Arana Gulch Habitat Management Plan
City of Santa Cruz

Year 9 (2022) Annual Report

Appendices

CDFW Permit No. 2081 (a)-13-013-RP
CDFW Permit No. 2081 (a)-18-016-RP
Coastal Development Permit No. 3-11-074 (Arana Gulch)

May 9, 2023

Appendix A AMWG Meeting Minutes, 2022

A-1: AMWG Meeting Minutes for:

January 19, 2022
March 14, 2022
November 7, 2022

Minutes
Arana Gulch Adaptive Management Working Group Meeting
Zoom Meeting
9:00 a.m. – 11:00 a.m. on Wednesday, January 19, 2022

VIRTUAL MEETING

Working Group Members present:
Travis Beck, City of SC Dept. of Parks and Recreation
Blake Woessner, City of SC Dept. of Parks and Recreation
Kathy Lyons, Biotic Resources Group
Alison Stanton, Botanist
Bill Davilla, EcoSystems West
Sylvie Childress, UCSC Greenhouses
Grey Hayes, CA Native Plant Society
Serena Stumpf, CA Department of Fish and Wildlife
Mark Ogonowski, US Fish and Wildlife Service
Todd Lemein, US Fish and Wildlife Service
Devii Rao, USDA
Lauren Garske-Garcia, CA Coastal Commission

Additional Attendees: Frank and Teresa Locatelli, Jean Brocklebank, Michael Lewis

AMWG Members Absent: None

Virtual meeting was held via a Zoom video call. Travis Beck facilitated the meeting, representing the City of Santa Cruz Department of Parks and Recreation.

Welcome and Meeting Objectives. Travis opened the meeting.

The minutes from the November 4, 2021 AMWG meeting were reviewed, with revisions. The revised minutes will be recirculated for AMWG review and approval

Meeting Agenda

1. Grey requested a discussion on the budget. Travis added this to The Wrap Up and Next Steps section of the agenda.
2. Mark requested a discussion on possible USFWS funding. Travis added this topic to the end of the agenda.

Public Comments

1. Jean indicated that she and the AMWG members were not able to see the public participants due to the Zoom settings. She requested that setting be adjusted to see all participants. No other public comments.

Research Updates and Discussion

1. Greenhouse Experiment. Sylvie reported on the UCSC Greenhouse plants where there are over 1,000 SCT under cultivation. The plants will be ready for outplanting in two batches – January and February. The first batch is hardening outside now. She reported that the SCT ray achene germination experiments will begin next week. All outplanting plants to date have been propagated from the disk achenes; however, on another project she had successful germination of ray achenes using gibberellic acid. The growing medium for the plants is standard potting mix.
2. SCT Outplanting Experiment. Alison gave a summary of the results from the SCT outplantings, to supplement information presented at the November 4, 2021 AMWG meeting. The goal of the outplantings was to facilitate the survival and reproduction of container grown SCT plants to introduce seed into the site. She provided a handout with the treatments conducted: control (no treatment, grazing release), mowing (Toro deck mower at 10" height), sheet mulch (B-flute cardboard with 3" depth bark mulch), demo (sheet mulch plots with mowing), and Juncus (plot with native Juncus in plot). Implementation was implemented by volunteers from UCSC, which Sylvie helped to organize. Plant survival was 75% in control plots and 78% in control plots that were mowed once. Average height in control plots was 7-9"; number of flowers per plant averaged 4 to 8. In the sheet mulch plots, survival ranged from 58 to 63% and was similar in weeded and un-weeded plots but lower survival was noted in the woodchip only (no cardboard) plot, likely due to gopher activity. Average plant height in sheet mulch plots was 14-18", with multiple stems. Flowerhead production ranged from a low of 52 in an unweeded subplot to a high of 582 in a weeded subplot. The City received approval for use of Sluggo (exemption from City IPM) and Sluggo was applied to the plantings in February. The sheet mulched plots provided the most success in introducing seed onto the site, as it was estimated that the sheet mulch plots produced 50,000 seeds, compared to 2,000 seeds in the control plots. In October and November 2021, wood chips in several of the sheet mulched plots was raked away to allow for SCT germination in native soil. SCT germination was observed in the sheet mulched plots at that time. In December, SCT seedling cover data was gathered from five subplots. Kathy presented her data, showing highest cover in mulched, then raked, plots, with cover by SCT ranging from 15% to 80% cover. In unraked sheet mulch plots, SCT cover was lower, from 0% to 1%. Bill asked if non sheet mulch plots were raked to remove thatch. Kathy

indicated no raking of thatch was done, but could be considered for 2022 plots. Alison reported that weeding the sheet mulch plots (with string trimmer and/or hand-pulling) is important, based on UCSC studies. Alison reported that she would like to do a simpler outplanting plan for 2022. Bill asked about Sluggo application and whether to apply to just a portion of the plots. Grey indicated that snail and slug herbivory is a concern and is in favor of using Sluggo. Grey also indicated that maintaining a low canopy cover will reduce slug and snail populations due to predation. Sylvie commented that Sluggo, not Sluggo+, should be used to avoid any potential impact to earthworms. Alison discussed other recommendations for the 2022 outplantings: use of browse cages not feasible due to cost and labor to install; mowing with a flail mower earlier in the season; consider a plot with cardboard only (held down with staples); sheet mulch with wood chip (vs. bark), with chips raked away when plants in full flower, and sheet mulch with native grass plantings. Sylvie indicated that only *Elymus glaucus* plants are available right now and the group agreed those would not be suitable. Group discussed keeping cattle grazing out of Area A again for 2022 unless a cross fence could be installed to allow grazing in the northern part of Area A. Grey suggested a consideration of using funds from the budget for grazing exclosure fencing in Area A, indicating no grazing in Area A will degrade the northern portion of Area A. Blake indicated he could use a flail mower in the northern portion of Area A. Mark indicated a focus on simple actions and to monitor 2021 macroplots for recruitment and progress through 2022. Jean would like to see Area A outplantings continue with no grazing for 1-2 more years to see a longer trend. Bill indicated that grazing should be considered as a maintenance regime for SCT and thought it was suitable when there is a viable seedbank; however, site is now in recovery mode for seedbank development. Yet grazing in the north part of Area A could prepare the area for SCT expansion, and suggested a sub meeting for grazing in the northern part of Area A.

3. USFWS SCT Study. Todd presented anecdotal input from the study which is funded by USFWS. The largest SCT colonies have a native prairie component that are suitable for passive management (mowing/grazing). The sites with less SCT density occur in areas with more non-native grasses and forbs. Seasonally-saturated soils are important. The USFWS study report is expected in late spring 2022.

Management Updates and Discussion

1. Grazing. Teresa reported that there are 5 animals in Areas C and D. They are hoping for more rain for good grass cover. Grey indicated the need for more bare ground and more cows may be needed. Artificial feeding could also be considered. Kathy reported that the canopy height measurements in Areas C and D from December 2021 are 2.4” and 2.6”, respectively, and are within the target range from the HMP. Alison showed the

table of bare ground from the spring vegetation assessment, indicated the current grazing pressure is not high enough to create enough bare ground. Teresa and Frank indicated they can put more cattle on site, in areas c and D, as well as Area A. The group discussed the goal is to control canopy height (2-3 inches), increase bare ground, and get SCT into the system.

