Saratoga Horticultural Research Endowment

Final Report

Project Title: Chaparral Ferns for California Landscapes

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Introduction

The majority of ferns used in California landscapes are non-native and water-loving; low-water-use alternatives are rarely available. Funding from the Saratoga Horticultural Research Endowment allowed the California Botanic Garden to begin a multi-year project with the goal of expanding the availability of low-water-use plants by introducing native, low-water-use fern species to the nursery trade. The target species for the project have broad ranges throughout California, and are found in chaparral habitats in our local San Bernardino and San Gabriel mountain ranges. These plants are uniquely adapted to California's Mediterranean climate. In this first year of the project, CalBG staff wild-collected, divided, and established 6 species of chaparral ferns that were then tested at the Garden for their horticultural viability. The project also began propagation trials so that the low-water-use ferns could be successfully introduced in its own retail nursery. Successful propagation protocols will also be made available to other native plant nurseries to encourage a wide range of availability of these ferns in southern California.

Materials and Methods

Fern Acquisition

Garden staff successfully collected 6 different species of chaparral ferns in the San Gabriel and San Bernardino Mountains: *Dryopteris arguta, Myriopteris covillei, Pellaea andromedifolia, Pellaea mucronata, Pentagramma triangularis,* and *Selaginella bigelovii* (fern ally). The collections were divided into over 20 plants of each species to over-summer in the nursery. The GPS location, habitat type, slope, associated species, collectors, and soil conditions were documented at the time of collection, and all of the material was accessioned in the CalBG database system.

Horticultural Trials

Two locations in the Garden were chosen for the horticultural trials in October. Both locations receive filtered light throughout the winter, and morning sun in the summer. The *Dryopteris arguta* and *Pentagramma triangularis* were found deep in the understories of larger chaparral shrubs on our collection trips, and so were planted in heavier shade, but still very close to the rest of the ferns in each location, maintaining a similar soil type. One location has quickly draining, rocky soil, and is little slope. The second location has a higher clay content, and is located on an east-facing slope.

Ten individuals of each species were planted in each location, save for *Myriopteris covillei* – only 17 of those survived over the summer in the nursery - and 9 and 8 were planted at each location, respectively. The plants were placed 2' apart from each other and each row was separated by 2.5'. Holes were dug for the ferns and filled with water which was allowed to drain completely before planting. Most of the ferns were found in very rocky sites, and companion rocks were placed by each fern. The planting took place in early November, which was one month later than scheduled due to unusually warm and dry weather in October. During the rainy season, the ferns were watered on an as needed basis, depending upon the rain fall, with rains continuing that year through late April. Once the rainy season ended and the soil dried, the horticulture staff implemented a summer watering regimen, with half of the ferns in each test plot receiving water weekly, and the other half in each plot receiving water every other week. The monthly growth index of each plant was recorded at the end of each month for the entirety of the trial. The growth index number was calculated using the formula developed by UC Davis ($h + [(l+w)/2]/2$), in their 2006 horticultural trials for native plants (Reid and Oki, 2008).

A brief survey, rating the vigor, foliage color, foliage texture, growth habit, and overall ornamental value of the ferns was developed, and administered to staff and volunteers at 4 points throughout the year-long trial. The participants were asked to rate each fern species on a 1-5 scale, with 5 being excellent, and 1 being poor. The total average score for each species was calculated for each survey.

Communication

A website dedicated to the chaparral fern project was created and updated monthly with the progress of the horticultural trials of the ferns. The website can be found here: https://www.calbg.org/collections/chaparral-fern-project. A podcast was also created during the initial COVID shut down in March and is also available on the website.

Signage was put in place at the site of each horticulture test plot to communicate the purpose of the project to garden patrons.

Propagation

Spores were collected from the acquired ferns both while they were in the nursery prior to planting and while they were in the ground for the horticultural trial. There were three primary methods of spore collection: a) fronds were collected and a series of sieves was used to separate chaff from spores, with the smallest sieve being #200; b) fronds were collected and spore material was scraped away from the sporangia using a precision knife or scalpel; c) fronds or leaflets were collected and stored in their entirety to dehisce into envelopes constructed from computer paper, and subsequently parchment paper; chaff was then tapped away from the spore material and discarded, leaving the spore material on the paper. Spores that were sieved or scraped away from the sporangia were stored in glassine envelopes.

The primary growing media was agar, and staff experimented with a range of nutrient solutions, including Parker Thompson Fern Basal Salt Mixture (PT), Murashige & Skoog Modified Multiplication Medium (555), Murashige & Skoog Basal Salt Mixture (524), Murashige & Skoog Modified Basal Medium (701), 6-Benzylaminopurine (BA), and Indole-3-Butyric Acid (IBA). Trials were also conducted on soil mixtures of 1:1 Sunshine Mix #5 and Peat Moss.

The propagation trials were conducted in a sterile lab environment with the use of an autoclave, flow hood, and in some cases, a centrifuge. The spores were sown into deli containers made of polypropylene plastic that were able to withstand the temperatures of the autoclave. The sown material was then stored in a growth chamber with 22 hours of light with a high temperature during the lit hours of 22^{o}C and a low temperature during the dark hours of 20^{o}C.

Results and Discussion

Horticulture Trials

The trials of the six species of ferns resulted in only four species being recommended for home gardens. The recommendations were made based upon the survey results that were administered at each season, as well as careful documentation of the ferns' growth throughout the trial. The four recommended species are *Pellaea andromedifolia, Pellaea mucronata, Dryopteris arguta*, and *Selaginella bigelovii*. Generally, the ferns located in the well-draining, rocky soil that were receiving weekly watering during the summer performed best out of all the ferns.

The *Dryopteris arguta* performed well in both soil types, and is the only species to maintain a lush green appearance under both summer watering regimens.

Pellaea mucronata was very adaptable; it was more tolerant of clay soil and greater sun exposure than other species in the trial. Watering once per week kept the foliage a deep grey-green, and broke its tendency toward dormancy in the summer. Those that received water every other week in the summer did not go fully dormant, but lost some of the vibrancy of their foliage color.

Pellaea andromedifolia prefers well-drained soil, and seems to perform better if it has less intense summer sun exposure. The two ferns that received the most sun in the well-draining soil site showed a tendency toward dormancy at a rate equal to that of those receiving water every other week. Many of the fronds tended to go dormant in both watering regimens, but most plants did not enter full dormancy if watered weekly. Those that received water every other week did go mostly dormant, but broke dormancy once the weather cooled. The dormant fronds do have some aesthetic value, with their deep red color.

