San Diego Thornmint Seed and Common Garden Study

Final Report

Prepared By:
Laurie Lippitt, Stacy Anderson and Bryan Endress
Applied Plant Ecology Division
Institute for Conservation Research
San Diego Zoo Global

Prepared For:
Center for Natural Lands Management

San Diego Thornmint Seed & Common Garden Study

Introduction

San Diego Thornmint (*Acanthomintha ilicifolia*) is a small herbaceous annual with a very restricted distribution (San Diego Co. and Northwest Baja) with federal Threatened status and state Endangered status in California. Within its restricted range, it is found on isolated patches of clay soils on gentle slopes in open areas surrounded by chaparral vegetation. Some research has been done on the species, but there is no information on the relatedness of the various populations. This would be useful in terms of prioritizing preservation efforts as well as deciding whether populations are distinct and seed from them should be kept separate or whether some populations share enough characteristics that seed could be combined to effectively increase the genetic base. Genetic analysis of plant material can show differences among populations, but not all of the differences might be adaptive. A common garden study will augment the laboratory genetic analyses and may have a reasonable chance of capturing some adaptive differences between populations. In a common garden study, seed from various populations are all grown in one environment, eliminating as much environmental variability as possible so that presumably, the differences that result are more likely to have a genetic basis. Characteristics of the plants, such as flowering date, top biomass, seed production and others, are measured to determine population differences. The common garden trial and supporting seed work is the portion of the project being done by the Institute for Conservation Research's Applied Plant Ecology Division.

Plant material collected from 9 populations, including subpopulations, was provided (Appendix 1). Note that two collections were made from Carlsbad North subpopulation 2, with one being called "Pilot" in case a pilot study was done prior to the main common garden study. Figures 1 and 2 show samples of the material that was provided. Note that some of the material is still green in color and likely somewhat immature.

Figure 1

Figure 2

Samples of San Diego Thornmint material provided for the study.

Processing, X-ray Analysis and Germination Testing

The seed processing protocol was determined and the seedlots processed. The processing protocol was to rub the material on a stack of sieves (U.S. testing sieves with the number referring to the mesh size) with a #20 on top, a #25 in the middle and a #40 on the bottom (Figures 3 & 4). The large debris remaining on top of the #20 was tossed. The material captured on the #25 might contain a seed or two, which were removed by hand, but the remaining material could be tossed. The bulk of the seed was captured on the #40 (Figure 5) and the small debris that passed through the #40 was tossed. The seed from the #25 and #40 were run through a small air separator (Figure 6) at a setting of 16 to remove debris but to retain all seed (Figure 7). The resulting seed was hand cleaned and counted. Table 1 shows the number of seed obtained from the material provided and the approximate number of seed on a per plant basis, given the number of plants they reported collecting from. Seed yield ranged from 170 to 2757 seed with a range of 10 to 183 average seed per plant.

Figure 3

Figure 4

Screens #20, #25 and #40 were used for processing. Material on screen ready to start processing.

Figure 5

Seed and debris captured on screen #40.

Figure 6
Small air separator.

Figure 7
Seed after air separation. Note mix of light and dark seed.

Table 1
Seed Yield Per Collection and Average Per Plant

Population	Seed Yield per Collection	Seeds per Plant Avg
Manchester	170	10.0
La Costa	302	25.2
Carlsbad North: subpopulation #1	685	57.2
Carlsbad North: subpopulation #2	2757	183.0
Carlsbad North “Pilot”: subpopulation #2	1482	82.3
Sycamore Canyon (Poway): subpopulation #1	551	42.4
Sycamore Canyon (Poway): subpopulation #2	975	65.0
Mission Trails Regional Park	588	49.0
Jamul (S.D. National Wildlife Refuge)	1369	105.3
Cleveland National Forest (Alpine)	1705	85.3