2. Monitoring for 2022. Alison presented data from the spring 2021 monitoring. There has been an increase in the cover of non-native forbs and no increase in native species from the baseline. The composition of grasses has changed, from *Avena* to lower stature *Festuca myuros.* Prominent non-native forb is *Erodium*. She suggests that there be an increase in sampling areas mapped as coastal prairie in Area A to better document vegetation composition compared to non-coastal prairie areas. She will conduct a power analysis to determine how many more transects are needed to more adequately document the coastal prairie. Kathy reported that RDM measurements are taken each fall and canopy heights are recorded in February and December. The coastal prairie maps were reviewed. Bill and Kathy indicated there has been no substantial change in the extent of coastal prairie since the onset of grazing, based on field observations. Lauren asked why there are monitoring transects in areas that are not coastal prairie. Alison indicated there could be a decrease in frequency of monitoring in non-coastal prairie areas, but would not like to abandon them. Consensus was to continue monitoring and Allison will update the Area A coastal prairie transects.

Wrap Up and Next Steps

1. Budget and Funding. Travis reported that funding is received from the City's annual operating budget, the Arana Gulch Trust Fund, and a grant from USFWS. Mark indicated that USFWS recovery funds are covering the UCSC greenhouse studies. Proposals for 2022/23 are due March 1, 2022. Proposal costs can range from $5,000 to $25-30,000.
2. Other. Jean asked about other resources, such as woodrats in Hagemann Gulch and implementing other habitat activities vs. all the coastal prairie work.
3. Zoom Chat. In the Zoom chat, Grey indicated at Arana and elsewhere, the sign that there is enough bare ground is a flush of *Juncus bufonius* (toad rush) which has a huge, long-term seedbank and co-occurs with SCT. Grey also expressed concern that any wood chip/bark is a long-term concern for SCT, suggest not using it at all or at least removing it before tarplant goes to seed. Grey indicated that the transects for native species cover and richness were supposed to be for the entire grassland, not just the coastal prairie. He suggested Alison discuss sampling with Coastal Commission as to original intent of monitoring.

Next Meeting: Not determined, but Travis indicated it may be soon to discuss funding and grazing in Area A.

Minutes

Arana Gulch Adaptive Management Working Group Meeting

Zoom Meeting

10:30 a.m. – 12:00 a.m. on Monday, March 14, 2022

VIRTUAL MEETING

Working Group Members present:
Travis Beck, City of SC Dept. of Parks and Recreation
Blake Woessner, City of SC Dept. of Parks and Recreation
Kathy Lyons, Biotic Resources Group
Alison Stanton, Botanist
Bill Davilla, EcoSystems West
Sylvie Childress, UCSC Greenhouses
Grey Hayes, CA Native Plant Society
Mark Ogonowski, US Fish and Wildlife Service
Todd Lemein, US Fish and Wildlife Service
Devii Rao, USDA
Lauren Garske-Garcia, CA Coastal Commission
AMWG Members Absent:
Serena Stumpf, CA Department of Fish and Wildlife

Virtual meeting was held via a Zoom video call. Travis Beck facilitated the meeting, representing the City of Santa Cruz Department of Parks and Recreation.

Welcome and Meeting Objectives. Travis opened the meeting.
The revised minutes from the November 4, 2021 AMWG meeting were reviewed, and approved, with correction of one typo on page 4, item 3. Word change from year to years.

The minutes from the January 19, 2022 AMWG meeting were reviewed and approved.

Public Comments

None.

Management Updates and Discussion

1. Coastal Prairie Restoration. Travis and Alison presented a review of the HMP objective #A-3E relating to coastal prairie. Alison's data indicates less than 10% native grass cover in all years. A map of the extant coastal prairie (Figure 7 from annual report) shows location of areas with native grass stands, yet the HMP goals apply to all mapped

grassland. There was discussion on the ability to enhance the extant prairie and how to manage and expand coastal prairie features to adjacent areas. Gray discussed findings from a study by Justin DeLoeng, a UCSC student, that indicates that coastal prairie restoration is feasible, using a simplistic approach that focuses on large weedy areas as a way to enhance coastal prairie and installation of native species (plugs of 6 coastal prairie species). Alison shows a graphic of the transect locations in Area A (Figure 27 in annual report). Data from Table 9 in annual report shows little cover by native species outside of areas mapped as coastal prairie. There was group discussion on compatibility of coastal prairie and Santa Cruz tarplant (SCT). There are opportunities for coastal prairie enhancement, yet limiting factor for SCT is depleted seedbank. There was discussion on identifying areas outside of historical SCT areas for creation of coastal prairie/native grassland, using grasses, such as *Elymus glaucus, Hordeum brachyantherum, and Bromus carinatus* outside of SCT areas. There were suggestions to install native grasses into the SCT planted areas to test microsite compatibility of native grasses and SCT. There was interest in investigating propagation of native grass plugs from UCSC. Sylvie indicated that may be feasible in coordination with Younger Lagoon growing system and possibly using their locally-collected seed, or the City could obtain a quote from Central Coast Wilds to harvest native seed from Arana and grow out plugs. Planting could occur in November/December 2022. Another option to consider is dividing existing on-site perennial grasses and outplant the divisions. The City indicated they will work on a grass planting program.

2. Area A Mowing. The existing mowing regime in Area was discussed. A new mower attachment can mow to 6-inch height. Its s expected that mowing will occur at 3-week intervals and will include all of Area A, including the 2021 SCT plots. A portion of the 2022 SCT plots will also be mowed. There was discussion to mow prior to flower heads on non-native grasses and to not mow when native species are in flower/ seed set. Travis requested field training for City staff on phenology to decide when to mow.
3. Fertilizer Experiment. The City reported that they received an offer from Craig Dremmen to conduct a fertilizer program in Area B, yet the City has yet to receive a quote for these services. There was discussion on the suitability of such as study, with consensus that existing soil nutrients are naturally low and a concern that fertilizer would encourage growth of non-native/weeds and, as such, there was not agreement on pursuing this study. There was discussion on mowing and selective weeding in Area B and possible installation of grass plugs. There is likely no viable SCT seed in Area B.
4. USFWS SCT Study. USFWS reported that their draft report is due soon. The report will have a characterization of SCT sites, including species composition, historical land uses, current management actions and the information will be used in developing a 5-year species review that is due in May 2022. There was discussion on sampling on first

coastal terraces, sharing information, and compare data on each site, such as in a workshop forum. A species status assessment will be done in 2023 to develop a species recovery plan in 2024. There is expected to be a recovery meeting after the assessment study is done in 2023/24.