Selaginella bigelovii is a very slow growing but attractive ground cover that would be great for smaller rock garden areas. We found that it performs best in well-draining soil, and watering once per week in the summer keeps its foliage bright green, and upright. Those receiving water every other week did not go completely dormant, but became a very pale, dull green.

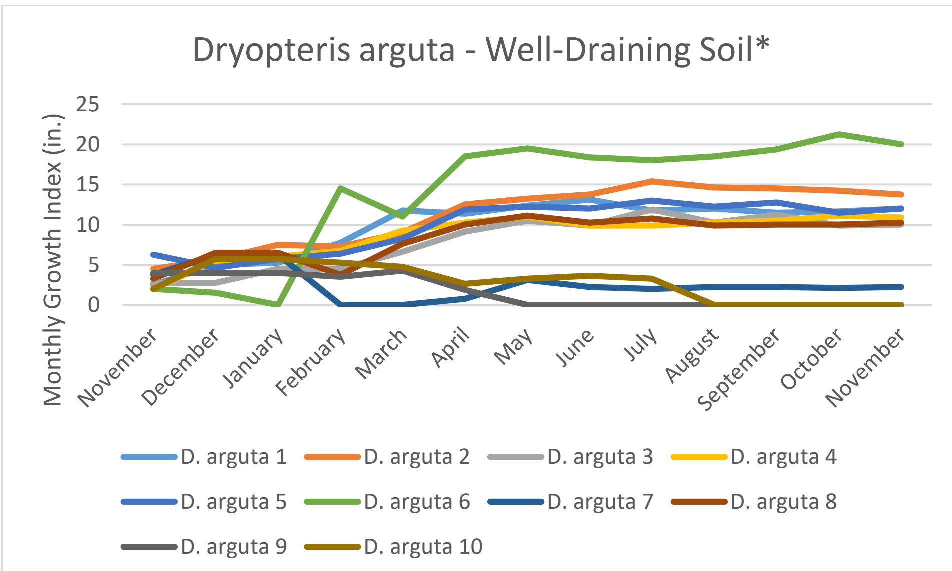
The *Pentagramma triangularis* started out very promising in the beginning of the trials in both locations. As summer approached however, they quickly began entering dormancy, with all specimens entering full dormancy by August when the summer temperatures began to get more extreme. All, except for three plants in the well-draining soil site, which have started to show signs of breaking dormancy, have remained dormant through the fall, and have remained so even after the first rains of winter. Although it was one of the favorites of all those surveyed in the spring time, it's prolonged dormancy, makes it less desirable for gardens.

Most of the Myriopteris covillei that were vigorous in the nursery, remained so during the trial and did not enter dormancy with the summer irrigation regimens. However, the average rating given to the plants by survey participants was, particularly in the last two seasons, in the poor performance range of 2. The ferns still have interesting leaflet texture and color and may be better suited as a potted plant rather than a garden specimen.

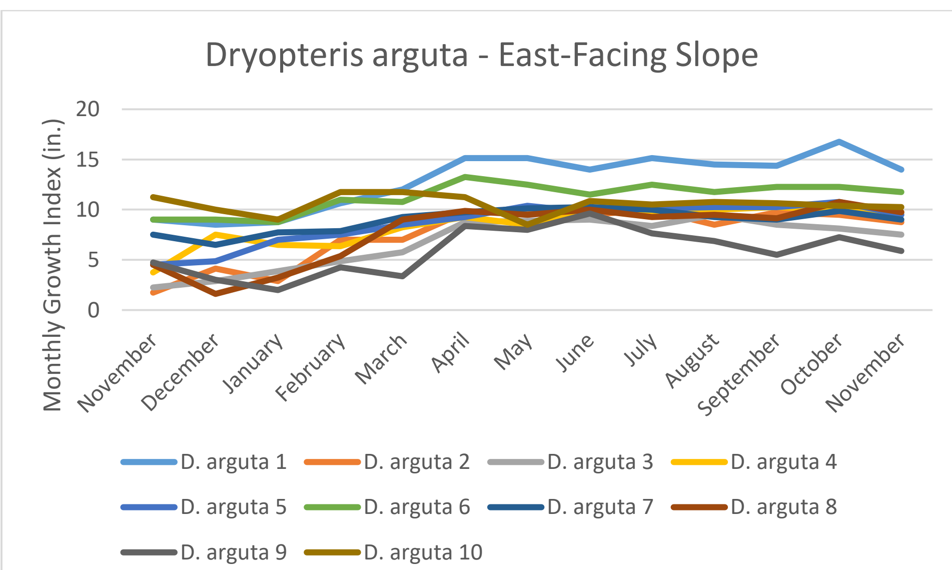
We had a decent rainy season during the trials, with 8.81 inches of rain fall from November-December, .45 inches January through February, and 10.09 inches falling very late in the season, in March and April. We experienced extreme summer temperatures in the summer, with temperatures in August hovering every day around 100°F. We experienced several days over 100°F, including a high of 115°F the first weekend of September. None of the ferns experienced signs of burn during the extreme heat. Nor were the ferns effected by frost, our lowest temperature was recorded at 28°F.

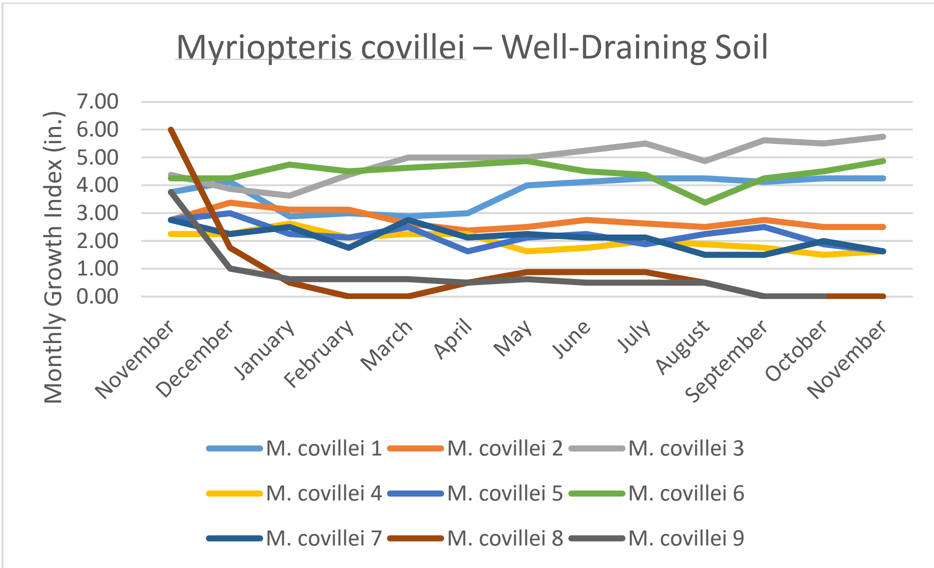
Monthly Growth Index Charts – Site Comparisons