The next step was to determine what level of filled seed had been produced, using non-destructive X-ray analysis done by Teri Griffis of CalFire’s L.A.Moran Reforestation Center in Davis, California. The seed was noted to be a combination of dark seed and much lighter seed. For other species, the lighter seed can often be less well-developed, possibly immature and have a lower filled seed percentage. To see if this was the case for San Diego thornmint, the seed was separated into dark and light fractions for each population prior to being sent for X-ray analysis. If there were fewer than 50 seed available following sowing for the common garden study, the seed were not sent for X-ray (indicated by a dash in Table 2). Seed from 8 populations was sent. Following X-ray analysis, the same seed were returned for germination testing. Germination test protocol involves soaking the seed for 24 hours in room temperature de-ionized water, draining the seed, placing it on 0.5% agar in dishes with 25 seed per replicate and putting the dishes into a germinator with alternating photoperiod and temperature regimes (11 hrs light at 25℃ and 13 hours dark at 10℃). Results can be seen in Table 2. Filled seed percentage for dark seed ranged from 88-100% filled while light seed ranged from 63-90% filled, a drop of 9-35% from darker seed of the same population. Germination rates for dark seed ranged from 84.8-100% while germination of light seed was 45-83.7%, making germination% for light seed 14.3-48% lower than for dark seed of the same population.

Table 2
Filled Seed Percent From X-ray and Germination Test Results

Population	Dark Seed Filled Seed%	Dark Seed Germination%	Light Seed Filled Seed%	Light Seed Germination%
Manchester	100	100	90	74.4
La Costa	98	98	-	-
Carlsbad North #1	88	84.8	-	-
Carlsbad North #2 "Pilot"	100	97	88	81
Mission Trails	-	-	-	-
Sycamore Canyon #1	98	98	63	70
Sycamore Canyon #2	98	98	89	83.7
Jamul (S.D. National Wildlife Refuge	97	93	80	45
Cleveland National Forest	88	85.9	-	-

Common Garden Study

Of the 9 populations provided, there was insufficient seed for three of them for the common garden study, so 6 populations were included in the trial. Table 3 shows the included populations and their relative location in terms of coastal versus inland and north versus south as well as elevation. The included populations were: Carlsbad North: subpopulation #1, Carlsbad North: subpopulation #2, Sycamore Canyon (Poway): subpopulation #2, Mission Trails Regional Park, Jamul (San Diego National Wildlife Refuge) and Cleveland National Forest (Alpine). This provided a suitable range of variability with three populations from low elevation, one from mid elevation and two from somewhat higher elevation as well as two populations from more northerly and coastal locations, one intermediate population and three populations from further south as well as further inland. Seed from two subpopulations of the Carlsbad population were included to provide some information on within population variation. In addition, since the literature noted that plant form was noticeably influenced by precipitation (plants taller, more branched and more seed in wetter conditions), both a lower water and higher water regime were applied to each population.

Table 3
S.D. Thornmint Populations Included in the Common Garden Study
Relative Location and Elevation

Population & Subpopulation	Coast/Inland	North/South	Elevation
Carlsbad North : Subpopulation #1	Coast	North	Low - 250'
Carlsbad North : Subpopulation #2	Coast	North	Low - 159'
Sycamore Canyon (Poway) : Subpopulation #2	Intermediate	Intermediate	Mid - 1120'
Mission Trails Regional Park	Inland	South	Low - 459'
Jamul (S.D. National Wildlife Refuge)	Inland	South	High - 2150'
Cleveland National Forest (Alpine)	Inland	South	High - 2310'