5. Coastal Prairie Reference Data. There was discussion on collecting data from others to get reference community information. Some information may be contained in Justin DeLoeng report. Work by Mark Stromberg suggested three types of coastal prairie: flat, sloping hillsides, and grassy balds.
6. Cattle Stocking. Cattle were added to the site after the January AMWG meeting. The City indicated that there was flexibility in moving animals on and off site and between pastures and could be moved based on SCT phenology.
7. 2021 SCT Outplantings and Mowing. Most weeds in 2021 plots is cats' ear (mulched plots; grasses and other forbs are present in the unmulched plots. Rattail fescue is weedy species of concern. There was consensus that it is okay to mow SCT plants once. The USFWS study indicates at Watsonville Airport they mow at 8-10" in spring and do a 2nd mow at 4" before Memorial Day. They also mow again in September/October. They mow very slowly.
8. 2022 SCT Outplantings. 1,145 SCT plants were installed on site; 600 on February 3 (Area A), 400 on February 24 (Area A), and 145 on March 7 (Area C). The goal of planting 1,000 plants was achieved. Drought conditions found hard soil conditions. Approximately 150 SCT plants remain at UCSC Greenhouses. They propose to allow student interns to do experiments, such as plant growth, seed dispersal, affects of clipping, etc., on these plants. There was consensus that this would be a good use for these plants.
9. Greenhouse Experiment. UCSC Greenhouse reported that the SCT germination study is in progress and they are getting good results on germinating the ray achenes.
10. USFWS Grant Funding Cycle: The City has submitted a funding request to USFWS for future SCT experiments for fall 2022/23.

Wrap Up and Next Steps

1. There is interest in some level of coastal prairie restoration/enhancement
2. The areas need to continue to have weed management, paying attention to the timing of mowing.
3. A more precise grazing plan should be developed for fall 2022.
4. SCT outplantings in fall/winter 2022/23 is recommended
5. There is no interest in the Area B fertilizer plan
6. Data on coastal prairie reference is still needed

Next Meeting: Not determined, but City indicated it will be in fall 2022.

Minutes

Arana Gulch Adaptive Management Working Group Meeting

Zoom Meeting

1:00 p.m. – 3:00 p.m. on Monday, November 7, 2022

VIRTUAL MEETING

Working Group Members present:
Travis Beck, City of SC Dept. of Parks and Recreation
Blake Woessner, City of SC Dept. of Parks and Recreation
Kathy Lyons, Biotic Resources Group
Alison Stanton, Botanist
Bill Davilla, EcoSystems West
Sylvie Childress, UCSC Greenhouses
Grey Hayes, CA Native Plant Society
Suzanne Schettler, CA Native Plant Society
Devii Rao, USDA
Serena Stumpf, CA Department of Fish and Wildlife
AMWG Members Absent:
Mark Ogonowski, US Fish and Wildlife Service
Todd Lemein, US Fish and Wildlife Service
Lauren Garske-Garcia, CA Coastal Commission

Virtual meeting was held via a Zoom video call. Travis Beck facilitated the meeting, representing the City of Santa Cruz Department of Parks and Recreation.

Welcome and Meeting Objectives. Travis opened the meeting.
The minutes from the March 14, 2022 AMWG meeting were reviewed and approved. Meeting objectives are to review and discuss monitoring and research, receive management updates, review grazing plan, and receive input on funding for next fiscal year.

Monitoring and Research Updates and Discussion

1. Santa Cruz tarplant Census, 2022. Kathy reported that there were no SCT plants within the historic SCT colony areas in 2022. There were 21 SCT plants in Area A in 2021.
2. Grazing Update. Blake reported that all cows were moved into the northern portion of Area A on April 7. This occurred after the Locatellis completed the cross-fence in Area A. There was no mowing within the northern portion of Area A prior to the cows being brought into the area. Dense stands of radish were observed in this area.

3. Transect Monitoring. Alison discussed the transect monitoring; no transect data was collected in spring 2022 due to the very dry site conditions. Instead she mapped the extent of native grassland and weed occurrences in the grassland/prairie. Blake reported that the southern portion of Area A was mowed four times in 2022 (February to May); the needlegrass patches were not mowed; however, the CA oatgrass areas were mowed. There was no change to management in Areas C and D in 2022, compared to 2021.
4. CNPS Conference. Alison presented information on the 2022 SCT outplanting program and summarized her presentation at the CNPS conference, which included a comparison of RDM maps from 2020 to 2021, change in species composition with grazing (increase in forbs), SCT seedbank viability, and 2021/22 outplantings.
5. SCT Outplanting Program, 2022. Alison reported that 600 SCT were outplanted into Area A on February 3 and 400 SCT were outplanted in Areas A and C on February 24. Macroplots included control, mowing, and sheetmulch. Sheetmulch included cardboard only, cardboard with wood chips, and cardboard with straw. 100 SCT were installed in clusters of 10 near macroplot 222; these plants were mowed on March 1. Plant survival was 20%. Gopher damage was prevalent throughout the area and SCT plants were smaller, likely due to drought conditions. AMWG members expressed support for raking mulch away from the surviving 2023 plantings prior to seed drop.
6. SCT Recruitment in 2021 Plots. An estimated 1,535 SCT naturally established in the 2021 plots, most occurred in the sheetmulch plots; only 6-7 SCT were observed in control plots. Sheetmulched plots that had mulch hand-raked away had higher recruitment of plants; there were 215 SCT plants in unraked plots and 1,320 SCT plants in raked plots. The 2021 plots were mowed 4 times and some SCT plants showed evidence of branching at 6”+; short SCT plants (below the mower height) were not affected by the mowing and these plants had less branching. Bill asked whether mowing would spread seed rain outside the 2021 plots.
7. SCT Germination Study. Sylvie reported on the SCT germination study she is doing at UCSC Greenhouses, under a USFWS grant. The study was to determine dormancy-breaking mechanisms for the ray seed. The tests included a control gibberellic acid, scarification, sulfuric acid, burned grass, burned charate (coyote brush), liquid smoke soak, heat, field soil, grass thatch, and cowpies. Treatments were in soaking solution and/or in petri dishes. Half of the samples had light incubation at 40 degrees (12 hours of light); half of samples had dark incubation (no light), with 5 replicates in each incubator. The petri dishes were monitored for 2 months, then heat was increased from 40 degrees to 60 degrees (F). Results indicated that the most germination (83%) was from gibberellic acid (110 pts./mi). Top four treatments were gibberellic acid, (2 strengths) and sulfuric acid (two strengths). The cold stratification was significant in the

gibberellic acid treatment; the sulfuric acid treatments did better in light than dark. Preliminary conclusions are that sulfuric acid affected the seed coat and increased its sensitivity to light. The gibberellic acid treatment allowed germination without the need for light and did better at 40 degrees than 60 degrees (cold stratification). Sylvie indicated a future study could evaluate the optimum rate of gibberellic acid as well as burn boxes and scarified seeds. AMWG members discussed results. Bill indicated the disk seed does not need cold stratification. Gray indicated the need for light seems reasonable as SCT in the wild come up in areas with no thatch. Questions were posed on the natural source of gibberellic acid. Sylvie indicated that the seed lab is testing seed viability rates and is awaiting that data before completing her report.

Public Comments

None received.

