THESIS

MIMULUS GEMMIPARUS AS A MODEL FOR THE EX SITU PROPAGATON AND STUDY OF PROPAGULE STORAGE FOR A SPECIES OF CONCERN

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ABSTRACT

MIMULUS GEMMIPARUS AS A MODEL FOR THE EX SITU PROPAGATON AND STUDY OF PROPAGULE STORAGE FOR A SPECIES OF CONCERN

Mimulus gemmiparus, W.A. Weber, budding monkeyflower, is a Colorado narrow endemic plant that could be at risk of extinction. Factors including rarity, a unique mode of clonal, vegetative, annual reproduction through petiole enclosed brood bulbils, specific microhabitat requirements, limited means of dispersal for migration, and vulnerability to stochastic events all play roles in *M. gemmiparus* survival. Conservation efforts by researchers and federal and state land managers have involved passive protection of existing known populations and active measures such as assisted establishment. A decision matrix to prioritize the active propagation of Colorado species of concern is presented that identifies *M. gemmiparus* as a high priority for action.

Ex situ propagation from field collected *M. gemmiparus* propagules can provide large quantities of plant material for active conservation efforts, however, short term to long term storage parameters for propagules have not been empirically investigated. Propagation of *M. gemmiparus* was undertaken in order to multiply propagules to produce material for a cold storage experiment to support active conservation efforts, also in order to document a reliable, repeatable propagation method that can be used to create banks of plant and propagule material for conservation, propagation, restoration, research, and storage.

Interactions between water, temperature, and desiccation tolerance characteristics of the vegetatively reproductive bulbil are key to determining optimal storage conditions. Categories of seed storage behavior, orthodox, recalcitrant, and intermediate, differentiated by seed characteristics such as

desiccation and cold tolerance, were used as a basis for exploration of bulbil storage behavior. The basis of knowledge used to guide cold storage of multiplied propagules was built on viability assays, measurements of bulbil moisture content, and storage temperature exposure and duration response.

An empirical study examined bulbil post storage temperature exposure germination response in 4 MIGE ex situ propagated populations at temperatures -20^o, -3^o to 0^o, and 3^o to 5^{o}C for 24 hours, 1 month, 3 months, and 6 months compared to a control storage temperature of 20^{o}C. Bulbil viability at moisture contents below 0.1 gH2Og-1DW, and longevity at subfreezing temperatures did not conform to typical orthodox or recalcitrant seed behavior. Propagules with a mean moisture content of 0.3877 $gH_20g^{-1}DW$ stored for 6 months at 3^o to 5^{o}C retained the highest rates of post storage temperature exposure germination observed over 12 weeks. A low moisture content threshold where germination observed over 12 weeks was ≤10% was found between 0.1350 and 0.1817 $gH_20g^{-1}DW$. There was not a higher post exposure germination response in low moisture content bulbils stored at subfreezing temperatures compared to high moisture content bulbils exposed to subfreezing temperatures. Storage behavior appeared intermediate in nature. Further study could confirm that the 3 to 5 year longevity at 5^{o}C storage temperature potential predicted for intermediate seeds is applicable to MIGE bulbils.

M. gemmiparus serves as a model that demonstrates the feasibility and efficacy of ex situ propagation and propagule storage for a species of concern. Creation and management of ex situ plant and propagule banks are intended as a compliment to, not a replacement for, traditional conservation of in situ populations. More study is needed using more controlled methods of desiccation or specialized pre treatments such as the application of cryoprotectants to bulbils or meristematic tissue to preserve germplasm for longer time frames at freezing temperatures.

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CHAPTER 1. A DESICION MATRIX TO IDENTIFY PRIORITY SPECIES OF CONCERN FOR PROPAGATION AND ESTABLISHMENT OF EX SITU PLANT BANKS

1.1 Introduction

Apparent extirpations of dozens of moisture loving, high elevation plants (Bowers and McLaughlin, 1987) and extirpations of rare plants from highly protected natural areas such as U.S. National Parks could forewarn of irreversible deterioration in the world's last bastions of biodiversity. Attempts to quantify threats to rare species can consider large scale stresses and broad threat categories such as habitat destruction or introduction of alien species (Wilson, 1992), or can consider threats on a smaller scale as they pertain to individual taxon in terms of level of protection or distance from other occurrences (Farnsworht et al., 2006). Precipitous declines in populations and permanent extirpations or extinctions of plants may evidence that natural communities are responding to greater pressure from stresses in rapidly changing climate and habitat conditions faster than science is quantifying and allowing for mitigation of those conditions.

Traditional conservation efforts that focus on passive protection of existing landscapes and habitats may not be enough to prevent loss of species in a rapidly changing climate. Ex situ propagation of rare plant species of concern can support active conservation efforts such as assisted establishment (Beardsley, 2016) and build valuable banks of plant material and propagules for on-going propagation, restoration, storage, conservation, and research. Landscape restoration as practiced by foresters such as Aldo Leopold does not have to conflict with traditional views of conservation. Ex situ propagation as a tool for use in landscape restoration may prove to be a viable complement to passive protection of habitats.

The first step when undertaking multiplication of plant material outside of natural habitats is to define priorities and consider constraints. Some species of concern such as rare orchids may have specific reproductive biologies requiring specialized horticultural methods of propagation. Others species, such as the 45 rare penstemons of Colorado, may have cultivated relatives that are widely propagated and multiplied, evidencing potentially fewer constraints for feasibility of propagation. A plant that is rare in a particular state, but abundant on a global scale may present a different priority for active ex situ propagation than a plant that is abundant in a particular state, but rare globally. There may be instances of subspecies with questionable taxonomic status, or ethical concerns regarding the impact of taking collections from native populations. Agencies tasked with protection of rare species may have budget constraints that limit the selection of plants that can be actively managed.