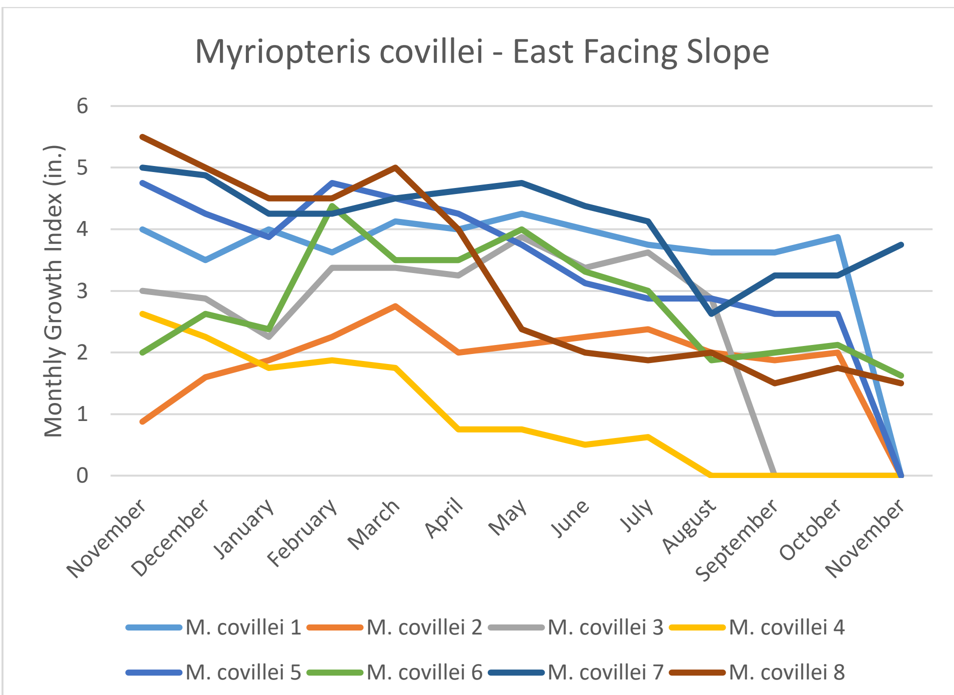
Below are side-by-side charts comparing the changes in Monthly Growth Index for each species at each site throughout the life of the horticultural trial, from November 2020 – November 2021. A Growth Rate Index of 0 indicates full dormancy, unless otherwise indicated. Declining trends in growth rate can be attributed to either the death of old fronds, or the dormancy of several fronds on the ferns.

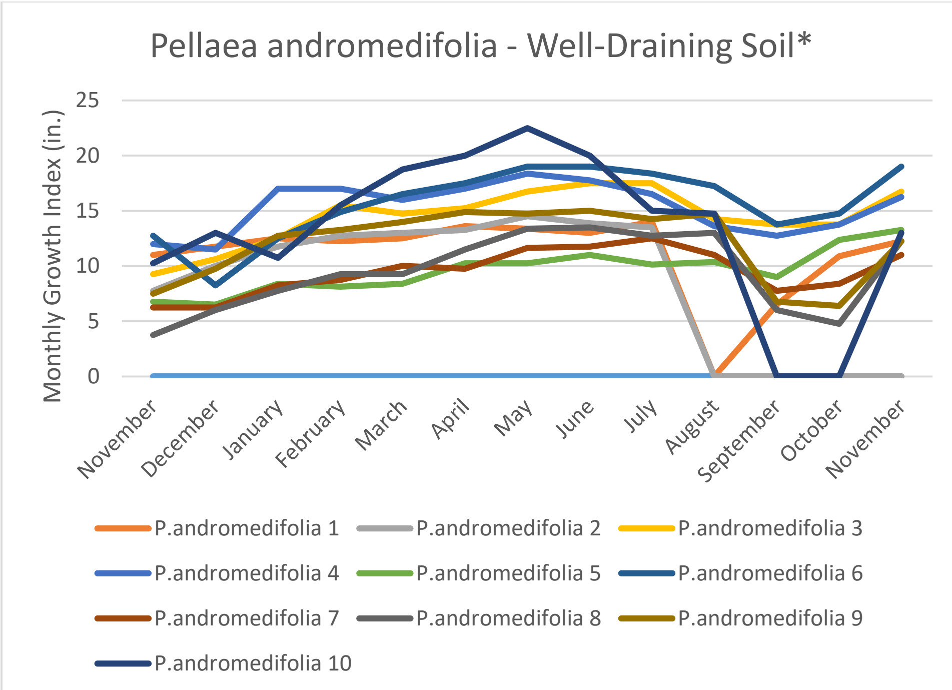


*D. arguta 6 died in January, and was replaced. D. arguta 9 and 10 died in May, and July, respectively.