Management Updates and Discussion

1. Santa Cruz Tarplant Outplanting for 2023. City reported that there are 1,000 plants in contract with UCSC for outplanting, AMWG members discussed locations, including within grazing plots, red RDM areas in Area C and D, red RDM areas in north portion of Area A, Area C in cattle exclosure, and more in southern portion of Area A. Alison reported that intensive sheetmulching is yielding the highest seed production. Sylvie reported she has approximately 100 flowerheads (maybe 5-10,000 seeds) that can be broadcast on site. AMWG members expressed support for that action.
2. USFWS Study. Preliminary results from the USFWS study indicate most SCT sites have soil with 19% clay; Arana was measured at 13% clay. The wet swales and depressions may have more clay. Perhaps these areas would be suitable for outplanting SCT, as well as the recently burned area in the very south end of Area A.
3. Grass Plugs. UCSC has approximately 300-400 grass plugs for installation. They are comprised on *Elymus, Danthonia, Hordeum brachyantherum,* and possible *Stipa*. AMWG members discussed planting locations, with suggestions for the northern portion of Area A, adjacent to extant native grass stands, wet areas in Area D (*Hordeum*), and seeps and/or eroded areas on lower Prairie Trail.
4. Grazing. Travis reported grazing will continue in 2023, following the 2022 program. Grey indicated his support for supplemental feeding to get cows to move around the site.
5. Other Recommendations. AMWG members suggested a public field trip to discuss cattle health and progress. Sign and interpretation are needed for the outplantings.

Next Meeting: Not determined, but City indicated it will be in March 2023.

Appendix B Photo Monitoring

Arana Gulch Photomonitoring 2022

June 6, 2022

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P1 3 2022

P1 4 2022

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Appendix C SCT Germination Study

A Germination Study:
Dormancy in ray achenes of *Holocarpha macradenia*, a rare coastal prairie forb

By Sylvie Childress

Greenhouse Director, University of California Santa Cruz

March, 2023

Prepared for the City of Santa Cruz under a 2021
Arana Gulch Santa Cruz Tarplant Population Recovery grant
from the US Fish and Wildlife Service

Introduction

The extensive efforts to restore the extremely rare plant *Holocarpha macradenia* are hampered by a lack of understanding of seed germination requirements, which were elusive before this work. This plant is a rare forb inhabiting coastal prairie habitats of California's central coast. It has been listed as Endangered by the State of California, and Threatened by the Federal Government.

Holocarpha macradenia flower heads are composite inflorescences with both disc and ray flowers. Both types of flowers are fertile, so each inflorescence is capable of producing both disc and ray seeds. (Image 1)

Image 1: Disc and ray seeds of *Holocarpha macradenia*

Disc and ray seeds (aka 'achenes') differ in their readiness to germinate, which may be an evolutionary adaptation to variable interannual growing conditions. The disc achenes are known to germinate readily with the addition of water under a wide range of temperatures. However, the ray achenes do not readily germinate under normal nursery or greenhouse conditions. They sit dormant, even after months in a moistened pot of soil. Presumably, these seeds form the long term seedbank for this plant. They are the plant's "insurance policy" against years with poor environmental growing conditions, or years when seed set is low for other other reasons (seed predation, plant death, etc). As an annual plant, if all seeds were to germinate the first year after they are produced, then one bad year without a successful seed set would decimate the population. In theory, at least some of these dormant ray achenes are able to persist for multiple years in the seed bank, waiting out the unfavorable years, and germinating when conditions are “right” for good plant performance. Past researchers have not been able to determine the precise environmental process or cue that stimulates these seeds to germinate, though some regeneration has occurred in the field after disturbance such as scraping and fire (Bainbridge, 2003).

Under a contract with the City of Santa Cruz, and funded by a grant from the United States Department of Fish and Wildlife (USFWS), the University of California at Santa Cruz (UCSC) Greenhouses have performed laboratory seed germination experiments in an attempt to understand this plant’s germination ecology and environmental triggers that initiate ray achene germination. Understanding this cue could add to our understanding of the year-to-year population dynamics and could inform future conservation activities.

We were able to determine the average baseline viability of ray achenes at 40%. The most successful experimental germination methods in this study achieved up to 43% germination of ray achenes. Thus, some of the methods discussed below appear to be sufficient in stimulating germination in the entirety of viable seeds.

An appendix to this document makes summary recommendations for those interested in germinating ray achenes, and a second appendix makes suggestions for future directions in Santa Cruz tarplant ray achene germination ecology research.

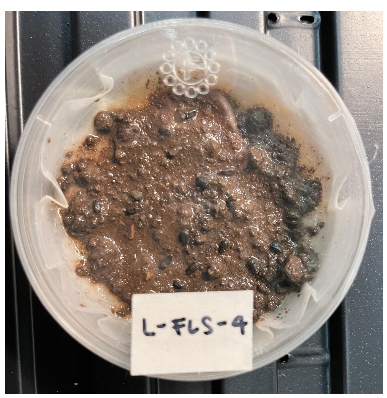
Materials and Methods:

The seed used for these experiments was harvested from 40 robust plants grown ex-situ at the UCSC Greenhouses in 2019 under contract with the City of Santa Cruz. The seed germination experiments described here were conducted in Spring 2022.

Seeds were sorted to ensure that only ray achenes were present in the samples tested. These seeds are morphologically distinct: disc achenes tend to be lighter in color, and linear in shape; ray achenes are darker, wider, wedge shaped, and proximally blunt-truncated. The location of the hilum differs slightly too: disc achenes attach to the receptacle straight underneath the proximal end, whereas ray achenes attach at the side of the blunter proximal end.

To assess baseline seed viability, a sample of disc and ray achenes was sent to the Oregon State University Seed Lab for tetrazolium testing. The sample of 208 ray achenes tested at 40% viability. For those curious readers: the disc achenes from this same seed batch were tetrazolium tested at 73% viability.

For experimental germination trials, small petri dishes (60mm x 15mm) were prepared with filter paper. Each petri dish contained 20 ray achenes under one of 15 treatments. Seeds were either pre-treated (e.g, soaked overnight) or treatment applied in the petri dish as appropriate (see Table 1 for a list of treatments). Dishes were then sealed with a strip of Parafilm to prevent excessive desiccation. (Images 2 and 3)



Images 2 and 3: Seed treatments inside of sealed petri dishes

Image 4: Incubator with a 12 hr photoperiod containing 5 replicates of each treatment

Each of these treatments was replicated 5 times under total darkness and 5 times under a 12 hour photoperiod inside of separate incubators. (Image 4) These petri dishes were the *cold stratified* set. Both incubators were held at 40ºF constant temperature for 2 months, then warmed to 70ºF constant temperature for an additional 2 months. At this point, a second batch of petri dishes identical to all of the treatments was sown and placed in these 70ºF incubators and monitored for 2 months. This second batch is the *warm temperature* batch, which was sown to see if there was a difference in germination under cooler temperatures. Figure 1 shows a representation of all 4 of the environmental conditions tested. All petri dishes were monitored 2x/week for germination and were randomly rotated within the incubator each time they were monitored.

Table 1: Treatments applied to seeds under all 4 environmental conditions. Treatments fit into 4 categories: Gibberellic acid (purple), scarification (blue), fire (red), and soil microbial activity (green).