A model decision matrix presented here using a frame of native plant species of concern in Colorado and neighboring states considered rarity, feasibility and ethics of propagation, and urgency of conservation need incorporating multiple agency management concerns to prioritize species to consider for propagation efforts. The results of this matrix helped to identify a species, *Mimulus gemmiparus,* that was chosen to serve as an example for a strategically precautious plan of ex situ propagation to support active conservation efforts. This decision matrix and scoring system was intended as a flexible, adaptive tool that can be revised and customized according to the status of knowledge, scale of interest, and objectives of land managers.

1.2 Methods and Logic

Taxa selected as the sample frame for a decision matrix identifying priority candidates for ex situ propagation were compiled from Threatened and Endangered Species (TES) Lists from USDA Forest Service (USFS) Regions 2 and 4 in Colorado, the Colorado Natural Heritage Program's (CNHP) tracking list of vascular plants, and rare plant lists from Colorado's 7 National Park Service (NPS) units and the

Bureau of Land Management (BLM). This Colorado state and agency centric collection of rare plant species of concern considered for ex situ propagation yielded 865 entries for Colorado native species, subspecies or varieties listed with CNHP, or USFS, NPS, or BLM rare or species of concern plant lists (CNHP, 1997+, CO Rare Plant Guide, www.cnhp.colostate.edu. Latest update: November, 2015). Candidates were listed alphabetically by scientific name and color coded by list from which species were derived, USDA FS Region 2, USDA FS Region 4, or CNHP vascular plants tracking list. Color codes were maintained to discern between tracking lists from which taxa were listed. Multiple listings of the same taxon from multiple tracking lists counted as one single candidate in the total matrix sample frame.

The Forest Service TES lists are composed of species within the administrative ranges of Rocky Mountain Region 2 and Intermountain Region 4 units of the USDA Forest Service. Plants were selected for inclusion on these lists based on an evaluation of specific criteria. The criteria that determine whether a species will be designated as sensitive, or a species of concern incorporate agency geographic and administrative unit scales, and plant and population information such as dispersal capability, abundance, population habitat trends, vulnerability of habitats, and life history related to demographics of reproductive rate and mortality that suggest how populations could respond to disturbance. (USDA Forest Service Manual, 2011).

National Park Service and BLM Rare Plant lists were summary documents that included species based on CNHP data. Agency management concerns are implicitly represented in an agency's choice of criteria, scale of interest, and geographical administrative range that selects for species of concern within a designated land management unit. Inclusion of species in the model decision matrix presented here represented the interests of multiple federal agencies, the US Department of Agriculture and the Department of the Interior, and selected rare species and species of concern documented in US Forests by region, NPS Intermountain Region units in Colorado, or land in Colorado managed by the BLM.

Parameters used to define priorities for consideration for propagation were Federal Status, presence in land managed by USFS Region 2, USFS Region 4, BLM, and CO NPS units, CONHP Global and State rarity rankings, a converted rarity ranking that accounts for different spatial scales, horticultural feasibility and ethics, alpine or at risk habitats, and Colorado endemism. Each category assigns a numerical score, with total scores calculated from 0 to 13, and a recommended (R), or not recommended (NR) designation. The highest scores that did not include a not recommended designation were chosen as priority candidates to consider for ex situ propagation. Plants from neighboring states were included in the original sample frame due to FS Regions 2 and 4 inclusions, but as not ranked by CNHP, received low and ambiguous ranking scores precluding designation as higher priority to consider for propagation.

Federal Status indicates if a plant has a legal designation listed by the Endangered Species Act (ESA) as Threatened (T) or Endangered (E). A designation Candidate (C) is a category that was previously used by the ESA to indicate species that were being considered for inclusion in the ESA list pending further study. C is no longer a legally recognized federal status; however, species that were previously identified as Candidates are included for scoring in the propagation priority matrix. Scores for this category were 0, indicating no Federal Status, or 1 indicating Federal Status or Candidate species.

Rarity of plants was initially quantified in this decision matrix according to designation under CNHP, which is a principle clearinghouse for periodically updated, expert monitoring and research information regarding rare plants in Colorado. CNHP standards for rarity follow the criteria of NatureServe, the parent organization of the Natural Heritage Program and its 75 member organizations located in all 50 U.S. states, Canadian provinces and 12 countries in Latin American and the Caribbean. A conversion chart produced decision matrix rarity scores that accounted for spatial scales for state and global rarity (Table 1.1). Matrix global rarity reverses the order of CNHP global rarity rankings, so that

Table 1.1. Conversion of Colorado Natural Heritage Program (CNHP) plant rarity rankings to propagation decision matrix rarity scores.