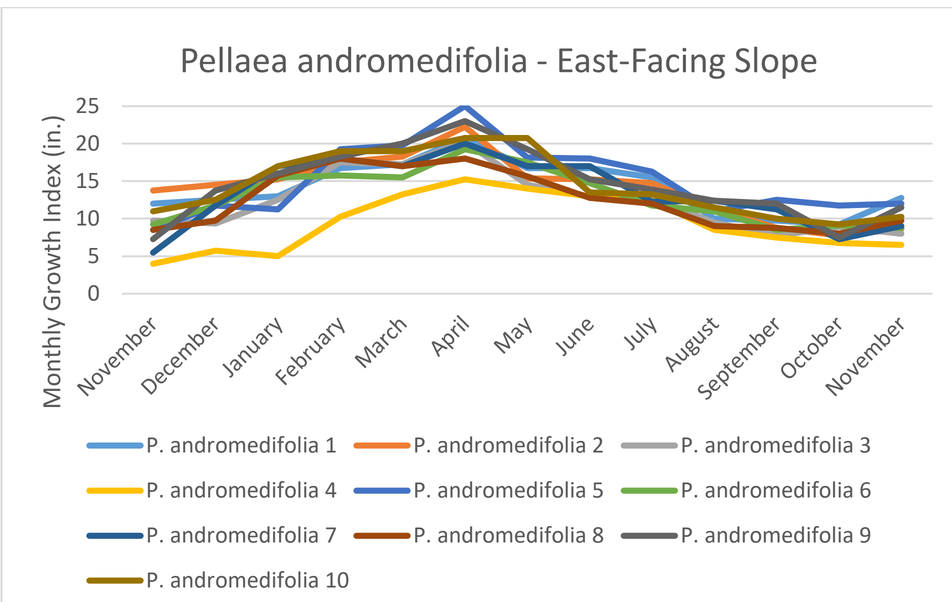


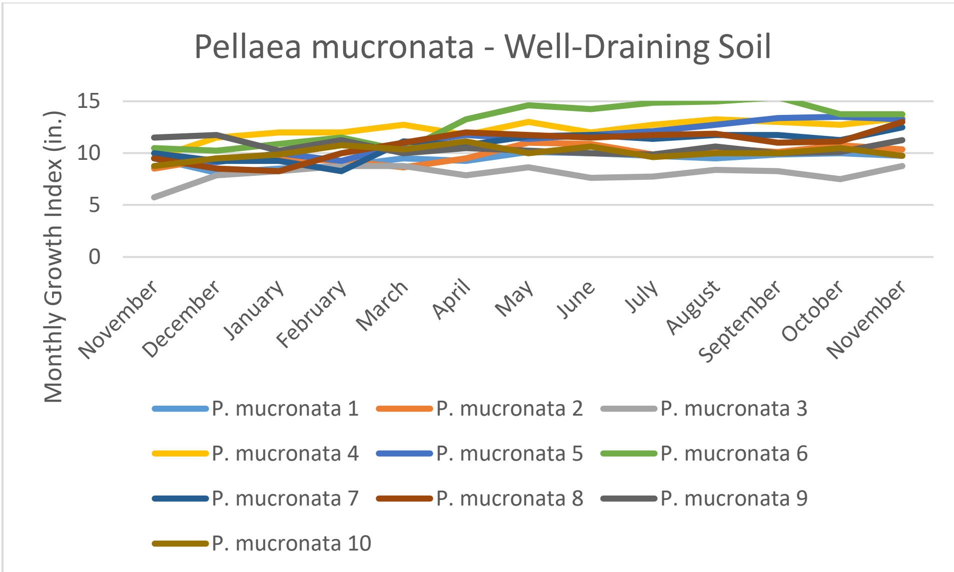

Myriopteris covillei – Well-Draining Soil
Monthly Growth Index (in.)
7.00
6.00
5.00
4.00
3.00
2.00
1.00
0.00
November
December
January
February
March
April
May
June
July
August
September
October
November
M. covillei 1
M. covillei 2
M. covillei 3
M. covillei 4
M. covillei 5
M. covillei 6
M. covillei 7
M. covillei 8
M. covillei 9

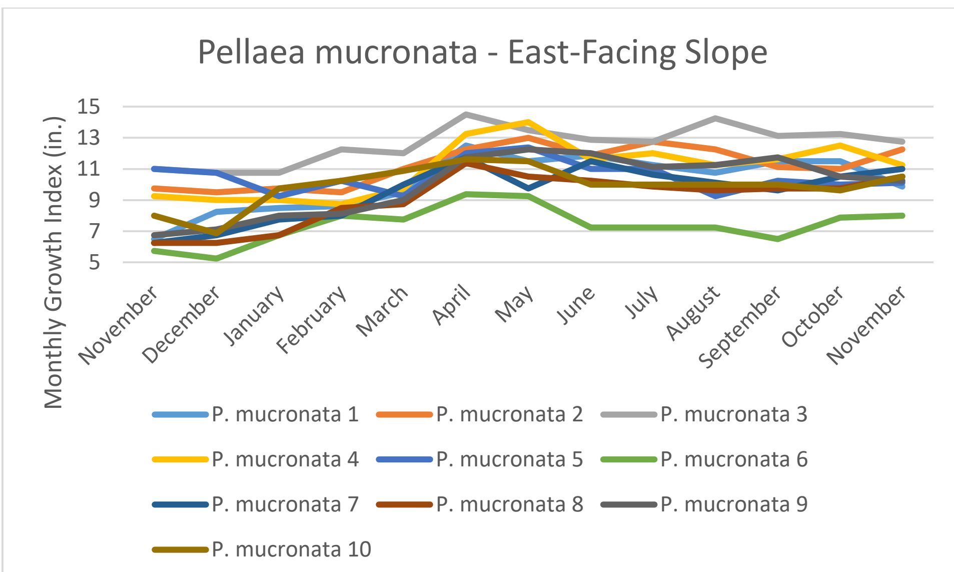

Myriopteris covillei - East Facing Slope
Monthly Growth Index (in.)
6
5
4
3
2
1
0
November
December
January
February
March
April
May
June
July
August
September
October
November
M. covillei 1
M. covillei 2
M. covillei 3
M. covillei 4
M. covillei 5
M. covillei 6
M. covillei 7
M. covillei 8