Treatment name	Details of pretreatment
Control	Control - no treatment, placed on moist filter paper in petri dish
Gibberellic acid 100ppm	Soaked in 100ppm gibberellic acid overnight; filter paper moistened with this solution
Gibberellic acid 250ppm	Soaked in 250ppm gibberellic acid overnight; filter paper moistened with this solution
Gibberellic acid 500ppm	Soaked in 500ppm gibberellic acid overnight; filter paper moistened with this solution
Sandpaper scarification	Scarified by gently rubbing between two sheets of sandpaper
Sulfuric acid 2 min	Soaked in concentrated sulfuric acid for 2 minutes
Sulfuric acid 5 min	Soaked in concentrated sulfuric acid for 5 minutes
Burned grass	½ tsp of burned grass included in petri dish
Charate	½ tsp of burned charate (coyote brush) included in petri dish
Smoke	Soaked in smoke water overnight, and filter paper soaked with this solution. Smoke water prepared with 1:500 concentration of Wrights liquid smoke
Heated to 50ºC	Heated at 50ºC for 17 hours before placing in petri dish
Heated to 65ºC	Heated at 65ºC for 6 hours before placing in petri dish
Field soil	½ tsp of field soil included in petri dish
Decomposing grass	½ tsp of dried grass included in petri dish
Cowpie	½ tsp of cow pie included in petri dish

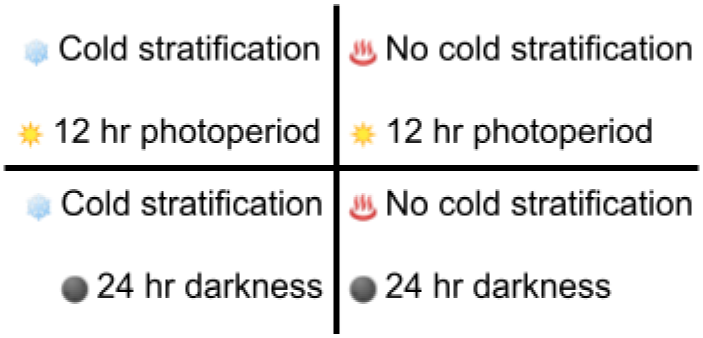


Figure 1: Graphic representation of the 4 environmental conditions under which all treatments in Table 1 were tested

Results and Discussion

While this experiment was able to find significant results from treatments, it would have been more ideal to use larger sample sizes for greater statistical power. This is difficult to do when using rare seeds. 5 replicates with 20 seeds each worked here, but it would be preferable to have 50 seeds per sample.

At first glance, it appears that germination was significantly better under the 40º cold stratification condition than under warm 70º temperatures ($t_{228.12}$=-5.37, p<.0001, Figure 2) and under the 12 hour photoperiod condition than in complete darkness ($t_{286.26}$=1.7716, p=.0388, Figure 3). However, the significance of these statistical trends may have been affected by the magnitude of individual seed treatments within these conditions. We will look at these interaction effects in more detail after we examine the individual seed treatments.

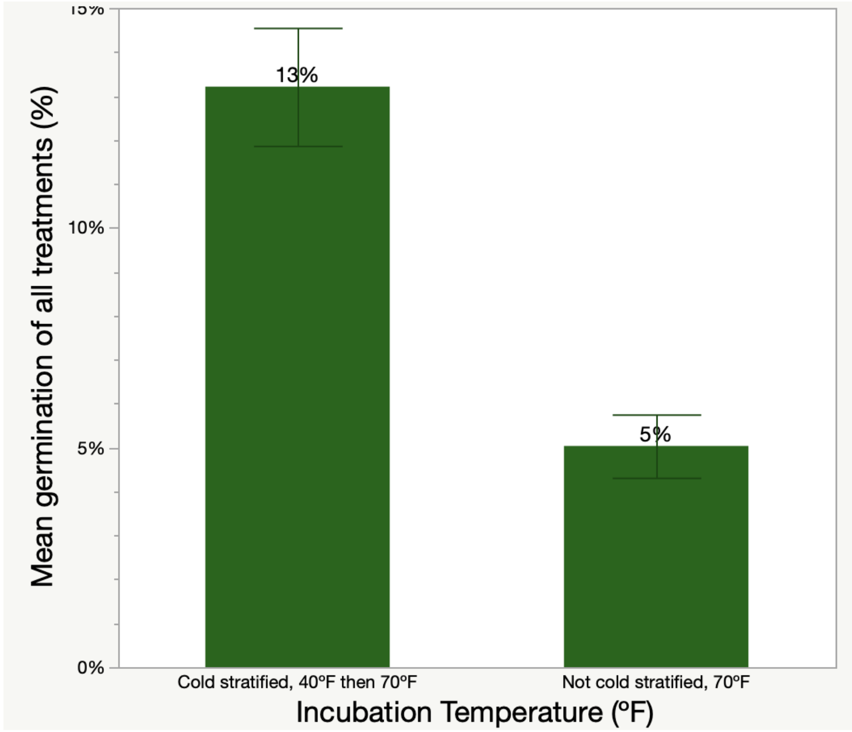


Figure 2: Total germination in cold stratified vs not cold stratified treatments.

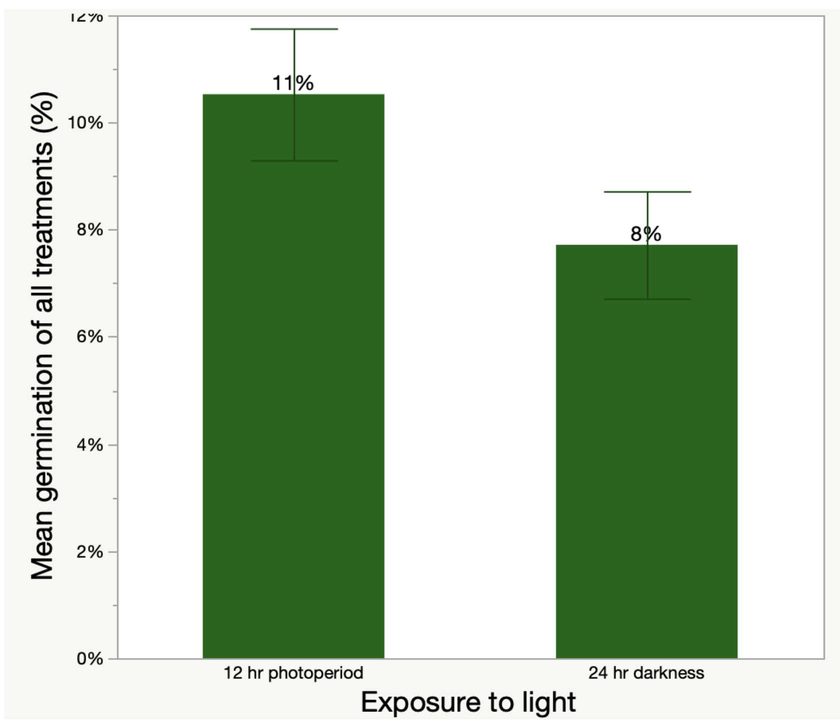


Figure 3: Total germination under 12 hour photoperiod vs total darkness

In addition to these trends in environmental conditions, the individual seed treatments had a significant effect on germination ($F_{14}=8.9676$, $p<.0001$). Specifically: compared to the control treatments, treatments of gibberellic acid at 100ppm ($p<.0001$), gibberellic acid at 250ppm ($p<.0001$), sulfuric acid for 2 minutes ($p=.0084$), and sulfuric acid for 5 minutes ($p=.0001$) all showed significant increases in germination. Those significant treatments are indicated with a gold star in Figure 4. No significant effects were found in any of the soil microbial activity treatments (field soil, cowpie, or decomposing grass) or grassland fire treatments (heat at 50ºC or 65ºC, smoke soak, burned charate, or burned grass.) Results of these treatments were similar whether under 12 hour photoperiod or complete darkness, and under cold stratification or warm temperatures.