	CNHP Global Rank		CNHP State Rank		Matrix Global Score		Matrix Raw State Score		Matrix Converted State Score	Matrix Total Rarity Score	
More Rare	**1**	**More Rare**	**1**	**Higher Priority**	**5**	**Higher Priority**	**5**	**Higher Priority**	**5/5 = 1**	**6**	**Higher Priority**
	1		**2**		**5**		**4**		**4/5 = 0.8**	**5.8**	
	1		**3**		**5**		**3**		**3/5 = 0.6**	**5.6**	
	1		**4**		**5**		**2**		**2/5 =0.4**	**5.4**	
	1	**Less Rare**	**5**		**5**	**Lower Priority**	**1**	**Lower Priority**	**1/5 =0.2**	**5.2**	
	2	**More Rare**	**1**		**4**	**Higher Priority**	**5**	**Higher Priority**	**5/5 = 1**	**5**	
	2		**2**		**4**		**4**		**4/5 = 0.8**	**4.8**	
	2		**3**		**4**		**3**		**3/5 = 0.6**	**4.6**	
	2		**4**		**4**		**2**		**2/5 =0.4**	**4.4**	
	2	**Less Rare**	**5**		**4**	**Lower Priority**	**1**	**Lower Priority**	**1/5 =0.2**	**4.2**	
	3	**More Rare**	**1**		**3**	**Higher Priority**	**5**	**Higher Priority**	**5/5 = 1**	**4**	
	3		**2**		**3**		**4**		**4/5 = 0.8**	**3.8**	
	3		**3**		**3**		**3**		**3/5 = 0.6**	**3.6**	
	3		**4**		**3**		**2**		**2/5 =0.4**	**3.4**	
	3	**Less Rare**	**5**		**3**	**Lower Priority**	**1**	**Lower Priority**	**1/5 =0.2**	**3.2**	
	4	**More Rare**	**1**		**2**	**Higher Priority**	**5**	**Higher Priority**	**5/5 = 1**	**3**	
	4		**2**		**2**		**4**		**4/5 = 0.8**	**2.8**	
	4		**3**		**2**		**3**		**3/5 = 0.6**	**2.6**	
	4		**4**		**2**		**2**		**2/5 =0.4**	**2.4**	
	4	**Less Rare**	**5**		**2**	**Lower Priority**	**1**	**Lower Priority**	**1/5 =0.2**	**2.2**	
	5	**More Rare**	**1**		**1**	**Higher Priority**	**5**	**Higher Priority**	**5/5 = 1**	**2**	
	5		**2**		**1**		**4**		**4/5 = 0.8**	**1.8**	
	5		**3**		**1**		**3**		**3/5 = 0.6**	**1.6**	
	5		**4**		**1**		**2**		**2/5 =0.4**	**1.4**	
Less Rare	**5**	**Less Rare**	**5**	**Lower Priority**	**1**	**Lower Priority**	**1**	**Lower Priority**	**1/5 =0.2**	**1.2**	**Lower Priority**

a matrix global rank of 5 indicates the highest rarity and 1 indicates the lowest level of rarity. In order to achieve a scoring system that takes different spatial scales of rarity into consideration, the decision matrix converted CNHP state rarity scores by expressing state rarity as a fraction of global rarity with a score of 0 to 5 divided by the highest possible matrix global rarity score of 5 for converted matrix state rarity values ranging from 0 to 1. Combined global and state total matrix rarity scores ranged from 1.2 to 6.

USFS Region 2 (Rocky Mountain Region) includes Arapaho, Bighorn, Black Hills, Grand Mesa, Gunnison, Medicine Bow, Nebraska, Pike, Rio Grande, Roosevelt, Routt, Samuel R. McKelvie, San Isabel, San Juan, Shoshone, Uncompagre, and White River National Forests and Buffalo Gap, Cimarron, Comanche, Fort Pierre, Oglala, Pawnee, and Thunder Basin National Grasslands in the states of Colorado, Kansas, Nebraska, South Dakota, and Eastern Wyoming. Scores for this category were 1 if a plant was documented in a TES List (USDA Forest Service Manual, 2011) within USFS Region 2, and 0 if the species was not documented.

USFS Region 4 (Intermountain Region) includes Ashley, Boise, Bridger-Teton, Caribou-Targhee, Dixie, Fishlake, Humboldt-Toiyabe, Manti-LaSal, Payette, Salmon-Challis, Sawtooth, Uinta, Wasatch-Cache Nation Forests within the states of Southern Idaho, Nevada, Utah, and Western Wyoming. Scores for this category were 1 if a plant was listed on a USFS Region 4 specific sensitive species list, and 0 if the species was not documented. Species were scored 1 if documented by CNHP as tracked on Colorado BLM land as sensitive or 0 if not documented as such. Species from USFS Regions 2 and 4 not found in Colorado and not tracked by CNHP were listed by Forest Service Region, but were eliminated from consideration for propagation by low and ambiguous scores that did not incorporate out of state rarity rankings.

Colorado Intermountain Region NPS units included Black Canyon of the Gunnison, Great Sand Dunes, Mesa Verde, and Rocky Mountain National Parks and Colorado, Dinosaur, and Florissant Fossil Beds National Monuments. Species received 1 point for documented presence within one or more of these Colorado NPS units, abbreviated in the matrix as B/G/M/R/C/D/F, respectively, for scores ranging from 0 to 1. Plants tracked by CNHP on land managed by the BLM that were documented as BLM sensitive species were scored 1. Plants tracked by CNHP on a mix of private and public lands documents rare plants in USFS Regions 2 and 4, BLM Colorado Field Offices Colorado River Valley, Grand Junction, Gunnison, Kremmling, Little Snake, Royal Gorge, San Luis Valley, Tres Rio, Uncompahgre, and White River.

Horticultural Feasibility considered ethical, biological or horticultural constraints such as lack of propagule source, sensitivity of native populations to collection of propagules, and likelihood of successful propagation. Questions that address possible horticultural constraints include whether there is an existing propagation protocol for a closely related taxa, the main mode of reproduction, life history and demographics, whether a species is annually reproducing or perennial, the longevity of propagules, and whether there is a reliable method of storage for propagules. This matrix parameter, intended to address ethical considerations concerning potential harm to extant native populations, resulted in an assignment as recommended or not recommended. A species that is difficult to propagate constrained a matrix recommendation for consideration. Potentially negative impacts to a population from which collections would be taken constrained recommendation. Questionable taxonomic status or rarity ranking also constrained recommendation. There was a high degree of suspicion to overcome in order to attain recommendation. A concern regarding feasibility of ex situ propagation or risk of harm to native populations resulted in a not recommended (NR) designation that over rides the numerical score.