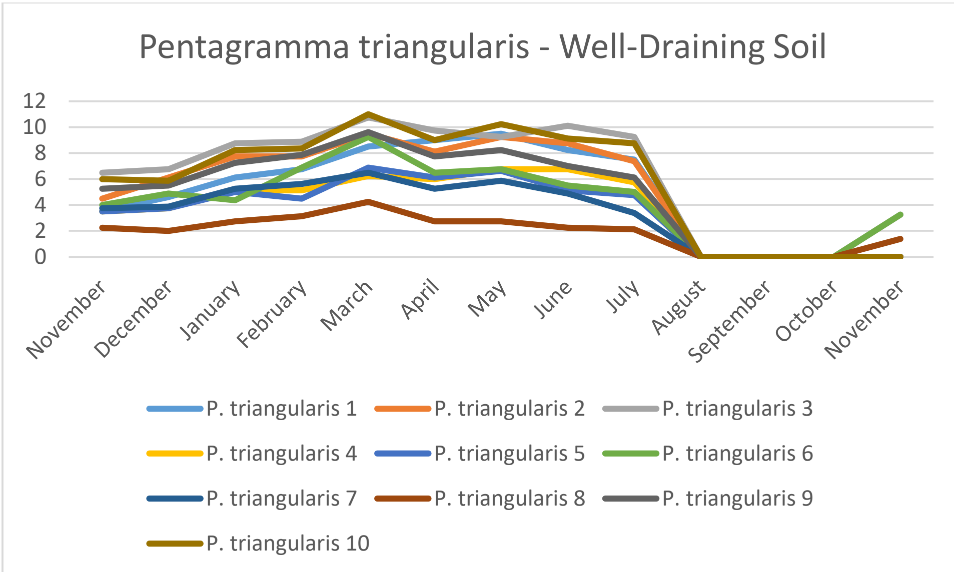


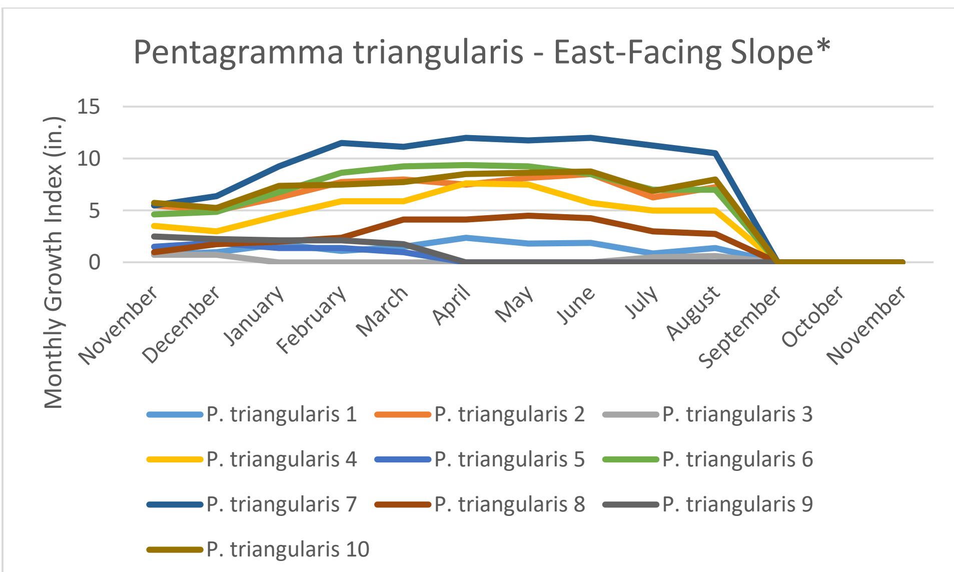
**P. andromedifolia*
2 died in August.



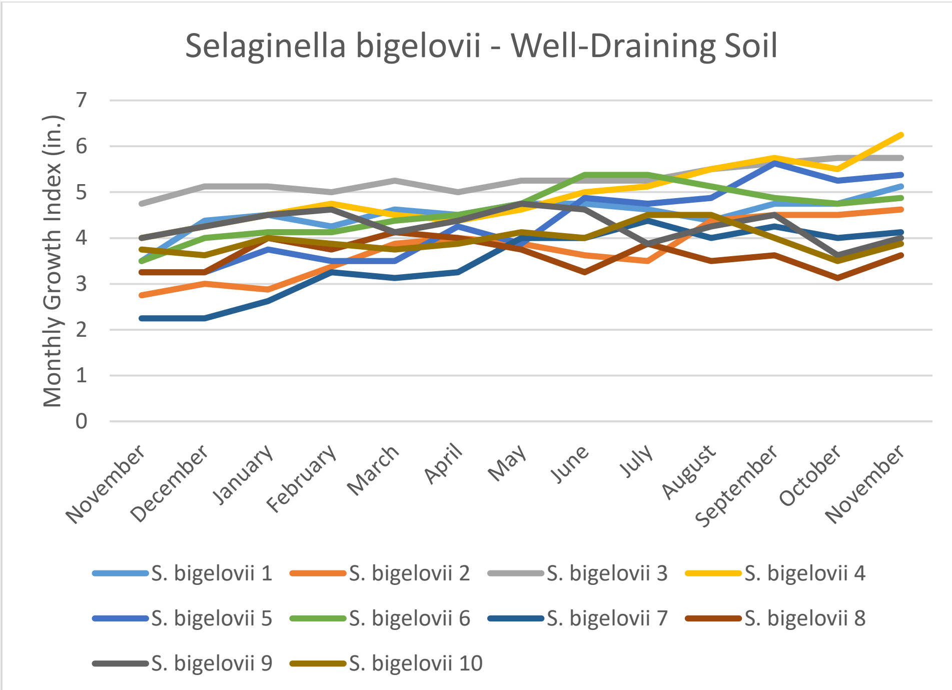

Pellaea mucronata - Well-Draining Soil
Monthly Growth Index (in.)
15
10
5
0
November
December
January
February
March
April
May
June
July
August
September
October
November
P. mucronata 1
P. mucronata 2
P. mucronata 3
P. mucronata 4
P. mucronata 5
P. mucronata 6
P. mucronata 7
P. mucronata 8
P. mucronata 9
P. mucronata 10

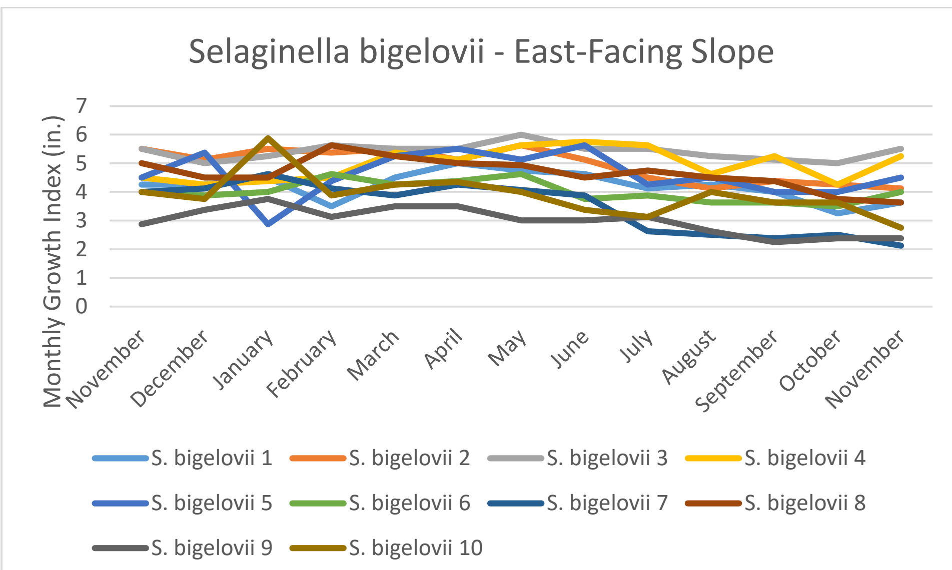

Pellaea mucronata - East-Facing Slope
Monthly Growth Index (in.)
15
13
11
9
7
5
November
December
January
February
March
April
May
June
July
August
September
October
November
P. mucronata 1
P. mucronata 2
P. mucronata 3
P. mucronata 4
P. mucronata 5
P. mucronata 6
P. mucronata 7
P. mucronata 8
P. mucronata 9
P. mucronata 10





*P. triangularis 3, 5, and 9 died on the east-facing slope.





