by vandalism within its native habitat. The recovery plan for this species states "In addition to increasing the number and geographical distribution of round-leaf birches in cultivation, making the plants available to the public was viewed as a way of heightening awareness of endangered species and possibly reducing vandalism to the natural population as the plant would no longer be perceived as rare." (USFWS 1990)

Rare plants which have not been listed have benefitted from commercial propagation, as well. *Franklinia alatamaha* (Franklin tree, named after Benjamin Franklin) was native to Georgia but not seen in the wild since 1803 (Merkle, 2015). It was a candidate for listing due to its rarity, but USFWS declined its listing considering it extinct in the wild (USFWS 1986). Seeds were propagated from a private collection of trees on a single family's property and are now widely available in the commercial market. Efforts are being made today to replant some of these trees within its historic range (Merkle, 2015).

USFWS often uses this technique applied to fish, as well. Threatened fish will be actively propagated and released to stock fisheries, not for commercial fisheries, but for recreational fishers. One example of this is the Gila Trout (*Oncorhynchus gilae*) in Arizona and New Mexico. USFWS (2006) asserts that making the species available for recreational fishing contributes to the species conservation by, among other things, by (1) allowing additional funding to be accessed through federal sport fishing funds, (2) aiding future management by enhancing their ability to monitor populations (e.g., creel censuses), and (3) the "creation of goodwill and support in the local community". These benefits mirror those that endangered plants would incur under conservation by dissemination.

Sections 4(d) and 10(j) of the ESA give USFWS further management flexibility in implementing species recovery. When potential "take" is involved, the 4(d) and 10(j) rules allow certain individuals in certain areas that would otherwise be protected as endangered, to be downlisted to threatened, therby relaxing taking prohibitions. This approach garners public cooperation and support from individuals who feel unfairly restricted by having an endangered species on their land. The species as a whole benefits because the pressure is reduced in the remaining areas as can be seen in the recovery of the gray wolf (*Canis lupus*). While a federal nexus is required to apply take prohibitions to plants, the 4(d) and 10(j) rules reiterate the intent of the ESA, to recover the species as a whole, while allowing certain individuals to be managed differently for the good of the species.

We must state the obvious. Plants are not animals. With this I do not seek to diminish the value of plants. My goal centers on the opposite. It is necessary to exaggerate this point for the same reasons Congress has chosen to treat plant protection increasingly the same as animal protection in the Endangered Species Act, to ensure we take their protection equally seriously. Habitat loss and degradation, collection pressure, climate change, land use and land cover change among others represent major threats to endangered species and more common species alike (Selwood et al. 2015). Plants and animals indeed share many of the same threats. Just because we believe plants should be equally protected from extinction does not mean we can protect them using equal methods. Many times we will employ similar mitigations, especially when we must implement conservation management plans under the ESA for USFWS to administer in a quantifiable and defensible way. Other countries with similar conservation laws have developed their own strategies.

International examples:

Australia has used commercial sales of at least one endangered plant to financially support and garner public excitement for endangered plant conservation. The Wollemini Pine, *Wollemia nobilis (*Araucariaceae), a prehistoric conifer from the Jurassic Era, was presumed extinct until it was rediscovered in 1994 in Wollemi National Park in the Blue Mountains, just outside Sydney, Australia (Wollemi Australia Pty Ltd 2014). It was listed as critically endangered under the *Environment Protection and Biodiversity Conservation Act 1999 (Federal)* (Jones et al 2014) and the New South Wales Threatened Species Conservation Act 1995. The federal *Environment Protection and Biodiversity Conservation Act 1999* is similar in many ways to the Endangered Species Act of the United States, including requiring development of a recovery plan for listed species. In its recovery plan for the Wollemini Pine, the Department on Environment and Conservation not only planned for the international commercial propagation of the tree, but specified that revenues from these sales would fund the trees other recovery actions in their entirety.

Royal Botanical Garden in Sydney, Australia was tasked with growing new plants from wild collected seeds and distributing propagules and cuttings to nurseries around the world for sales. (NSW DEC 2006) The first commercial sales commenced within Australia in 2005, and around the world in 2006, with amazing success. Plants are marketed to be used from everything from potted Christmas trees and indoor décor for school classrooms to veranda plants or garden features (Thornton 2013; Wollemi Australia Pty Ltd 2014). As of November 2014, plants and seeds are still popular enough that waiting lists to buy them still persist on online websites.