Urgency of conservation need was addressed by rarity ranking, Colorado endemism, and consideration of at risk habitats. Some alpine and wetland habitats may be negatively impacted by potential changes in climate while other habitats could potentially be degraded or lost due to land use practices such as oil and gas development or off road vehicle use. A taxon with published research documenting a risk to its local habitat or an elevated risk of loss due to population demographics scored 1. The standard for documentation of risk to habitat was a citation from a native habitat specific published study that identified a habitat threat. This standard relied heavily on CNHP Rare Plant Guide links to research. If only large scale threats not documented on site in question such as invasive species, land use practices, or climate change were documented, an at risk score of 0 was recorded. Research specific to Colorado species of concern occurrences that identified localized habitat risks scored 1 for alpine/at risk habitats. Lack of documented evidence of a habitat threat specific to the habitat of specific plant occurrences resulted in a 0 score. Plants with extant in situ habitat ranges reaching alpine elevations scored a 1.

Endemism was partially accounted for by rarity rankings, as a plant found in a single state or region will be documented as rare on a global basis by the criteria of Natural Heritage Program and NatureServe. An additional parameter scoring specifically for Colorado endemic plants was included because risk of potential extirpation of plants with geographically limited ranges is elevated, and because states could be more invested in preventing the loss of species specific to their state heritage. While the margins of plant populations will extend as continuous groups or in discrete patches beyond state borders, the limit of Colorado endemism was chosen as a spatial parameter for practical reasons. State and federal agency management budgets are allocated by regions, subregions, and units. The entire state of Colorado is included within a single NPS Intermountain Region, for instance, and budgets for management of rare plants in Colorado national parks units are allocated from this Intermountain

Region budget.It may be easier in some cases to predict budgetary constraints when considering ex situ propagation across mutlitple agencies by setting state borders as a de facto or de jure parameter.

A framework that includes plant occurrences on a regional rather than state scale would be preferred if decision priorties included data that could support ecological studies. A framework to prioritize Colorado native plant ex situ plant bank establishment could be useful to local, municipal, county, state, and federal agencies tasked with and budgeted for native plant and vegetation management. Taxon endemic to Colorado were scored 1. Taxa not identified as endemic to Colorado were scored 0. A summary of the decision matrix design, the matrix itself, and list of species identified as priority for consideration for ex situ propagation are presented in Appendices 1, 2, and 3, respectivey.

1.3 Results and Discussion

This Colorado state and agency centric decision matrix pulled from a collection of rare plant species of concern yielding 812 candidates for consideration for ex situ propagation. The total number of unique taxon was 812 species, subspecies, or varieties. Of those, 349 unique taxon scored R for recommended to consider for propagation. Taxon not recommended (NR) for propagation numbered 463. No candidate attained the highest possible score of 13. Scores rated R ranged in matrix values from 1.8 to 10 with a mean of 4.. Candidates not recommended (NR) ranged in total matrix scores from 1 to 7 with a mean score of 1.5. Multiple, questionable, or undocumented rarity rankings constrained an R rating for the majority of those deemed NR. The 12 highest ranking scores for species or interspecies taxon were globally rare CHNP G1 or G2 species. Of the 45 CNHP G1 taxa represented in this matrix, 23 were not recommended for propagation.

The majority of G1 plants deemed NR were so designated due to ambiguous rarity rankings (AR). Ambiguous rarity rankings could be due to lack of sufficient information or conflicting information. If a plant is not tracked by the Colorado Natural Heritage Program it lacks a key indication of rarity, so resulted in an ambiguous ranking justification for a NR designation. *Aquilegia chrysantha var. rydbergii, A. flavescens var. rubicunda, A. grhamii, A. micrantha var. mancosana* were designated AR due to lack of CNHP rarity rankings, while *Aquilegia saximontana* that had been assigned a CNHP rarity rank G3S3 was recommended for propagation with a matrix score of 6.6. *Aletes latilobis* scored 6-7 in the decision matrix and was a candidate species for the ESA, but is NR due to questions reqarding global rarity as G1 or G2.

Rarity of interspecific taxon scored with a 5 compared to a score of 2 for the species rarity elevated the total score of Aquilegia chrysantha var. rydbergii to 10 and made it a high priority to consider for propagation. *Mimulus gemmiparus* total matrix score of 10 resulted from points for global and state rarity, endemism, risks due to population dynamics, and documentation as of concern within multiple agency management units. Other highest scoring R taxa scored points for alpine habitats, Federal ESA or Candidate status, and identified threats to habitats.

FS Regions 2 and 4 plants not known to occur in Colorado may be globally rare, but are not ranked by CNHP. Incorporating neighboring state Natural Heritage Program rarity rankings would increase the pool of potential propagation candidates by 234. The highest priority candidates with scores of 8.8 - 10 with R designation were *Aquilegia chrysantha var. rydbergii, Astragalus microcymbus, Mimulus gemmiparus, Gutierrezia elegans, Draba weberi, Oreoxis humilis, Physaria pulvinata, Draba grayana, and Penstemon degeneri* (Table 1.2).

National Parks provide a more conservative standard for land use than multi-use USFS and BLM units, and may be less vulnerable to potential changes to designation of lands into Federal, State, or private ownership. Oil and gas development, grazing, off-road vehicle recreation, and timber harvesting are permitted on USFS and BLM lands. Budgets that would support ex situ propagation of plants on land managed by federal agencies are tied to the priorities of those agencies. Negative impacts to species on multi-use lands may be greater and land use priorities and potential changes in ownership of FS and BLM lands may indicate a greater urgency in risk of loss while at the same time potentially constraining the ability to conduct long term, costly plant collection and propagation projects.

The entire state of Colorado is included within a single NPS Intermountain Region, with 7 Colorado NPS units. There are 38 National Forests within just 2 of the FS Regional Districts incorporating Colorado, and the BLM manages more land than any other federal agency. An attempt to weigh the relative importance of various agency spatial scales would add a layer of complexity and finer scale measure of factors such as risk to habitat, the potential negative impacts from collections.