Horticulture Trial Survey Results Average species score				
Species	December	April	July	November
Dryopteris arguta	3.9	3.76	4	4
Pentagramma triangularis	4.7	4.03	1	1
Myriopteris covillei	3.9	2.88	2.1	2
Selaginella bigelovii	4.1	3.47	3.35	3
Pellaea mucronata	3.6	4.1	3.88	3.75
Pellaea andromedifolia	3.3	3.77	3.4	3.6

Photos of horticultural trials

Myriopteris covillei in well-draining soil in August (left) and October(right)

Pellaea mucronata on the east-facing slope in May (left) and September (right)

Pellaea andromedifolia in well-draining soil in November (left) and August (right)

Dryopteris arguta on the east-facing slope in February (left) and August (right)

Pentagramma triangularis in well draining soil in December (left) and September (right)

Selaginella bigelovii in well-draining soil in February (left) and August (right)

Propagation Trials

Additional work needs to be done to find the optimal propagation protocols for the recommended chaparral ferns. Initial trials were largely unsuccessful, with little to no germination recorded and mold and bacterial contamination taking over the inoculated plates. Our trials showed that nutrient agar solutions were much more effective for germination, than soil mixes, which did not yield germination. Throughout the grant period, the propagation team achieved germination of all of the species, although *P. triangularis* and *D. arguta* have yielded higher levels of contamination and less consistent germination than the rest of the species. The period from sowing to germination tended to vary widely, ranging from 2 weeks to well over 3 months. Growth into gametophytes has generally been slow, and contamination tends to increase as the gametophytes mature, which necessitates that they be transplanted into fresh containers prior to becoming sporophytes. After transplanting, many gametophytes have died, especially those that have been transplanted onto soil mixtures. All of the gametophytes that have survived the transplanting process thus far have stagnated growth. We will continue to experiment with this process, so that we will eventually be able to produce ferns on a scale suitable for retail sale, and share that information with others.

The charts below document the trials and observations of the various experiments conducted during the grant period.

Description: *D. arguta* spore germination was observed to be frequently overgrown with contamination in trials prior to the grant period. The spores in the trials below were collected from very mature fronds, and sporangia was separated from spores using a 200# sieve. All Spores were prewet 24 hours with nonsterile DI water, then sterilized using the different agents at the concentrations and exposure times specified below. A centrifuge was used to triple rinse the spores with sterile DI water prior to sowing into plates of sterile nutrient agar solution. This series of experiments was unsuccessful and yielded no germination.

Date	Fern species	Spore sterilization	Media	Germination	Transplant date	Media
2/3/2020	Dryopteris arguta	70% Ethanol @ 6 minutes, .25% NaDCC @ 10 minutes	1x PT, +2.5% sucrose, .7% agar, pH Not adjusted	No germination. Contamination present.	N/A	N/A
2/3/2020	Dryopteris arguta	70% Ethanol @ 6 minutes	1x PT, +2.5% sucrose, .7% agar, pH Not adjusted	No germination. Contamination present.	N/A	N/A
2/3/2020	Dryopteris arguta	70% Ethanol @ 6 minutes, .12 % NaDCC @ 10 minutes	1x PT, +2.5% sucrose, .7% agar, pH Not adjusted	No germination. Contamination present.	N/A	N/A

2/3/2020	Dryopteris arguta	.12% NaDCC @ 10 minutes	1x PT, +2.5% sucrose, .7% agar, pH Not adjusted	No germination. Contamination present.	N/A	N/A
2/14/20	Dryopteris arguta	.12% NaDCC @ 7 minutes	1x PT, +2.5% sucrose, .7% agar, pH Not adjusted	No germination. Contamination present.	N/A	N/A
2/14/20	Dryopteris arguta	.12% NaDCC @ 12 minutes	1x PT, +2.5% sucrose, .7% agar, pH Not adjusted	No germination. Contamination present.	N/A	N/A
2/21/20	Dryopteris arguta	.5% NaDCC @ 10 minutes	1x PT, +2.5% sucrose, .7% agar, pH 6-7	No germination. Contamination present.	N/A	N/A
2/21/20	Dryopteris arguta	.5% NaDCC @ 10 minutes, 50% Ethanol @4 minutes	1x PT, +2.5% sucrose, .7% agar, pH 6-7	No germination. Contamination present.	N/A	N/A
2/25/2020	Dryopteris arguta	.25% NaDCC only	1x PT, +2.5% sucrose, .7% agar, pH 6-7	No germination. No contamination.	N/A	N/A
2/25/2020	Dryopteris arguta	.25% NaDCC, 50% Ethanol @4 minutes	1x PT, +2.5% sucrose, .7% agar, pH 6-7	No germination. Contamination present.	N/A	N/A

Description: Fertile fronds were collected and stored in computer paper to allow them to dehisce for two weeks. The lighter chaff material was tapped off of the paper, leaving the spores behind. A minimal amount of sporangia was present with the remaining spores. The spore material was then flicked from the paper onto the sterilized media. Several spore stuck to the paper and could not be removed. Germination was observed in the *D. arguta, M. covillei,* and *P. mucronata*, with germination occurring much later for the *D. arguta*, and *M. covillei* than was expected. Once gametophytes were visible, they were transplanted to new sterile containers. Most of the gametophytes died after transplanting. Those that survived, no significant growth has been observed in those gametophytes that survived the transplant process.

Date	Fern species	Spore sterilization	Media	Germination	Transplant date	Media	Notes
2/5/20	Dryopteris arguta	None	8g/L agar, .385 g/L PT	Germination on 6/13/20. Contamination present.	11/10/2020	8g/L agar, .385 g/L PT	very slow continued growth of gametophytes
						1:1 peat moss, sunshine mix #5	gametophytes died
			1:1 peat moss, sunshine mix #5	No germination.	N/A	N/A	
2/5/20	Myriopteris covillei	None	8g/L agar, .385 g/L PT	Germination on 5/13/20	11/10/2020	8g/L agar, .385 g/L PT	gametophytes died
						1:1 peat moss, sunshine mix #5	gametophytes died
			1:1 peat moss, sunshine mix #5	No germination. Contamination present.	N/A		
2/5/2020	Pellaea andromedifolia	None	8g/L agar, .385 g/L PT	Germination on 7/13/20	Very slow growth. To be transplanted		
			1:1 peat moss, sunshine mix #5	No germination.	N/A	N/A	N/A
2/5/2020	Pellaea mucronata	None	8g/L agar, .385 g/L PT	Germination on 3/12/20	11/10/2020	8g/L agar, .385 g/L PT	very slow continued growth of gametophytes
						1:1 peat moss, sunshine mix #5	gametophytes died