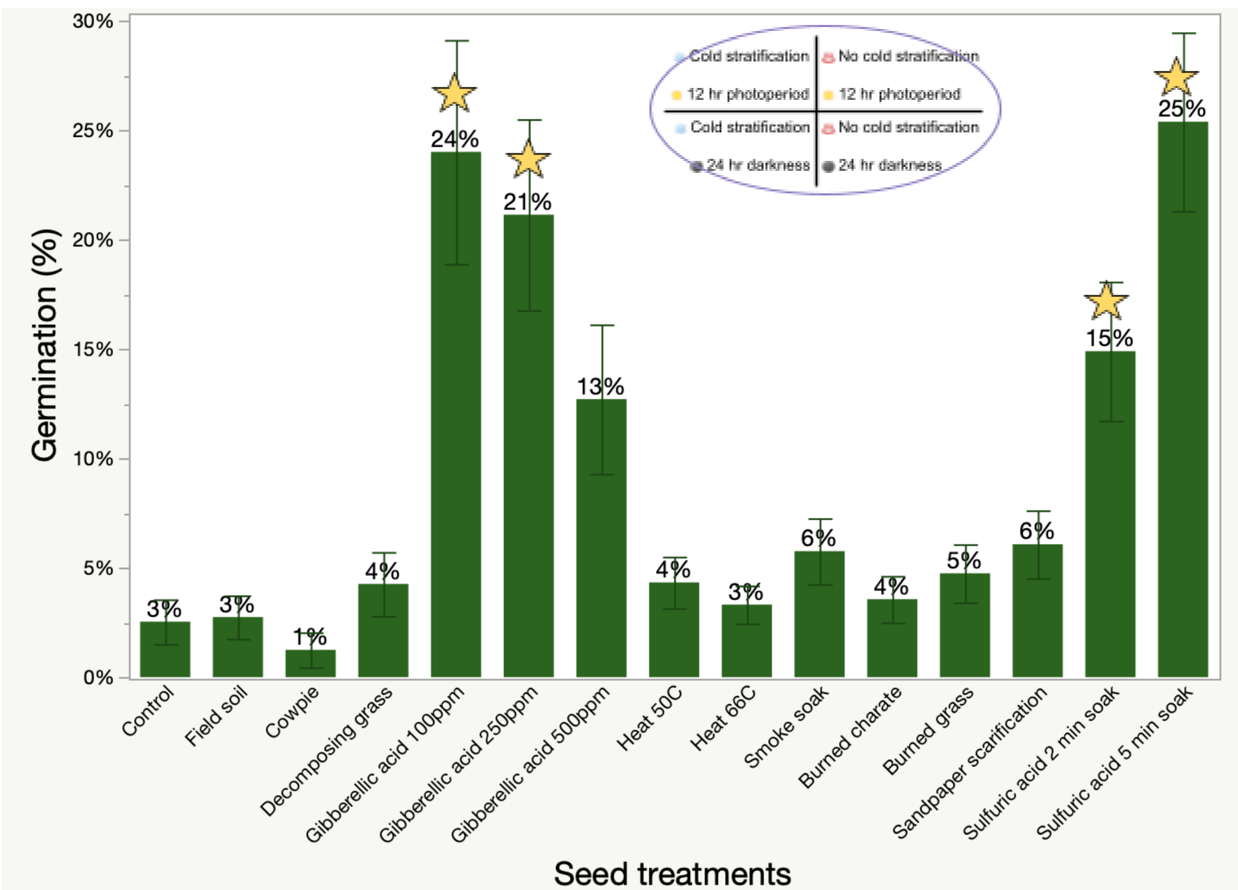


Figure 4: Mean germination of each seed treatment, averaged across all four environmental conditions

Among the three different scarification treatments, only the 2 minute and 5 minute soaks in sulfuric acid were effective; the sandpaper treatment was not sufficient to stimulate a significant amount of germination under any of the growing condition variables. We can speculate that the sandpaper treatments may not have worn away enough of the seed coat, or perhaps the right *region* of the seed coat, to allow sufficient water and gas exchange. A more precise method of mechanical scarification may provide a different result. Research shows that some species of seeds with impermeable seed coats are known to imbibe from the hilar region of the seed coat (Jaganathan et al, 2019) but this sandpaper treatment was most likely to impact the broad side of the seeds instead. The success of the 5 minute acid scarification treatment is interesting because it suggests that these seeds may be able to survive passing through the gut of an animal. If this is the case, perhaps these ray achenes aren't only the long-term seed bank of the plant, but could also have a survival advantage in years with high seed predation, or even a dispersal advantage.

The applications of three strengths of gibberellic acid (which all resulted in a significant increase in germination compared to control) are not to be construed as scarification treatments or mimicking natural processes. The success of these methods is exciting for those who may grow this plant in the future, however does not pinpoint an ecological trigger behind germination of these seeds in the field. Gibberellic acid is a plant hormone that naturally occurs (endogenously) in seeds, and plays a role in germination. The mechanism by which exogenous applications of this hormone stimulate germination are not very well understood. It seems as though gibberellic acid plays some role in softening seed coats and initiating enzymatic activity (Gupta and Chakrabarty, 2013). Thus, the *ex situ* success of this treatment in stimulating germination in disc achenes does not point towards a particular environmental process whereby germination is stimulated *in situ*.

Now that we have discussed the overall trends and successes with treatments overall, we can examine the significant *interaction effects* between the seed treatments and the growing condition variables. Two interactions stand out:

1. All three strengths of gibberellic acid produced comparable germination whether exposed to light or not, but performed significantly better when sown under cold stratification temperatures. (Figures 4 and 5) Perhaps a germination inhibitor related to these seeds' need for light exposure was overridden by the signal from the gibberellic acid.

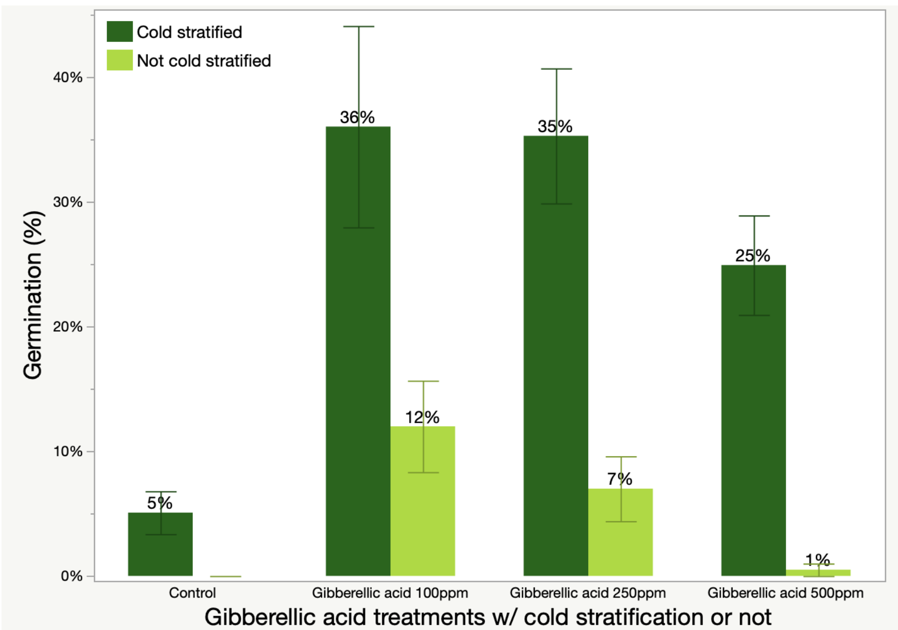


Figure 5: Gibberellic acid treatments germinated better under cold stratification than warm temperature