Table 1.2. Taxon with total matrix scores of 8.8-10 in a decision matrix prioritizing rare Colorado plants and species of concern to consider for ex situ propagation. The matrix scored for rarity, horticultural feasibility, ethics, and urgency of conservation need incorporating multiple agency management concerns. *Penstemon debilis*,is one of 12 taxa with total matrix scores of 8. G=global rarity, T=interspecific taxon rarity, S=state rarity, 1=most rare, 5=least rare.

	Total Matrix **Score**	CNHP Rarity
Aquilegia chrysantha var. rydbergii	10	G4T1 S1
Astragalus microcymbus	10	G1 S1
Astragalus tortipes	10	G1 S1
Mimulus gemmiparus	10	G1 S1
Gutierrezia elegans	10	G1 S1
Draba weberi	9	G1 S1
Oreoxis humilis	9	G1 S1
Physaria pulvinata	9	G1 S1
Draba grayana	8.8	G2 S2
Penstemon degeneri	8.8	G2 S2
Aliciella sedifolia	8.8	G1 S1
Penstemon degeneri	8.8	G2 S2
Penstemon debilis	8	G1 S1

While taxa that are rare state wide but globally secure received lower matrix scores, there may be benefit to assessing all recommended candidates. *Salix serissima,* and *Aster alpinis var.vierhapperi* could be good candidates to choose for propagation, although their scores were 5 and 3 respectively. Existing NPS and FS protocols for vegetative reproduction of willow stakes and propagation by seed of native Colorado asters could encourage choice of candidates such as these to increase chances for ex situ propagation success (RMNP Propagation Protocols, 2011.)

1.4 Conclusion

Scoring was intended as a measure of relative priorities to narrow candidate species to consider for proactive ex situ propagation in agencies with limited funds and resources. Many lower scores could be raised as monitoring efforts are increased in agency lands, or as more risks are recognized and identified. Parameters are flexible and may be adjusted to account for different agencies or different geographical locations. The matrix framework presented here is Intended as a tool for use in adaptive management and can be updated and revised regularly to meet the selection objectives of matrix users.

To address the issue of scale when assigning points for both global and state rarity, global rarity scoring was given higher power than state rarity. Other methods of prioritization of spatial scale could be devised depending on management objectives and total area under consideration. Differences that may develop in spatially distinct populations may necessitate the careful differentiation of rare genotypes. Unresolved questions of taxonomy should constrain any consideration of ex situ production.

Mimulus gemmiparus was identified as a high priority for propagation. It received the highest score of 10 due to its global and state rarity, endemic designation, and presence in two federal agency management units. Vegetative propagation was recommended for horticultural feasibility. Greenhouse grown *Mimulus gemmiparus* has shown high fecundity from ex situ sown bulbils (Chu, 2016), and it has been reproduced ex situ successfully at Colorado State University periodically since 1997 (Beardsley, 1997, Steingraeber, personal communication, 2015, Chu, 2016).

A model Priority for Propagation Decision Matrix can serve to justify the selection of a rare Colorado plant to consider for ex situ propagation (Appendix I, II). *Mimulus gemmiparus*, was selected to serve as a model species of concern that was used to demonstrate a plan of action for the creation and management of ex situ plant material. Chapter 2 describes a repeatable method for the multiplication of propagules and proposes a propagation protocol. Chapter 3 describes studies in viability assays, moisture content, and storage temperature exposures to guide best practices for storage of propagules. Taken together, these chapters provide a model for the ex situ propagation of a rare Colorado species of concern in order to create a bank of plants and propagules for conservation, restoration, and research.

CHAPTER 2. OBSERVATIONS IN GREENHOUSE PROPAGATION OF *MIMULUS GEMMIPARUS*

2.1 Introduction

Propagation of the rare native plant *Mimulus gemmiparus* W.A. Weber, henceforward referred to as MIGE, was undertaken in order to multiply propagules to produce material for a cold storage experiment that supports active conservation efforts, also in order to document a reliable, repeatable propagation method that can be used to create banks of plant and propagule material for conservation, restoration, research, and storage. While MIGE has been grown ex situ under various methods at Colorado State University periodically since 1997, a document that recommends methods to achieve specific plant product goals has not been published. The basis of knowledge used to guide cold storage of multiplied propagules was built on viability assays, measurements of bulbil moisture content, and storage temperature response (Chapter 3).

Reproduction in MIGE appears to be nearly exclusively from vegetative propagules, classified as brood bulbils. These grow at the base of the plant's leaves, enclosed in saccate petioles which act as a kind of seed coat. Bulbils can be thought of as analogous to the seeds of an annual plant (Beardsley, 1997), and are treated as such for purposes of propagation. The plant uses a form of asexual reproduction that resembles somatic embryogenesis but is actually organogenesis, with the formation of a completely formed plantlet arising from the leaf and stem. This unique petiole enclosed propagule has not been found in any other species.

Consideration of life history and ecology of extant populations is an important aspect of a conservation directed native plant propagation protocol. Specific in situ climate or habitat conditions of the parent material can help guide the selection of light, moisture, and temperature parameters to initiate growth and maximize development, or to determine what if any pre-treatment is needed to break a dormant period. Inferences into extant population characteristics or risk factors may emerge from observations in characteristics of ex situ propagated plants.

The methods described here, based on previously used methods (Steingraeber, personal communication 2015) combined with the author's experience in native plant propagation, are intended for the production of robust plants capable of producing on average 1000 or more bulbils per plant. These methods are not intended to simulate natural growing conditions in the wild, however, options for growth at higher density and under alternative light regimes that may more closely resemble natural growth conditions are discussed.