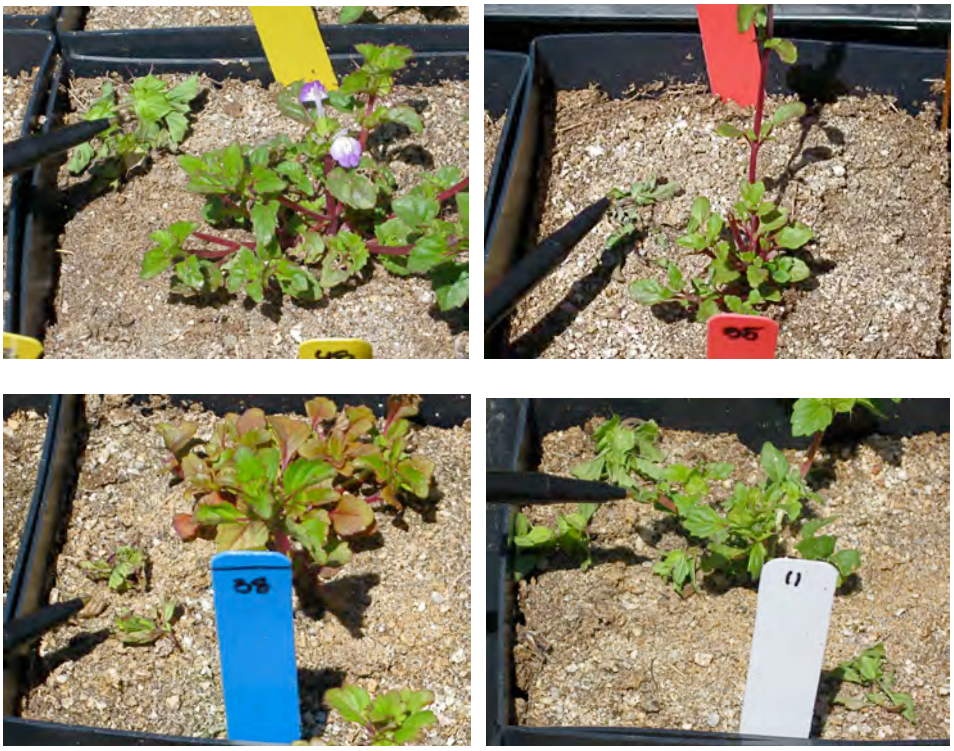
On February 6, fifty-five pots with 5 seed per pot were sown for each population for each watering treatment. Anderson Plant Bands AB58 (5” width by 8” depth and nine per tray) were used with a substrate of potting mix and washed sand (3:1) that had been previously used in the literature. To achieve as much uniformity as possible, the two individuals did all the potting mix preparation, each doing the same steps and a single individual filled all the containers. All pots were watered in with 500ml of water and all plants were misted twice a week during the germination phase. Subsequent irrigations were 1000ml per pot. The San Diego Zoo Safari Park Horticulture Department graciously provided bench space for the trial in an area where all pots would be on raised benches and get full sun. Plants were randomized within a tray and tray locations were randomized. Plants in the high water treatment received water every 2 weeks while the lower water treatment was irrigated every 4 weeks. When the plants started to show signs of water stress, the frequency was adjusted to once a week for the higher water treatment and every 2 weeks for the lower water regime. Irrigation continued until all plants senesced and were harvested. Figures 8- 17 (Appendix 2) show potting mix preparation, filling of containers, flagging trays to mark watering regime, starting to randomize trays and watering.

Germination was noted in early March and number of seedlings per pot was monitored once a week from March 12 through March 26 (Figures18 & 19). The maximum number of germinants per pot was recorded. Following completion of germination, seedlings were thinned to one per pot. Though specific data wasn’t taken on these features in the early growth phase, plants started to show population differences in growth form fairly early on with some more upright and others more low and spreading. There were differences in foliage color as well. Appendix 3 has a series of photos from April 9 that show some plants from each population. Plants were observed for date of first flowering (Figures 20 - 23). Data was taken 5 days per week starting April 16 and continued until June 7 when all but 1 plant had flowered.

Figures 18 & 19
Early germinants and young seedlings prior to thinning.

Figures 20-23
Observations were made to determine date of first flowering.

Note that from germination onward, there was some herbivory (figures 24-27) by snails and insects that affected both the number of surviving seedlings and the extent of foliage on some seedlings, until a treatment was performed around and under the bench area. Extent of herbivory appeared to be similar regardless of population.



Figures 24-27
The pen points to material removed by herbivory in each case.

Plants were checked for senescence 5 days per week starting in late April. The first plants senesced on May 2 and continued through July. Figures 28-31 show aspects of this phase of the project. When plants completed their life cycle, data was taken on plant height, plant width and number of inflorescence whorls. The top of the plant was harvested and dried to obtain dry biomass data. The roots were so fine that it proved impossible to separate them from the potting mix, so it was not possible to collect data on root biomass. Seed production data was obtained by processing twenty-five dried tops per population per watering treatment (for a total of 300 samples) to extract the seed. Seed was separated into dark, light and damaged fractions and the number of each was recorded. Total number of seed produced and total weight of seed produced per plant were also recorded.