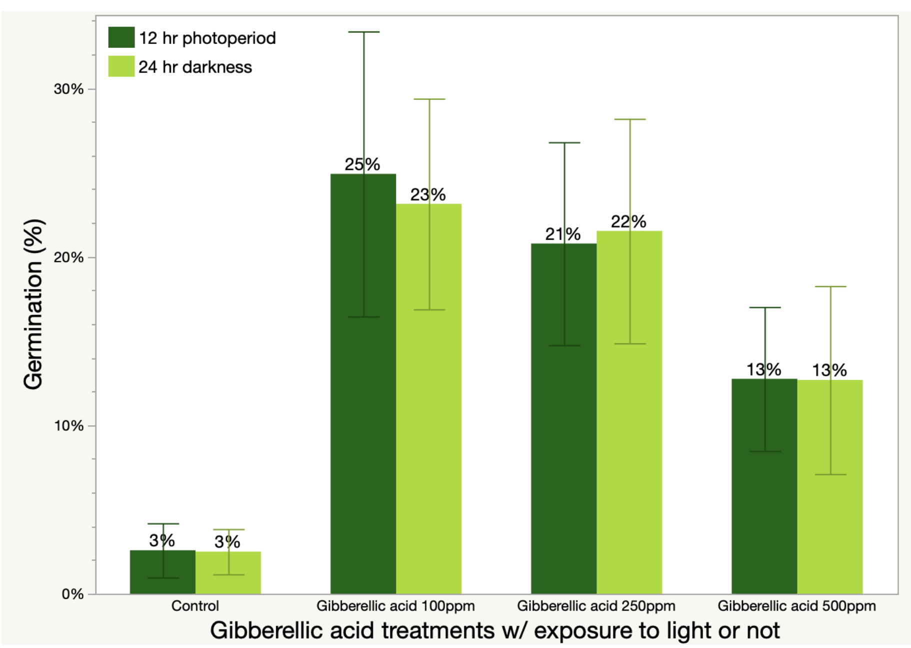


Figure 6: Gibberellic acid treatments showed similar germination rates with 12 hr exposure to light or in 24 hr darkness

2. Treatment with sulfuric acid produced significantly more germination in the 12 hour photoperiod than in 24 hour darkness, but did not require cold stratification temperatures. (Figures 6 and 7) This result is particularly interesting because it indicates that even once seeds have been scarified, they still may not germinate unless they have access to light. Perhaps this indicates that if seeds fall to the ground after scarification or pass through the gut of an animal but land under high amounts of thatch or duff, they may "know" they are in an unsuitable location for growth.

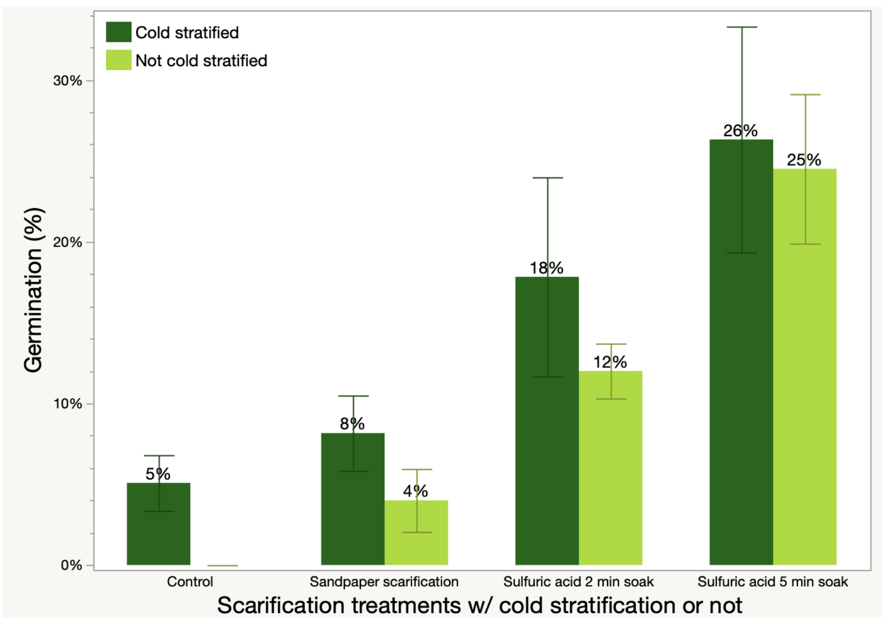


Figure 7: Scarification treatments germinated similarly with cold stratification or warm temperature

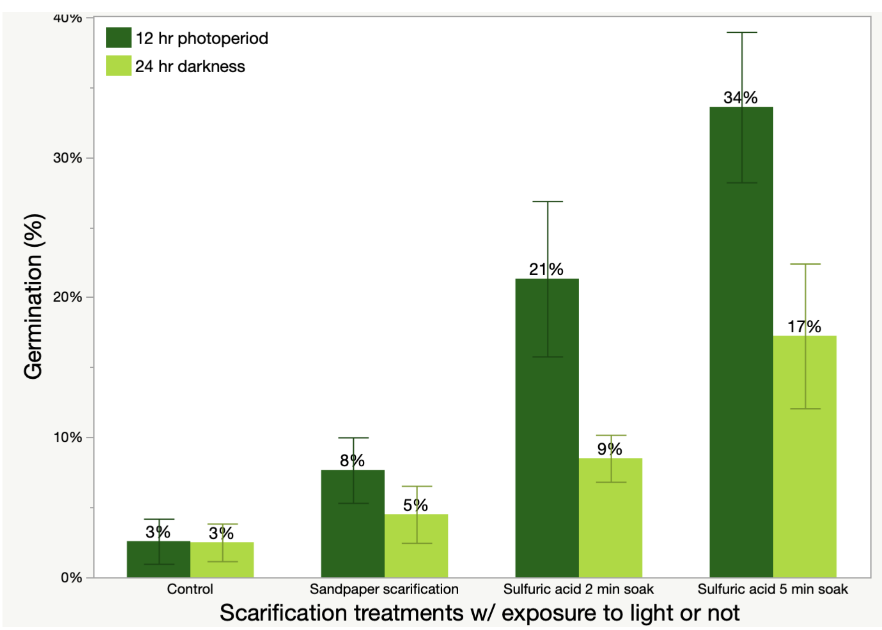


Figure 8: Scarification treatments germinated better with 12 hr exposure to light than they did in 24 hr darkness

Finally, we can examine which treatments and environmental factors *combined* worked the best. If we look only at the data collected from plants in both 40º cold stratification and 12 hour photoperiod conditions, we see that our highest mean germination rates occurred in this set. Figure 9 shows the mean germination of these treatments under only these combined environmental conditions, and excludes the other ¾ of the data collected in this study. Under these conditions, the 5 minute sulfuric acid treatment produced a mean germination of 36%, and the seeds treated with gibberellic acid at 100ppm germinated at 43%.