2.2 Methods and Materials

Populations of greenhouse propagated plants were grown from August 2015 field collections of bulbils from native populations Guanella Pass (GP), Hankins Gulch (HG), and Saint Vrain (SV) (Figure 2.1).The Guanella Pass native population is located in Pike National Forest near Georgetown, Colorado in Clear Creek County on the east side of the Continental Divide at an elevation of 3,390 m. The Hankins Gulch collection came from Pike National Forest in a valley between Lost Valley Park and Lake Park in Jefferson County at an elevation of 2,560 m. The Saint Vrain collection came from the Middle Fork area in Arapaho-Roosevelt National Forest in Boulder County at elevations ranging from 3,085 m to 3,127 m. Saint Vrain is the only extant group of these 3 native populations found on National Forests considered a stable metapopulation. The other 2 groups, GP and HG, are considered remnants unlikely to establish new patches or disperse new colonies (Beardsley, Steingraeber, 2013). Small subsamples remitted from these collections were intended for multiplication of propagules for study of storage parameters.

A population of 4[th] generation (4G) propagated plants were grown from a tray of live Elk Creek (EC) 3[rd] generation (3G) parent plants obtained in September, 2015 (Figure 2.2). Elk Creek was originally collected from its native habitat in Staunton State Park, Colorado in 2013, and underwent several generations of ex situ propagation for research purposes. Elk Creek plant material consisted of one flat of live plant material, numbering 20 individual plants at various stages of development from flowering to senescing. Bulbils from this 3G material were planted to produce 4G plants used in a cold storage study (Chapter 3).

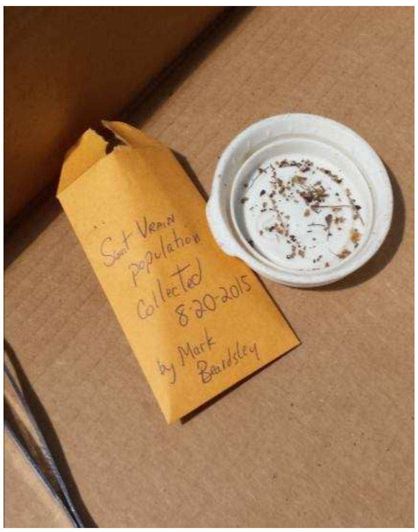


Figure 2.1. Field collection from native Saint Vrain population of *Mimulus gemmiparus.*

Figure 2.2. *Mimulus gemmparus* bulbils harvested from a tray of 3[rd] generation ex situ propagated Elk Creek population parent plants.

Bulbils were sown on September 1, 2015 at a density of 2 bulbils per 10 cm^2 in moistened Fafard 4P potting mix consisting of 45-55% Canadian Sphagnum peat moss, vermiculite, bark, dolomite lime, and a wetting agent (Figure 2.3). Fafard 4P soiless mix has negligible nutrient content measured in parts per million of 100 ppm N, 25 ppm P, and 100 ppm K. Bulbils were placed on the surface of the potting medium and sparsely covered with Fafard Super Fine Germinating Mix. For each of the 4 populations EC, GP, HG, and SV, 25 10 cm x 10 cm pots sown with 2 bulbils were arranged on plant trays in a checkerboard pattern with trays placed in 34 L mixing tubs for bottom watering (Figure 2.4). A second sowing using same methods was performed to achieve desired number of mature plants, ≥ 50 for each population, EC, GP, HG, and SV.

Plants were grown at CSU University Greenhouse on a south facing bench adjacent to a pad and fan cooling system (Figure 2.5). Temperatures were set at 17^o to 22^oC (62^o to 72^oF) day and 16^o to 21^oC (61^o-68^oF) night. Plants had 16 hours of light each day supplemented with high intensity sodium discharge lamps in the early morning and late afternoons. Photosynthetically active radiation (PAR) was measured at morning light of 290 – 320 watts per m^2, 600-700 watts per m^2 in the late afternoon.

Pots were bottom watered in the mixing tubs 2-3 times per week, and thoroughly drained between waterings to keep substrate consistently moist, but not permanently saturated. Watering needs of plants were closely monitored. For plants displaying loss of turgor, yellowing foliage, stunted growth, or other indications of failure to thrive, a layer of clean pea gravel was placed in the plant trays under individual pots to regulate water uptake.

After 8-12 weeks of growth and development, plants were hardened off in preparation for harvest by gradual reduction in watering, from 2-3 times per week to 1 time per week. Timing of full senescence was manipulated by maintaining water regime to extend plant life or allowing the plant to

Figure 2.3. Bulbils sown in Fafard 4P at density of 2 per $10cm^2$.

Figure 2.4. Pots arranged on tray in checkerboard pattern bottom watered in 34 Liter tubs.

Figure 2.5. MIGE (*Mimulus gemmiparus)*: 4 Populations, Elk Creek, Guanella Pass, Hankings Gulch, and Saint Vrain 1st and 4th generation ex situ propagated plants at Colorado State University Greenhouse, October 2015.

dry to encourage senescence. When desired harvest dates approached, and plants showed signs of senescence, with browning of vegetation and bulbils red to brown in color, watering was completely stopped. 1-2 weeks before harvest, unwatered, potted plants were placed upright in open paper bags to capture shattering bulbils.

At harvest date, plants were pulled from soil and assigned a unique identification number within each population. Shattering bulbils were separated from inert material and packaged in paper bags and envelopes labeled with population, plant identification number, and harvest date (Figure 2. 6).Bulbil sample weights of 100 were used as basis to estimate total number of bulbils produced per plant. Bulbils were stored at 20^{o}C and 38-42% RH, and utilized for moisture content, viability, and cold storage tests.