Figure 28
Plants senesced at different rates.

Figure 29
Almost ready-still a few green leaves at the base.

Figure 30
Four plants in this tray ready for data & harvest.

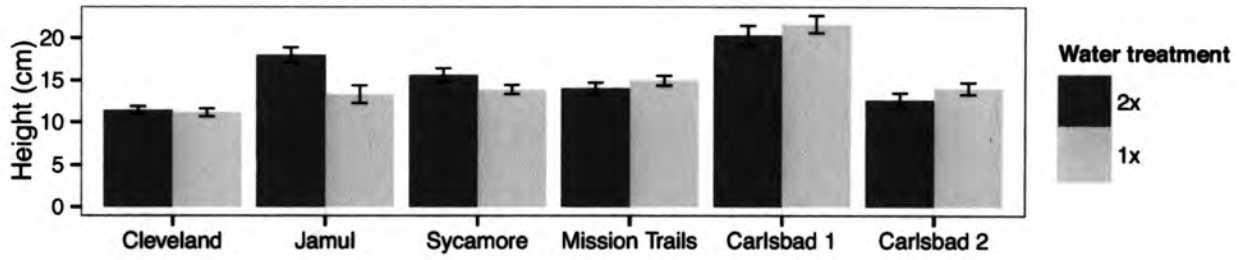
Figure 31
Data taken and tops harvested.

Common Garden Results

Results for the morphological traits of plant height, width, number of inflorescence whorls and biomass can be seen in Figures 32 through 35. Plant height ranged from an average of 11-20 cm

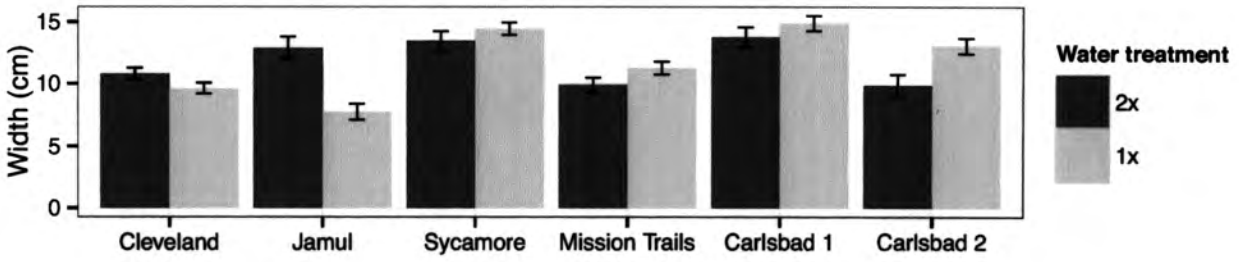
for the higher water treatment and from 11-21 cm for the lower water treatment. Plant height did not vary much by population with the exception of taller plants for the Jamul and Carlsbad 1 populations for the higher water treatment and the Carlsbad 1 1ower water treatment. Plant height was not even routinely higher for the higher water treatment as might have been expected from the literature where taller plants resulted in higher rainfall years. The Jamul population had the greatest difference (approximately 4 cm) between the higher and lower water treatments. It was interesting to note that there was as much variability between the two closely situated Carlsbad subpopulations (approximately 8cm difference between a given water treatment) as there was between populations at much greater distance and elevation difference.

Figure 32
Plant Height by Population and Water Treatment



Average plant width ranged from 10-13 cm for the higher water treatment to a range of 7-15 cm for the lower water level. Plant width varied by population within a given water regime. The Jamul, Sycamore and Carlsbad1 populations had the greatest plant width with the higher water regime while Sycamore, Carlsbad 1 and Carlsbad 2 had the widest plants under the lower water regime. Plant width did not vary much between water treatments with the exception, again, of Jamul which had the greatest difference between the two water treatments (approximately 5 cm). Again there was a fair amount of difference (approximately 3 cm difference between a given water treatment) between the two Carlsbad subpopulations.