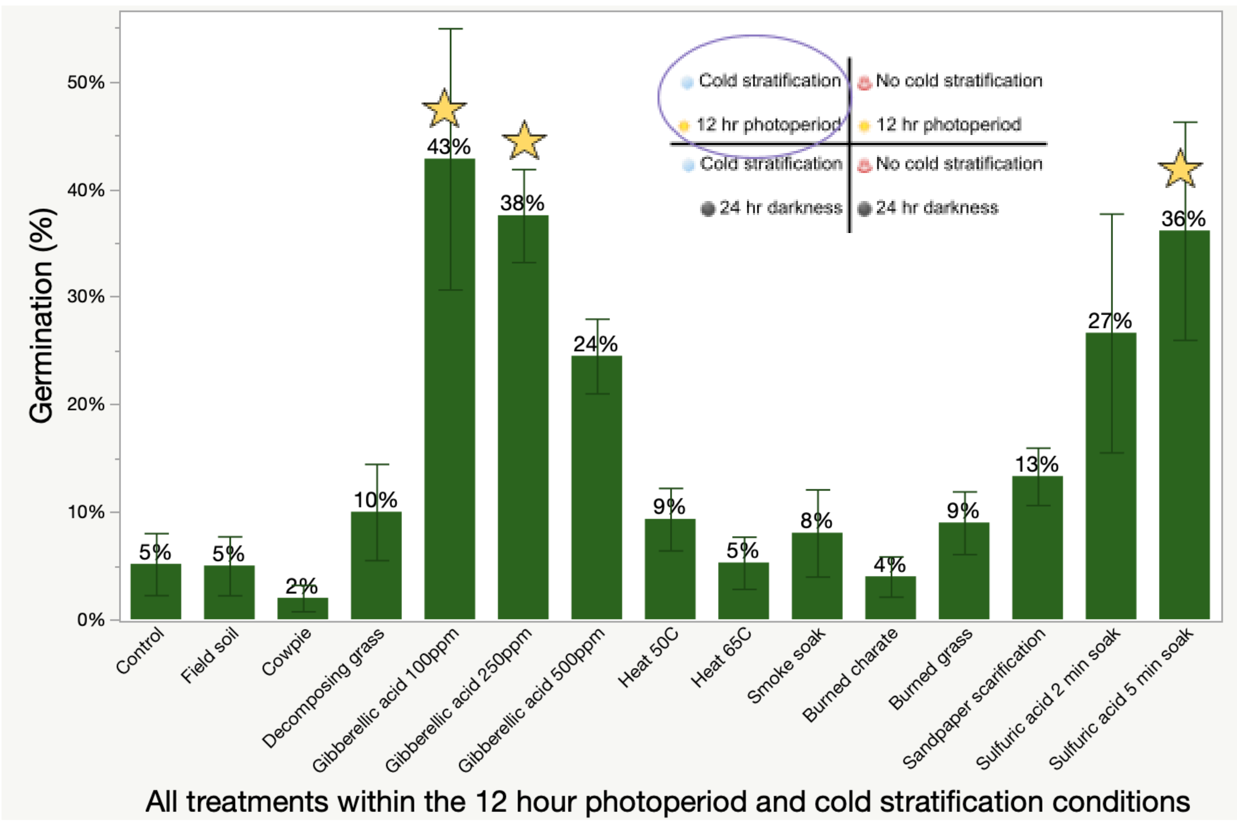


Figure 9: Some treatments in the 12 hr photoperiod and 40ºF cold stratification environmental cohort achieved significant mean germination rates between 36-43%. This represents the highest mean germination achieved by this experiment.

Conclusion

In summary, the best germination rates occurred under cold stratification temperatures of 40ºF with 12 hr diurnal exposure to light when seeds were either scarified with sulfuric acid for 5 minutes or pretreated with gibberellic acid at 100ppm. Under these conditions, mean germination rates ranged between 36% (sulfuric acid 5 minute soak) and 43% (gibberellic acid 100ppm), which are both reasonably close to their tested batch viability rate of 40%.

Under field conditions, scarification of the ray achene seed coats is the most likely trigger for germination. However, seeds treated with sulfuric acid germinated better when exposed to light, indicating that seeds have both a physically impermeable seed coat, *as well as* some sensitivity to light to stimulate embryonic development. The gibberellic acid treatments did not require exposure to light, but this finding is consistent with other research where gibberellic acid overcame photosensitive species light requirement (Deno, 1993). Thus, it tracks that *Holocarpha macradenia* ray achenes are photosensitive and require light exposure for germination.

This work underscores the need for more research to understand the unique germination ecology of this plant, and especially how its seeds interact with its field environment and ecosystem.

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Appendix A:

Notes for practitioners who wish to germinate ray achenes:

- You'll first need to sort ray achenes from disc achenes. The best method I have found for sorting disc achenes from ray achenes is to first do a rough sorting using an Oregon Seed Blower to remove the majority of the disc achenes from your sample. It won't be perfect, and you will need to follow up with a careful manual sorting. Accidental treatment of *disc* achenes with either gibberellic acid or concentrated sulfuric acid is likely to produce an undesirable result: Unnatural elongation of seedlings may occur in disc achenes treated with gibberellic acid; concentrated sulfuric acid is likely to damage the embryo of disc achenes, which do not have as robust of a seed coat.
- Each seed batch may have a different baseline viability. I recommend tetrazolium testing to establish baseline viability of your seed lot prior to further research into effectiveness of seed treatments.
- For growers looking to produce germination in ray achenes in the simplest and most accessible way, I recommend treating ray achenes with 100ppm gibberellic acid and placing seeds in cold stratification temperatures. Germination may take multiple months under these conditions. Scarification in concentrated sulfuric acid may produce results more rapidly, however I do not recommend that most growers attempt scarification with concentrated sulfuric acid. This chemical can be dangerous to handle and should be done by trained laboratory professionals with adequate protective equipment. Since each seed batch may have variable viability, the expected result may vary widely.

Appendix B:

Suggestions and questions for future seed germination research

- Pinpointing the optimum rate of gibberellic acid may be useful for growers, especially those who may be working with a limited supply of seeds and wish to maximize their germination. Do concentrations under 100ppm provide significant benefit?
- Did the sulfuric acid treatment destroy a germination inhibitor in the seeds that mechanical scarification did not?
- Once seeds are mechanically scarified, do other treatments impact their germination? For example, does additional treatment with smoke, field soil, or cowpie affect germination compared to scarification on its own?
- Can these seeds pass through a rodent, bird, or cow’s digestive tract and remain intact? How does this compare to disc achenes survivability? Does digestion break ray achene dormancy?
- How long do scarified (opened) seeds maintain viability? Does this differ between acid scarification, which may wear away much of the testa, and mechanical scarification, which may be targeted to one area?

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