Australia boasts other benefits of its commercialization program including greater education on threatened biodiversity, heightened appreciation for National Parks and their role in conservation, and "Establishment of a new international standard for successful, case-specific blending of recovery and commercial actions." (NSW DEC 2006) While the commercial marketing of this endangered species has indeed funded its recovery, it has also deterred would be collectors of wild plants and seedlings and allowed for its main objective, the conservation of the few remaining wild plants to persist undisturbed by potentially damaging site visits.

The conservation approach known as *circa situm* has similarities and differences to conservation by dissemination. In *circa situm* conservation, people are encouraged to plant a rare plant or tree in private or commercial gardens or farms outside of, but in the general vicinity of the plant's natural range in attempt to artificially extend the boundaries of the species current habitat and allow for increased pollinator interactions (Ojeda & Santos-Guerra 2011). Although only useful (Barrance et al. 2003) for plants that are popular as landscape plants, it has been found to be an effective conservation strategy in Mesoamerica (Ojeda & Santos-Guerra 2011), Mexico (Barrance et al. 2003), Brazil in agroforestry (Dawson et al. 2013), Kenya and Niger in coffee crops (Pinard et al. 2014), and the Canary Islands (Ojeda & Santos-Guerra 2011).

One example is a species of Lotus vine *(Lotus berthelotii*) endemic to the Canary Islands that had been nearly extirpated in its native habitat due to habitat fragmentation, human disturbance from tourism, and goat grazing (Ojeda & Santos-Guerra 2011). Hybrids of this species have been commercially available locally and internationally for 35 years and continue to be popular decorative plants including in the United States

(Ojeda & Santos-Guerra 2011). The local government in the Canary Islands began promoting the planting of the endemic rare species in the local area instead of its commercial hybrids in hopes it would re-establish in its native range, facilitate greater pollinator interactions with its wild counterparts, and increasing genetic variability among the same (Ojeda & Santos-Guerra 2011). It was feared the wide variety of hybrids being planted prior to employing this method would contribute to extirpation of the wild plants.

This program was successful, and hundreds of thousands of the rare species are now grown in private gardens in the Canary Islands (Ojeda & Santos-Guerra 2011). However, further studies revealed that since these commercially available rare plants are generally propagated clonally, their genetic variability is probably only a small subset of that found throughout the species range (Ojeda & Santos-Guerra 2011). This presents a paradox and valid argument opposing this method. While it certainly can prevent a rare species from going extinct, it can also create genetic bottlenecks making these plants unuseful candidates for reintroduction purposes. This is assuming of course that reintroduction would be their purpose. As I discuss conservation by dissemination, I am making the assumption that commercially available rare plants would not be used for reintroduction into their native wild habitats.

Assess the risks and benefits of conservation by dissemination as it applies to *Amsonia kearneyana*.

Risks:

Could *Amsonia kearneyana* escape cultivation and become invasive in another area? Ornamental horticulture has been identified as perhaps the largest pathway for worldwide plant invasions (Bradley et al 2012, Dehnen-Schmutz 2011). However, species moved within North American regions are generally not problematic (Bradley et al 2012). In fact, all plants are not equal opportunity invaders. As any home gardener or ecologist could attest, different species of plants require different combinations of environmental factors to grow or become established, let alone become invasive. There is abundant science investigating the factors influencing invasiveness risk. For example, Ruprecht et al. (2013) found some alien congeners had a greater ability to germinate earlier than native species in lower light and lower nitrogen conditions. However, the greater plant functional trait plasticity seen in the alien species did not confer a significant competitive advantage over native species (Ruprecht et al. (2013). It is also difficult to identify potential invaders based exclusively on plant traits because traits of invaders often depend on characteristics of the invaded habitats (Funk 2013). Therefore, a species likelihood to invade should evaluated based on the biological traits of the species and how those relate to the considered environment. Similar analysis can help predict if a species would be unlikely to naturalize in a new environment outside of human care, and therefore inform the horticultural trade (Bradley et al 2012, Dehnen-Schmutz 2011).

One method to inform risk-of-spread was developed by Dehnen-Schmutz (2011), She compiled a "Green List" in Britain evaluating factors relating to spread of long-term commercially available species. Factors positively associated with spread were residence time in the area, propagule pressure or the extent plants were exposed to the area, whether the plant or close congeners ever became invasive in another area, and the plants hardiness to climate change (Dehnen-Schmutz 2011). While proxys to some of these factors would have to be used for rare plants new to the market, like *A. kearneyana*, this serves as a good base for analysis. Dehnen-Schmutz (2011) did assess one species of *Amsonia* (*A. tabernaemontana var. salicifolia*) widely available in England and the United States. It was determined to be one of the least likely species to become invasive in Britain. Many species of *Amsonia* are commercially popular in the United States, including *A. tabernaemontana*. *A. Hubrichtii*, and *A. ciliata var. filifolia*. I could find no studies indicating that any of them had become, or had risk to become, invasive anywhere. Most southwestern US *Amsonias* are rare or at least not overly common anywhere in the wild (Topinka 2006). As many similarities exist between *A. kearneyana* and its southwestern U.S. congeners, there is no reason to believe *A. kearneyana* is likely to become invasive, even if it did escape cultivation.