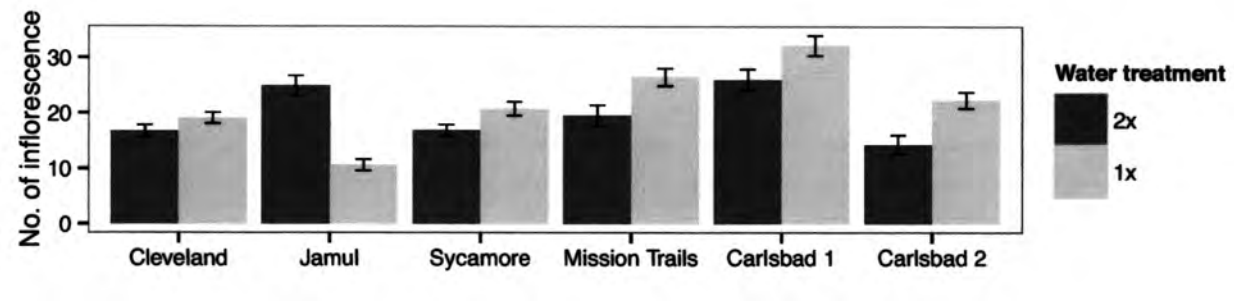
Figure 33
Plant Width by Population and Water Treatment



Average number of inflorescence whorls per plant ranged from 14-24 for the higher water regime and 10-31 for the lower water regime. There were fewer whorls with the higher water treatment for all populations except Jamul, where the pattern was reversed. Jamul again had the biggest difference (approximately 14 whorls) of any population between the value for the lower

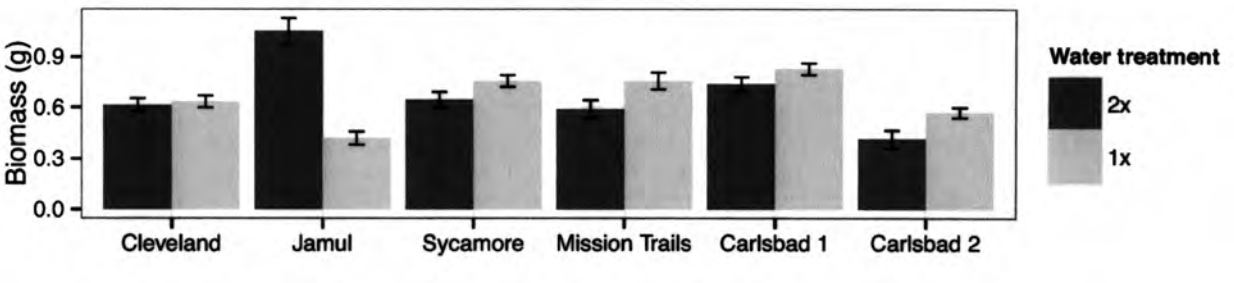
and higher water treatment with Carlsbad 2 also having a large difference. Values for Carlsbad 1 and Carlsbad 2 subpopulations were very different for both watering levels.

Figure 34
Number of Inflorescence Whorls by Population and Water Treatment



Average biomass ranged from 0.4-1.0 g for the higher water level and 0.4-0.8 g for the lower water level. Most populations had biomass that was similar between water treatments for a given population as well as similar between populations. Again, Jamul was an exception with the greatest difference between water treatments (0.6 g) with the highest biomass of any population with more water and the lowest biomass with less water. There were again marked differences between the two Carlsbad subpopulations (approximately 0.3 g difference between a given water treatment).