2.3 Results

50-60 mature 1G plants grew from GP, HG, and SV bulbil sowing and 125 mature 4G plants grew from EC sowing. Greenhouse emergence rates were 38-60% of sown bulbils. Plants on average from all populations grown in 4” pots produced > 1,000 bulbils. Highest rates of viability measured by germination test (Chapter 3) were EC 92%, GP 92.5%, HG 92% 5-13 days post harvest , and SV 94.5% 57 days post harvest (Table 2.1).

Transplanting was successful with >50% survival to maturity when performed on newly emerged plants. Minimal success was achieved with transplants of plants that had already developed multiple nodes, and long branching root systems that may have entangled with adjacent plant roots.

Most plants reached maturity in 8 to 10 weeks. Timing of senescence was manipulated by withholding or extending watering, with maximum life span extended to 12 weeks. Plants continued to grow, branch, and flower throughout life span until onset of senescence. Flowers are indeterminate emerging from axillary branches. Plants were large and profusely flowering with marked differences in morphology and growth habit between populations.

Foliar application of water damaged plants, causing leaf wilt and stunted growth. Plants left in dry substrate for > 5 continuous days during growth and development progressed to early senescence before full growth potential or underwent sudden mortality. Standing water in tubs and moist substrate invited pests, notably the fungus gnat. Consistently wet soil grew algae which can prevent soil aeration. Overwatering resulted in stunted plants that failed to thrive or underwent premature mortality, symptoms of damping off. There was no appreciable change in tendency for damping off or occurrence of pests between plants grown in Promix HP with fungicide compared to Fafard 4P when recommended watering and drainage procedures were followed. Promix HP had such high porosity that when potted

Table 2.1. The percentage of Mimulus gemmiparus (MIGE) sown bulbils that emerged and survived to harvest during greenhouse propagation. Viability determined by germination test of bulbils observed for 12 weeks in Elk Creek (EC), Guanella Pass (GP), Hankins Gulch (HG), and Saint Vrain (SV) populations of ex situ propagated plants EC, GP, and HG, 5-13 days post harvest and SV 57 days post harvest. The average number of bulbils produced per plant sampled from n=30 from each population, EC, GP, HG, SV. Average moisture content level of bulbils on a dry weight basis (MCDWB) in $gH_2Og^{-1}DW$ for EC, GP, HG, and SV at 5, 10, 13, and 57 days post harvest, respectively, n=10.

		EC	GP	HG	SV
Percent greenhouse emergence		54	42	38	60
Viability by germination test		92	92.5	92	94.5
MCDWB	mean	0.6618	0.644	0.5522	0.5204
	minimum	0.4706	0.5368	0.4988	0.4861
	SD	0.1634	0.1028	0.0522	0.0295

Figure 2.6. Fully senesced bulbils shattering from plant during harvest.

plants were bottom watered, water pooled at the top of the pots dislodging newly sown bulbils from the soil.

Plants grown by these methods displayed marked differences in morphology and growth habit between populations (Figure 2.7). HG grew tall and upright with clustered bulbils. SV had small, fine leaves and an ethereal appearance. EC flowered profusely and grew short and densely compact. GP displayed long internodes and a vinning habit. Variation was also observed between plants within the same populations and on the sub-individual level. Individual plants produced 300-2,500 bulbils averaging > 1,000 propagules per plant.

Figure 2.7. Populations ex situ propagated MIGE (*Mimulus gemmiparus*); Hankins Gulch (HG), Saint Vrain (SV), Elk Creek (EC), and Guanella Pass (GP) displaying differences in morphology and growth habit.

Results of storage research (Chapter 3) indicated fresh bulbils held for < 2 months before use can be stored at 20^{o}C, 38-42% RH for retention of excellent (>90%) viability. Reduction over time in moisture content of bulbils equilibrated to ambient storage conditions is correlated with reduction over time in viability. Bulbil moisture content levels below .2 g H20 g-1 DW showed poor viability (<50%). Moisture contents >.3 g H20 g-1 DW showed fair to good viability (50-75%). Bulbils stored for 3 months retained good (>75% viability) stored at 3^{o}-5^{o}C. Sub 0^{o}C storage temperature is not recommended without use of special protective procedures. Storage of bulbils or meristems in liquid N using cryoprotectants has not been investigated, but may be a promising avenue for long term preservation of germplasm.

2.4 Alternative Methods and Results

Numerous alternative potting methods were explored. Watering regime and drainage were essential elements of successful MIGE propagation in containers. Nested round black plastic pans, and clear rectangular tubs, both with drainage/uptake holes in bottom of the container holding potting medium and plant proved effective alternatives. Round plastic pans were used to start growth of bulbils intended for transplant into larger pots (Figure 2.8). Clear triangular tubs were lined with a layer of clean pea gravel under the potting medium and planted at a density of 4 bulbils per 10 cm^2 (Figure 2.9, 2.10).

An alternative potting mix, Promix HP, is a high porosity growing medium with a biofungicide and mycorrhizae, 65-75% sphagnum peat moss, perlite, limestone, and a wetting agent. Trials were grown in 100% Promix HP, 50/50 or 75/25 Fafard 4P/Promix HP.Potted and tub sown plants were grown at the CSU Horticulture Center on a southeast facing bench backed by a pad and fan cooling system, at temperatures of 18^{o}-24^{o}C with 16 hours of light supplemented by light emitting diode (LED) lamps. The red and blue light spectrum of LED lights can be manipulated to more closely approximate the long wave partial shade conditions of native populations.

Figure 2.8. Round pan to start plants for transplant.

Figure 2.9. Alternative container for high density planting.

Figure 2.10. Pea gravel for improved drainage.