Another risk that would need to be considered is the potential for *A. kearneyana* to hybridize with and potentially harm wild *Amsonia* species. For any natural hybridization to occur among plants cross pollination must occur. This requires both species to be in flower at the same time and in close enough proximity for pollinators to interact with both. *Amsonias* do generally flower at similar times, April to May. However, although many morphological traits can blur between closely related taxa, *A.*

kearneyana has the longest corolla tube of any *Amsonia*, which could potentially exclude some pollinators. Additionally, even if a capable pollinator was present, the chance of it then finding another *Amsonia* in the wild would be slim. Of the twenty recognized *Amsonia* taxa, half are rare. Especially in the southwestern U.S., *Amsonias* tend to be geographically isolated and distant from populated areas. For a pollinator to find and pollinate a wild *A. kearneyana* plant would be virtually implausible since all wild *A. kearneyana* plants are found in remote areas of federal land, in a single mountain range in southern Arizona and far from any populated area likely to house a cultivated *A. kearneyana*.

The potential to spread disease to wild taxa is another concern. However, no diseases have been reported in *Amsonias*. If any were developed in a cultivated stock, for the same reasons stated, it would be unlikely to be spread to wild plants. Though generally cultivated plants may commonly hybridize their wild congeners, the life history traits of *Amsonias* would likely mitigate this risk.

Benefits:

We cannot deny that there are times when human intervention may be an endangered species' last chance at survival, especially in light of climate change, habit loss, loss of pollinators, and loss of genetic diversity in wild populations (Guerrant & Kaye 2007). However, the research necessary to mitigate these threats can be expensive for rare plants. When a plant is listed, the already overburdened US Fish and Wildlife Service is essentially handed the responsibility of either conducting or funding this research. The citizen science potential that would accompany conservation by dissemination could help fill in these gaps while not straining USFWS budget. Data could be collected from registered customers and participating nurseries which would supply a needed wealth of information about how the species might react to new conditions that may reflect imminent future conditions of the species wild habitat (Shirey 2011).

Promoting commerce of endangered plants in a responsible way would promote education and public support for plants in general. People love to collect things that are inherently rare from limited edition stamps to antique costume jewelry. It is already evident from “crazy cacti collectors” that this can definitely extend to plants in the U.S (Goettsch et al. 2015). Harnessing the fervor seen in cacti collectors could return needed funding and public excitement to plants without endangering the plants in the wild. This approach has traditionally been employed to discourage wild collection of endangered plants by flooding the market with legally obtained privately grown stock (Thornton 2013). This would continue to be a benefit. However, the focus should shift. People should not be discouraged from possessing native plants, endangered or not, as long as the survival of their wild congeners are not threatened.

Marketing would be key, but a part of the profits from commercial sales could be returned to conservation efforts for the wild populations. Non-listed *Amsonia* species are popular garden plants in the eastern US. Many use them to line their gardens. All *Amsonias* are unpalatable to wildlife due to their bitter milky sap (like Oleanders in the same *Apocynaceae* family). This keeps wildlife, particularly deer and small mammals, out of gardens but they do attract butterflies. The flowers and foliage are both attractive throughout spring and summer, and the foliage turns a rich orange-yellow in the fall, adding to its appeal. Additionally, even when the stems die back over winter, they dead leaves tend to stay on the stems for a while making them easy to clip off and clean up before new stems emerge in the spring. They are also considered drought tolerant and easy to grow in back yard gardens or containers. *Amsonia kearneyana* shares all of these qualities and could be a welcome addition to southwest gardens. As growing tips and tricks become widely available on the internet as garden plants increase in popularity, this information, even at this informal level, could serve to be informative to USFWS as indicators of the species climate tolerances and general life history trends in varying habitat conditions. By encouraging a system of registering commercially obtained endangered plants and collecting specific plant data, these data could be further exploited to inform conservation efforts of wild plants. For example, data could be obtained from commercial customers of *A. kearneyana* regarding its tolerance to different climate regimes, watering needs, pollinator visits, life span, rooting-depth, and regeneration niche, all of which remain poorly understood. This will be vital knowledge to understand how this species will adapt to climate change.