Figure 35
Plant Biomass by Population and Water Treatment

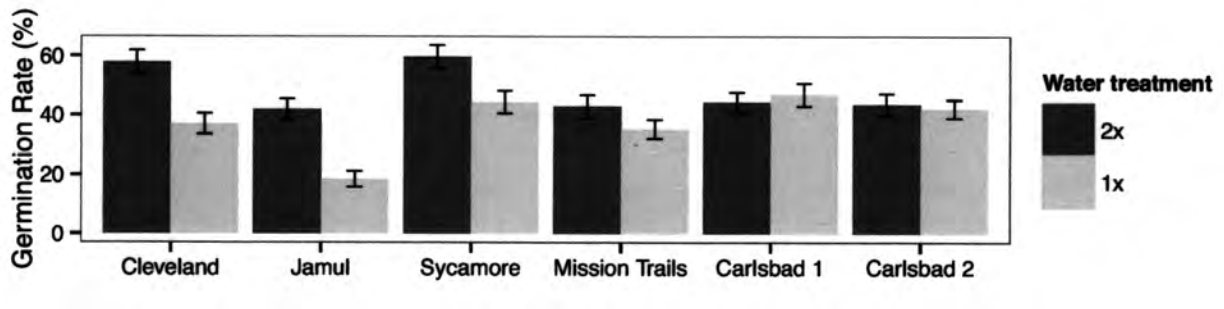


For the four related plant growth characteristics of plant height, width, number of inflorescence whorls and biomass, there was no consistent difference between inland populations and coastal ones, between higher versus lower elevation or between populations from the southern part of the county versus the north.

Results for the suite of characteristics related to reproduction can be seen in Figures 36 through 39 for germination rate of seed sown in the pots, days to first flowering, number of inflorescence whorls, number of seed produced and weight of seed produced.

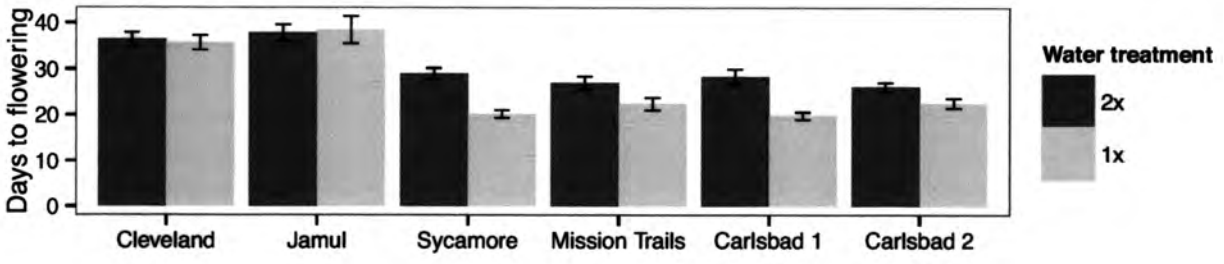
Average germination rates ranged from 40-60% with the higher water treatment to 17-45% for the lower water treatment. The Cleveland and Sycamore populations had the highest germination with more water, while the other populations had very similar but lower rates. With lower water, most populations had similar germination rates with the exception of Jamul's much lower rate. The two Carlsbad subpopulations had very similar results for this characteristic.

Figure 36
Seed Germination Rate by Population and Water Treatment



As might be expected, the two high elevation populations, Cleveland and Jamul, had the longest number of days to flowering for both the high and low water treatment. The other populations had very similar but fewer days to flowering, with a longer time to flowering for the higher water treatment and a shorter time to flowering with the lower water treatment. Again, the two Carlsbad subpopulations had very similar results for this characteristic.

Figure 37
Days to Flowering by Population and Water Treatment



Seed production and seed weight results were quite similar and will be discussed together. In general the number and weight of seed produced was greater for the lower water treatment across all populations. The Cleveland population produced significantly more seed and seed weight under both water regimes than any other population. There was generally more seed and seed weight produced for the higher elevation and more inland populations versus the coastal, low elevation Carlsbad populations. The two Carlsbad subpopulations both produced almost no seed under the higher water regime, though Carlsbad 1 produced somewhat more seed under the lower water regime than Carlsbad 2.