			1:1 peat moss, sunshine mix #5	No germination.	N/A	N/A	N/A
2/5/2020	Pentagramma triangularis	None	8g/L agar, .385 g/L PT	No germination.	N/A	N/A	N/A
			1:1 peat moss, sunshine mix #5	No germination.	N/A	N/A	N/A

Description: Leaflets from fertile fronds were collected and sown the same day. Entire leaflets from each species were sterilized in a 10% bleach solution for 15 minutes, and triple rinsed with DI water. The sterilized leaflets were placed on top of sterile media. There was relatively good germination on the agar plates for *M. covillei, P. andromedifolia,* and *P. mucronata*. Contamination was not present for most species at the time of germination, but did develop as gametophytes were very slowly beginning to form. Growth has been very slow, with transplanting to take place in the future for *M. covillei* and *P. andromedifolia*. The successfully transplanted *P. mucronata* has exhibited stagnant growth.

Date	Fern species	Spore sterilization	Media	Germination	Transplant date	Media	Notes
3/18/20	Dryopteris arguta	Entire leaflet - 10% bleach @ 15 min. Triple rinsed in DI water	8g/L agar, .385 g/L PT	No germination. Contamination present.	N/A	N/A	N/A
			1:1 peat moss, sunshine mix #5	No germination. Contamination present.	N/A	N/A	N/A
3/18/20	Myriopteris covillei	Entire leaflet - 10% bleach @ 15 min. Triple rinsed in DI water	8g/L agar, .385 g/L PT	Germination 6/1/20	Very slow growth - to be transplanted	N/A	N/A
			1:1peat moss, sunshine mix #5	No germination. No contamination	N/A	N/A	N/A
3/18/20	Pellaea andromedifolia	Entire leaflet - 10% bleach @ 15 min. Triple rinsed in DI water	8g/L agar, .385 g/L PT	Germination on 4/20/20	Very slow growth - to be transplanted	N/A	N/A

			peat moss, sunshine mix #5	No germination. No contamination	N/A	N/A	N/A
3/18/20	Pellaea mucronata	Entire leaflet - 10% bleach @ 15 min. Triple rinsed in DI water	8g/L agar, .385 g/L PT	Germination on 5/31/20	7/21/2020	8g/L agar, .385g/L PT	Very slow growth
			1:1 peat moss, sunshine mix #5	No germination. No contamination	N/A	N/A	N/A
3/18/20	Pentagramma triangularis	Entire leaflet - 10% bleach @ 15 min. Triple rinsed in DI water	8g/L agar, .385 g/L PT	No germination. Contamination present.	N/A	N/A	N/A
			1:1 peat moss, sunshine mix #5	No germination. Contamination present.	N/A	N/A	N/A

Description: Spores were collected by lightly scraping the underside of fertile fronds with a precision knife or scalpel into a glassine envelope. Spores collected two weeks prior to sowing and were pre-wet for 3 days in a sterile tube with nonsterile DI water. Five different sterilizing techniques were attempted, and spores were then rinsed with DI water in the centrifuge. While germination was still elusive in this trial, the spore collection method yielded less chaff being mixed in with the spore material and likely contributed to the decrease in contamination.

Date	Fern species	Spore sterilization	Media	Germination	Transplant date	Media
5/24/2020	Dryopteris arguta	1% NaDCC @ 14 minutes	1/2x 701 + 3% sucrose+.7% agar, pH 6-7	No germination. No contamination.	N/A	N/A
5/24/2020	Dryopteris arguta	70% Ethanol @ 4 minutes, 1% NaDCC@ 14 minutes	1/2x 701 + 3% sucrose+.7% agar, pH 6-7	No germination. No contamination.	N/A	N/A
5/24/2020	Dryopteris arguta	3% H202 @ 6 minutes, 1% NaDCC@ 14 minutes	1/2x 701 + 3% sucrose+.7% agar, pH 6-7	No germination. No contamination.	N/A	N/A
5/31/2020	Dryopteris arguta	50% Ethanol @ 2 minutes ,1% NaDCC@ 10 minutes	1/4x555 +3% sucrose+.7% agar pH 6	No germination. Contamination present.	N/A	N/A

5/31/2020	Dryopteris arguta	70% Ethanol @ 2 minutes ,1% NaDCC@ 8 minutes	1/4x555 +3% sucrose+.7% agar pH 6	No germination. No contamination.	N/A	N/A

Description: With some successful germinations in the first trial of flicking non-sterilized spores directly onto sterilized media, the experiment was repeated. The fronds were stored to dehisce in parchment paper instead of computer paper, which the spores tended to stick to, despite vigorous tapping in the previous experiment. The results were somewhat inconsistent, with germination occurring for all species except for *Pellaea mucronata* and *Pentagramma triangularis,* which both germinated in the previous trial. Germination occurred slightly faster than in the first experiment, but growth of the gametophytes continues to be very slow, and will be transplanted after the end of the grant period.

Date	Fern species	Spore sterilization	Media	Germination	Transplant date	Media
6/5/20	Dryopteris arguta	None	8g/L agar, .385 g/L PT	Germination on 8/25/20	To be transplanted	N/A
			1:1 peat moss, sunshine mix #5	No germination. Contamination present.	N/A	N/A
6/5/20	Myriopteris covillei	None	8g/L agar, .385 g/L PT	Germination on 8/25/20	To be transplanted	N/A
			1:1 peat moss, sunshine mix #5	No germination. No contamination	N/A	N/A
6/5/20	Pellaea andromedifolia	None	8g/L agar, .385 g/L PT	Germination on 8/25/20	To be transplanted	N/A
			1:1 peat moss, sunshine mix #5	No germination. No contamination	N/A	N/A
6/5/20	Pellaea mucronata	None	8g/L agar, .385 g/L PT	Germination on 7/21/20	To be transplanted	N/A
			1:1 peat moss, sunshine mix #5	No germination. No contamination	N/A	N/A
6/5/20	Pentagramma triangularis	None	8g/L agar, .385 g/L PT	No germination. Contamination present.	N/A	N/A
			1:1 peat moss, sunshine mix #5	No germination. Contamination present.	N/A	N/A

Description: Fertile fronds were collected from both *Pellaea* species; the rolled margins were pried open and spores scraped out using a combination of a precision knife and metal tweezers. Spores were prewet for 3 days in a sterile tube with nonsterile DI water. Spore sediment was then sterilized as indicated in the chart below, and then rinsed with sterile DI water using the centrifuge. Germination was observed but was overtaken by contamination before gametophytes could be formed. The sterilization time was subsequently lengthened which successfully reduced the contamination, but it also prohibited spore germination.