Southwestern U.S. *Amsonias* are generally described as drought tolerant once established. The wide spectrum of soil moisture observed in wild populations of *A. kearneyana* (Chapters 1 and 2) supports this idea. As hardiness zones are shifting northward, the commercial demand for drought tolerant species is increasing (Bradley et al 2012), and *Amsonias*, including *A. kearneyana* could grow in popularity. Considering its low invasiveness risk, this could be a good option for consumers. In fact, one study found U.S. native plants comprised nearly all new drought tolerant species made available in the nursery trade between 2005 and 2011(Bradley et al 2012).

Commercial propagation and the internet

Many listed species are available on the commercial market via the internet (Shirey 2011). However, the vast majority of these vendors are not permitted by USFWS (Shirey 2011). No monies obtained through sales are returned to conservation efforts toward the species recovery. Also, any potential citizen science type data are not being collected toward these efforts. Additionally, potential risks as discussed above cannot be assessed or mitigated through USFWS controls. This is a devastating trend for an already cash strapped USFWS. For example, unregulated and unpermitted sales of black lace cactus (*Echinocereus reichenbachii* var. *albertii*) is estimated over $10,000 (Shirey 2011). This is a species where commercial propagation was suggested in its recovery plan, but never implemented through the agency. Kuenzler hedgehog cactus (*Echinocereus fendleri var. kuenzleri*) is another cacti for which commercial propagation was suggested in the recovery plan but not implemented by the agency (USFWS 2005). While, here too, not retaining any monies, data, or control, USFWS states in its 2005 5-year review, "This plant is readily available from commercial growers, who are probably satisfying much of the demand from cactus hobbyists. There are no published data on the popularity of this cactus among hobbyists, or its demand on the world market (USFWS 2005)". Kuenzler hedgehog cactus had $171,768 in estimated commercial sales (Shirey 2011).

A. grandiflora, another rare *Amsonia* from the southwest U.S. is currently available at least five commercial nurseries in the Phoenix metropolitan area alone and widely available on the internet for about $18.00 (USD) for a seedling. USFWS declined to list this species due to a lack of information (USFWS 1993c). However, species information including growth conditions and planting tips can be found online and in social media sites.

Conclusion

While it is difficult to predict potential sales of *A. kearneyana*, USFWS has a unique opportunity to take the lead in that venture and reap the benefits both financially and intellectually for the conservation of this endangered plant. Admittedly, with the shortfalls, loopholes, and lack of enforcement evident with the current commercial propagation system, this is not a long term fool proof plan. However, considering the risks to the species' survival are negligible and the benefits are probable, its implementation should be seriously considered.

The prevailing USFWS policy is the *Policy Regarding Controlled Propagation of Species Listed Under the Endangered Species Act* (2000). The requirement that controlled propagation be included in the species recovery plan is already satisfied for *A. kearneyana*. The recovery plan would therefore not need to be amended to include commercial propagation. Not only was it included in the recovery plan (USFWS 1993b), but controlled propagation and reintroduction has already been implemented according to the Center for Plant Conservation Guidelines (USFWS 2000) as directed. Permitted controlled propagation and seed accessioning of *A. kearneyana* continue at Arizona Sonoran Desert Museum in Tucson, Arizona and at Desert Botanical Garden in Phoenix, Arizona to maintain refugia plants.

It would be straightforward to begin, since permits for sales could be granted to the aforementioned botanic gardens currently holding stock. The botanic garden would affix the required state permit tag to each plant. Initial plants to be sold could be genetically profiled prior to sale to establish the maternal lines. This information would allow a more informative study of how progeny from different maternal profiles establish and reproduce differently under varying conditions (Guerrant 2007). Purchasers of these initial plants could be easily tracked and asked for their participation such as registering on a website. In registering, buyers could be asked about their geographical location (to establish climate regime), what soil type they will use, if transplanting, and what shading conditions will be provided. Participation could include logging in at some predefined intervals and reporting certain information on their plants such as their height, number of stems, number of fruits, any pollinators observed, and any seed germination and seedling establishment. These data could be collected and analyzed by USFWS over time and compared to results of scientific studies of wild populations. Even if a small portion of purchasers were diligent in reporting, this citizen science data could be vital in informing future reintroduction events, climate change scenarios, and generally contributing to knowledge of the species. Almost all gardening websites provide some version of reporting opportunity on plants they sell, and are always ripe with responses. If people feel they can be part of the bigger picture of conservation of rare species, even if the program is voluntary, I predict many will embrace the opportunity.