Figure 38
Number of Seed Produced by Population and Water Treatment

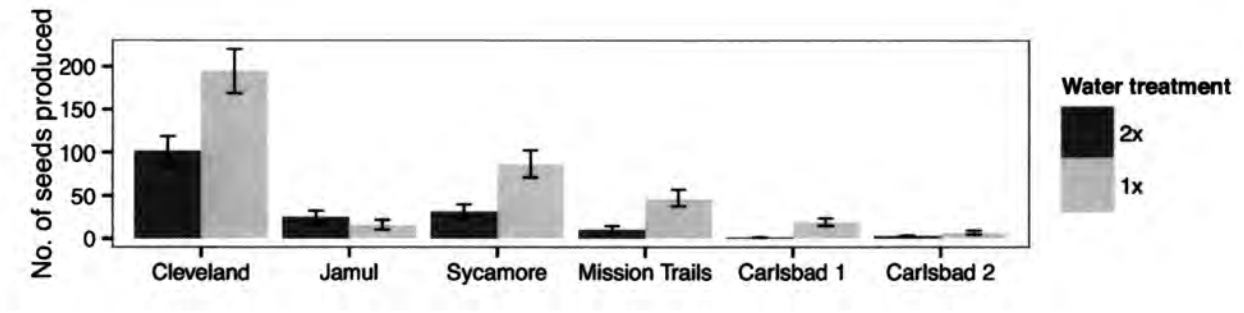
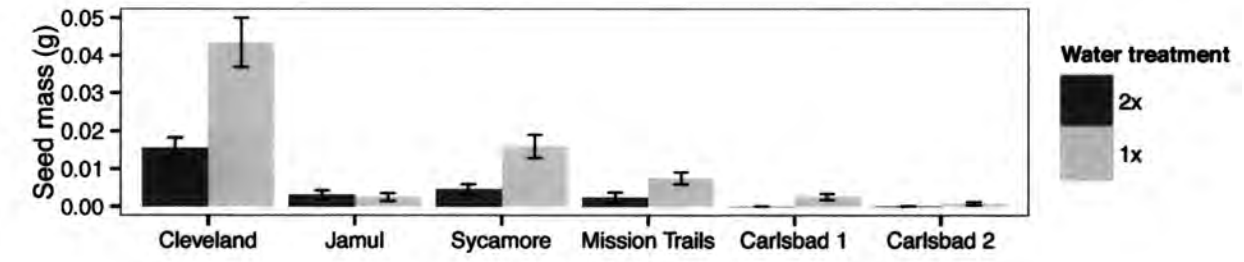


Figure 39
Weight of Seed Produced by Population and Water Treatment



For the four characteristics related to reproduction, there was a general tendency for the inland and higher elevation populations to have a higher germination percent, take longer to flower and produce a greater number and weight of seed as compared with the coastal populations. The Jamul population was not as distinct with regard to these traits as it had been for the morphological characteristics. The two Carlsbad subpopulations were similar with respect to these characteristics rather than differing as they did for the morphological traits.

Summary

The seed processing protocol was determined for San Diego thornmint and the provided samples were processed. Cleaned seed was separated into dark and light fractions to determine if there was any difference. If there was sufficient seed, the resulting seed received X-ray analysis to determine filled seed percent. The x-rayed seed was tested for germination. Dark seed had both a higher filled seed percent and a higher germination percent across all populations. The lighter colored seed may not have been fully mature.

The common garden study used seed from 6 of the populations provided. These covered a range of coastal versus inland, low versus higher elevation, northern versus southern populations and seed from two subpopulations of the same location. For the four related plant growth characteristics of plant height, width, number of inflorescence whorls and biomass, there was no consistent difference between inland populations and coastal ones, between higher versus lower elevation or between populations from the southern part of the county versus the north. Plant

heights and biomass were similar both between populations and within water treatments for a given population. Plant heights were not routinely greater with more water, though there were fewer inflorescence whorls with the higher water treatment. Plant widths were somewhat more varied than heights. For all four traits, there was considerable within subpopulation variability between values for the two closely situated Carlsbad subpopulations; often more than between populations located at considerable distance from each other. For each of the four traits, the Jamul population was unusual in having the greatest difference between the two water treatment levels.

For the four characteristics related to reproduction, the inland and higher elevation populations tended to have a higher germination percent, take longer to flower and produce a greater number and weight of seed as compared with the coastal populations. The Jamul population was not as distinct with regard to these traits as it had been for the morphological characteristics. The two Carlsbad subpopulations were similar with respect to these characteristics rather than differing as they did for the morphological traits.

Findings from the genetic analysis component of the current project will contribute to the understanding of population differences and similarities. However, it would be good to do additional common garden work on more populations across the range of coastal/inland, north/south, low/high elevation as well as another set of subpopulations to see if any of the trends and differences found in this study become clearer. Ellen Bauder and Paul Kemp's 1994 report "Surveys and Assessment of known *Acanthomintha ilicifolia* Populations" lists additional thornmint populations that could augment the number of populations in each category. For example, the relatively large and stable Viejas Mountain and McGinty Mountain populations would be excellent for bolstering data for inland, higher elevation populations, while testing several of the Viejas Mountain and Sycamore Canyon subpopulations would increase data on the extent of variability between subpopulations. With sufficient seed, the Manchester and La Costa populations would augment the data for coastal populations, given that they were included in the genetic analysis in this first round. The Poway/Sabre Springs population would add an additional intermediate population in terms of distance inland and elevation.