Date	Fern species	Spore sterilization	Media	Germination	Transplant date	Media
6/14/2020	Pellaea mucronata	70% Ethanol@ 2.5 minutes, 1% NaDCC @ 10 minutes	1/10x 524+ 3% sucrose.7% agar, pH 6	Germination 6/28/20	N/A - Overtaken by contamination.	N/A
7/1/2020	Pellaea andromedifolia	70% Ethanol@ 2.5 minutes, 1% NaDCC @ 11 minutes	1/10x m524 +3% sucrose,.7% agar, pH 6-7	Germination 8/22/20.	N/A - Overtaken by contamination.	N/A
7/12/2020	Pellaea andromedifolia	70% Ethanol@ 3-4 minutes, 1% NaDCC @ 12 minutes	1/10x m524+ 3% sucrose,.7% agar, pH 6-7	No germination. No contamination.	N/A	N/A

Description: Fertile fronds were collected and stored in parchment paper for 2 weeks, and allowed to dehisce. Chaff material was tapped off of the paper, leaving the spores behind. Instead of tapping the spores from the paper into sterile media, they were rinsed off of the paper with DI water, it was hypothesized that additional water may facilitate germination and growth, especially in the soil mixture. However, no germination was observed in either media.

Date	Fern species	Spore sterilization	Media	Germination	Transplant date
3/12/20	Dryopteris arguta	None	8g/L agar, .385 g/L PT	No germination. Contamination present.	N/A

			1:1 peat moss, sunshine mix #5	No germination. Contamination present.	N/A
			8g/L agar, .385 g/L PT	No germination. Contamination present.	N/A
3/12/20	Myriopteris covillei	None	1:1 peat moss, sunshine mix #5	No germination. No contamination	N/A
			8g/L agar, .385 g/L PT	No germination. Contamination present.	N/A
3/12/20	Pellaea andromedifolia	None	1:1 peat moss, sunshine mix #5	No germination. No contamination	N/A
			8g/L agar, .385 g/L PT	No germination. No contamination	N/A
3/12/20	Pellaea mucronata	None	1:1 peat moss, sunshine mix #5	No germination. No contamination	N/A
			8g/L agar, .385 g/L PT	No germination. Contamination present.	N/A
3/12/20	Pentagramma triangularis	None	1:1 peat moss, sunshine mix #5	No germination. Contamination present.	N/A

Description: The whole leaflet trial was repeated to confirm if relatively good germination could be replicated. The sterilization time of *D. arguta* and *P. triangularis* was lengthened to 20 minutes to potentially decrease contamination. Leaflets from fertile fronds were collected and sown the same day. The fronds collected from *P. triangularis* were dormant. Leaflets from each species were again sterilized in 10% bleach solution for 15 or 20 minutes, and triple rinsed with DI water. Leaflets were placed on top of media on two sterile media types described below. The results were somewhat inconsistent with the first trial, with a greater degree of contamination observed in the plates, but germination was observed in all species except for *P. mucronata*, which germinated in the previous trial. The gametophytes will be transplanted after the grant period.

Date	Fern species	Spore sterilization	Media	Germination	Transplant date	Media
9/16/20	Dryopteris arguta	Entire leaflet - 10% bleach @ 20 min. Triple rinsed in DI water	8g/L agar, .385 g/L PT	Germination on 12/30/20	To be transplanted	N/A
			Pure peat moss	No germination. Contamination present.	N/A	N/A
9/16/20	Myriopteris covillei	Entire leaflet - 10% bleach @ 15 min. Triple rinsed in DI water	8g/L agar, .385 g/L PT	Germination on 12/30/20	To be transplanted	N/A
			Pure peat moss	No germination. No contamination	N/A	N/A
9/16/20	Pellaea andromedifolia	Entire leaflet - 10% bleach @ 15 min. Triple rinsed in DI water	8g/L agar, .385 g/L PT	No germination. Contamination present.	N/A	N/A
			Pure peat moss	No germination. No contamination	N/A	N/A
9/16/20	Pellaea mucronata	Entire leaflet - 10% bleach @ 15 min. Triple rinsed in DI water	8g/L agar, .385 g/L PT	Germination on 11/14/20	To be transplanted	N/A
			Pure peat moss	Germination on 12/30/20	To be transplanted	N/A
9/16/20	Pentagramma triangularis	Entire dormant leaflets - 10% bleach @ 20 min. Triple rinsed in DI water	8g/L agar, .385 g/L PT	Germination on 12/15/20	To be transplanted	N/A
			Pure peat moss	No germination. Contamination present.	N/A	N/A

Decription: Description: Vegetative reproduction techniques were attempted for *Selaginella bigelovii*. Cuttings of mature stems were taken of *Selaginella bigelovii* and placed in agar solutions, as well as a sand/verminculite/pumice mixture. Some of the cuttings were dipped in the rooting hormone, Dip n' Grow prior to being stuck in the agar or sand mixture. This was not carried out in a sterile environment. Contamination was present, but did not seem to hinder vegetative growth of the stems. Taking cuttings, in combination with dividing mature specimens may prove a viable option for the propagation of *S. bigelovii*.

Date	Species	Treatment	Media	Observations
12/13/2020	Selaginella bigelovii	No treatment	8g/L agar	New vegetative growth within 1 week
12/13/2020	Selaginella bigelovii	12% Dip n' Grow rooting hormone @ 1 min	8g/L agar	New vegetative growth within 1 week; rooting structures observed at time of report
12/23/2020	Selaginella bigelovii	12% Dip n' Grow rooting hormone @ 1 min	1:1:1: #20 Fine Sand /Vermiculite/Pumice	New vegetative growth within 1 week
12/23/2020	Selaginella bigelovii	No treatment	1:1:1: #20 Fine Sand /Vermiculite/Pumice	No new vegetative growth

Conclusion

The horticultural trials conducted indicate that our native, local chaparral ferns do present a viable option for low-water-use alternatives to the water-loving ferns that are more commonly used in landscapes today. Additional work on propagation is needed to move these ferns into the nursery trade. While germination for all of the species has been achieved, it continues to be somewhat inconsistent, and growing the ferns from gametophytes to sporophytes has proved challenging. However, this is a multi-year project, and we will continue to work on improving propagation protocols so these plants can be moved into the retail trade.

Works cited

Reid S., and L. Oki. (2008). Field trials identify more native plants suited to urban landscaping. *California Agriculture* 62(3):97-104.https://doi.org/10.3733/ca.v062n03p97.