Further, this would be a great opportunity for USFWS to connect positively with the public and educate them directly about the work they do and why it is important. Propagules from sold plants would be difficult to track and may eventually succumb to a broader commercial availability. However, the ability to track genetic information from future generations of these *A. kearneyana* plants using that collected from the parentals may be further informative for genetic studies and evaluating the breadth of the commercial market itself. In conclusion, conservation by dissemination of *A. kearneyana* is a ground floor opportunity for USFWS to use the modern age of technology and instant communication to work for endangered plant research and conservation. While this approach may not be suitable for all endangered plants, the life history and characteristics of *A. kearneyana* make it an excellent candidate for consideration.

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APPENDIX A

LANGUAGE IN RECOVERY PLANS REGARDING COMMERCIAL PROPAGATION.

For most of these species, including the following examples, over-collection of wild plants was identified as a predominate threat to the species survival.

- *Ancistrocactus tobuschii* (Tobusch Fishhook Cactus), Texas, 1987, threats of commercial trade, habitat, grazing “Develop a program to provide propagated plants and seeds to the commercial market.” (USFWS 1987)
- *Coryphantha minima* (Nellie Cory Cactus), Texas, 1983, threats commercial trade, habitat, grazing “Determine the feasibility of reducing the collecting pressure on the wild populations by promoting a commercial artificial propagation program.” (USFWS 1984)
- *Coryphantha ramillosa* (Bunched cory cactus), Texas, 1990, threats commercial trade, habitat, grazing, “Mesa Garden of Belen, New Mexico maintains parent plants for the commercial sale of seeds of bunched cory cactus.” “Refine propagation techniques to provide nursery s-tocks and seeds to reduce collecting pressure.” (USFWS 1990b)
- *Echinocactus horizonthalonius var. nicholii* (Nichol’s Turk’s Head Cactus), Arizona,(USFWS 1986a): (recovery plan 1986):threats copper mining operations, urban development, off road vehicle use and over collection, “A commercial artificial propagation program may remove some of the collecting pressure on the cacti in the field. Some collectors enjoy raising their own plants from seeds or seedlings if these are easily and economically available.” (USFWS 2009b): (5 year review 2009):“Cactus collection for profit and seed collection by commercial nurseries may still pose a potential threat to NTHC”
- *Echinocereus chisoensis var. chisoensis* (USFWS 1993a): (Chisos Mountain Hedgehog Cactus), Texas, 1993, threats over collecting, habitat degredation, etc “…by making cultivated material available to satisfy the desire of enthusiasts to own and cultivate the variety” “Foster horticultural development of cultivated material to address the commercial demand for horticultural specimens.
- *Echinocereus reichenbachii var. albertii* (Black Lace Cactus), Texas, over collection, (USFWS. 2009a): (5 year review): “The species’ recovery plan objectives included cultivation of stocks for commercial distribution, as well as seed collection to aid in propagation studies (USFWS 1987). The development of seed stock by authorized responsible, and/or licensed agencies was considered a potentially practical method of reducing collection pressures.” “The demand for rare cacti by collectors has escalated in the United States, and in other countries, including Japan and Germany (Westlund 1991). The demand for export of BLC to these countries is primarily attributed to the attractive blooms of the species (Westlund 1991). In 1987, during the course of collecting field data for preparation of the recovery plan, Gardner and O’Brien found no evidence of collecting pressure on any of the three extant

populations (USFWS 1987). In 1991, the TPWD published a report on the cacti trade, monitoring impacts by investigating 72 individual collectors, family nurseries, and commercial nurseries (Westlund 1991). Although many of these collectors/growers had less than 50 individual cacti plants representing only three to four species, one collector had more than 1,000 freshly dug cacti of 13 subspecies. Among the three subspecies most heavily collected was the E. *reichenbachii* var. *fitchii* . Due to taxonomic confusion, it is unclear how many of these may have actually been BLC. The report concluded that the already established monitoring of the trade of these flowering cacti needs to be increased. Another finding was that other species in the genus *Echinocereus* have been exploited by smaller dealers, as well as commercial nurseries, without permits (Westlund 1991). Information on the level of threat due to field collecting of this species since TPWD's 1991 report is lacking. Sporadic site visits to the Refugio and Kleberg populations over the last 10 to 15 years have not produced reports indicating that illegal collection is ongoing at either site."

- *Betula uber* (Virginia round leaf birch tree), Virginia, vandalism and over collection. USFWS encouraged commercial sales of these trees, but interest from commercial nurseries was scant in the early 1990s. The recovery plan for the species states "In addition to increasing the number and geographical distribution of round-leaf birches in cultivation, making the plants available to the public was viewed as a way of heightening awareness of endangered species and possibly reducing vandalism to the natural population as the plant would no longer be perceived as rare." (USFWS 1990)