Without strong data, it is usually best to consider each population distinct and not transfer seed between them. However, if management decisions on seed transfer guidelines for transplant operations were to be made solely on the basis of the current common garden study, it would be prudent to consider the higher elevation/further inland populations separate from the coastal populations and not transfer seed between them. The populations intermediate in terms of distance inland and elevation seem intermediate in terms of their characteristics and seed likely could be moved in either direction. Conservatively it would also be wise to consider the Jamul population as distinct from the others.

Appendix 1
San Diego Thornmint Material Provided for Study

Population	Collection Date	Coast/Inland	North/South	Elevation	# Plants Collected	Lat/Long UTM
Cleveland National Forest (Alpine)	7/26/12	Inland	South	2526’	20	325123.23N 1164437.56W
Jamul (S.D. National Wildlife Refuge)	6/13/12	Inland	South	2150’	13	512024E 3623595N
Mission Trails Regional Park	6/25/12	Inland	South	502’	12	493134E 3631926.7N
Sycamore Canyon (Poway) – subpopulation #1	6/13/12	Intermediate	Intermediate	1120’	13	502042E 3643518N
Sycamore Canyon (Poway)- Subpopulation #2	6/13/12	Intermediate	Intermediate	1120	15	501618E 3644307N
Manchester (Encinitas)	6/14/12	Coast	North	161’	17	476687E 364732?N
Rancho La Costa	6/14/12	Coast	North	102’	12	475247E 3664418N
Carlsbad Oaks North – Subpopulation #1	6/14/12	Coast	North	250’	12	475447E 3666487N
Carlsbad oaks North – Subpopulation #2	6/14/12	Coast	North	174’	15	475231E 3665873N
Carlsbad Oaks North “pilot” – Subpopulation #2	6/14/12	Coast	North	174’	18	475231E 3665873N

Appendix 2

Figures 8 & 9
Commercial potting mix and washed sand were used in a 3:1 proportion.

Figures 10 & 11
Mixing was done in a cement mixer in small batches. The same two individuals performe
same steps in the mix preparation, in order to achieve as much uniformity as possible

Figures 12 & 13
The Anderson Plant Bands AB58 were all filled by the same individual, for uniformity

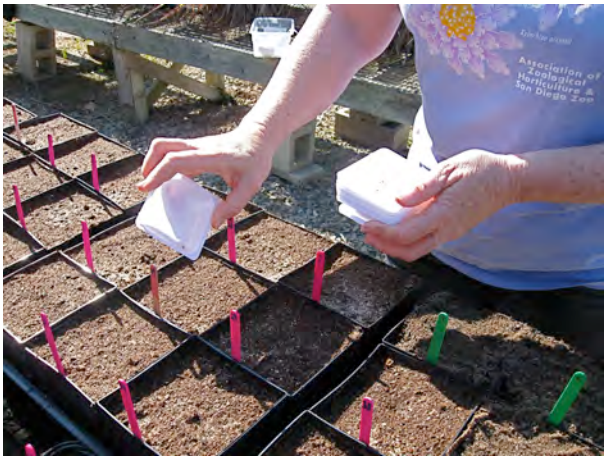


Figure 14
Sowing 5 seed per pot.

Figures 15 & 16
Seeds sown and trays being flagged for higher or lower watering regime.

Figure 17
Starting to randomize the trays.

Appendix 3
Photos of Early Growth - April 9

Cleveland National Forest (Alpine) – Green Tags

Jamul (S.D. National Wildlife Refuge) – Blue Tags

Mission Trails Regional Park – White Tags

Sycamore Canyon (Poway) Subpopulation #2 – Yellow Tags

Carlsbad North Subpopulation #1 – Red Tags

Carlsbad North Subpopulation #2 – Orange Tags