2.5 Discussion

A preferred method was identified for > 1,000 per plant bulbil production with large, fully expressed plants displaying marked morphological and growth habit differences between populations. This method, described in a propagation protocol (Appendix IV) is useful for the production of propagules for field out planting to support restoration and assisted establishment efforts, and for establishment of a bank of material for continued propagation, storage, and research.

Alternative methods of propagation can be useful for specific research questions. Root and rhizosphere investigations might be best served using clear tubs planted at high densities, while alternative light regimes could be useful to investigate factors influencing flower production and root-shoot growth. Studies at Purdue University have investigated manipulation of red and blue light ratios and energy consumption and differences in root dry mass and growth patterns between plants grown under LED or high-pressure sodium lamps (Poulet et al., 2014, Gomez et al., 2013).

Watering is the key factor to achieve successful MIGE propagation, and must be monitored carefully. Entire crops can be lost from damping off mortality caused by overwatering. Foliar application of water is not recommended. Recommendations for application of water vary with light and temperature conditions in greenhouses on a daily and seasonal basis. Propagators can use condition of soil, which should remain consistently moist but not fully saturated for long periods of time, and condition of plants to gauge any needed adjustments in watering. 24-48 hour tub water soakings 2-3 times per week with replacement of water is recommended. Drain time following tub watering can range from at least 12 hours up to 2 days depending on temperature and PAR conditions of greenhouse. Overnight drainage is less likely to result in accidental over drying of plants.

The moist substrate needed for successful MIGE propagation invites damping off of plants and pests such as fungus gnats. Overwatering is the main cause of damping off, symptoms of which include stunted growth, loss of turgor, and premature mortality of young plants. Potted plants showing signs of overwatering should be more thoroughly drained between waterings. A layer of pea gravel placed between the outer bottom of pots and the plant tray that holds pots in watering tubs can facilitate better drainage.

Fungus gnats were an ever present pest during MIGE greenhouse propagation. Typical species *Orfelia, Bradysia*, and *Scaridae* are small delicate flying insects with segmented antennae. They remain close to soil, plants, or wet organic material. The white or clear, black headed larvae hatch in soil of greenhouse pots and feed on fungi and organic material. Fungus gnat larvae feeding on root hairs can potentially reduce crop success. These insects have a rapid life cycle of 17 days at 75^{o}F (Dreistadt, 2001). Potato slices placed on the surface of potting medium act as a trap for larvae and yellow sticky pest strips can reduce the number of adult flies.

No fertilizers or soil amendments were used, in accordance with previous propagation methods. No chemical pesticides were applied. The effect of pesticide application on MIGE is not known and therefore is not recommended for use. Greenhouse sanitation and non-chemical means of pest control were adequate to control pest infestations. Yellow sticky traps were used to reduce the number of adult fungus gnats. Potato slices placed in substrate then removed and disposed of outside of the greenhouse and replaced weekly were used to reduce the number of larval fungus gnats. Other methods used to reduce the incidence of pests and disease included frequent replacement of water in mixing tubs, removal of any stagnant water, and general greenhouse sanitation.

2.6 Conclusion

A preferred method was identified for MIGE propagation that will reliably produce > 1,000 per plant bulbil plant product with large, fully expressed plants displaying marked morphological and growth habit differences between populations. This method, described in a propagation protocol (Appendix IV), is useful for the production and multiplication of propagules for field out planting to support restoration and assisted establishment efforts, and for establishment of a bank of material for continued propagation, restoration, and research.

Propagation protocol templates from the Native Plant Network, University of Washington, Rocky Mountain National Park, and Glacier National Park were selected from as the framework for a MIGE propagation protocol. This protocol is intended for use by USDA Forest Service, or other approved rare native plant propagators. Propagators using the preferred methods described in this protocol will be able to reliably multiply hundreds of thousands of viable propagules from hundreds of original bulbils. A proven method for ex situ multiplication is essential for active conservation efforts such as assisted establishment. The propagules produced in this study were used to investigate storage behaviors to guide the next step in establishment of a strategic MIGE ex situ plant material management plan (Chapter 3).

In most cases where flowering has been reported in the wild, as few as 1 flowering individual in a location is common (Beatty et al., 2003). Flowering had not yet been observed in HG, in Staunton State Park (EC, Black Mountain Creek) as late as 2003 (Beatty et al., 2003). Greenhouse propagated plants are capable of producing prodigious flower displays and are generally larger, with more branching, greater numbers of propagules, and more full expression in growth habit than plants from extant, wild populations. Propagation provides an opportunity to observe plant characteristics that are difficult or impossible to observe in rarely occurring, climate and habitat limited extant populations. Apparent differences in morphology between repetitive parts, internodes, leaves, bulbils, and flowers than can be observed in controlled greenhouse environments may lead to better understanding of MIGE, and may suggest there are differences in genetic /phenotypic expression between various wild or ex situ propagated populations (Figures 2.11-2.14).

Figure 2.11. Elk Creek (EC) 4th generation (4G) ex situ propagated plants with profuse flowering.

Figure 2.12. Guanella Pass (GP) 1st generation (1G) ex situ propagated plants with long internodes.

Figure 2.13. Saint Vrain (SV) 1st generation (1G) ex situ propagated population with finespun leaves.

Figure 2.14. Hankins Gulch (HG) 1st generation (1G) ex situ propagated population with upright habit and clustered bulbils.

CHAPTER 3. STUDIES TO GUIDE STORAGE OF *MIMULUS GEMMIPARUS* BULBILS

3.1 Introduction

Mimulus gemmiparus W.A. Weber, henceforward referred to as MIGE, is one of the rarest Colorado endemic plant species of conservation concern (SCC). SCC is a U.S. Forest Service specific term applied to species for which there is evidence or concern regarding the ability for continued long term existence within a landscape[1]. The Colorado Natural Heritage Program (CNHP), which tracks rare and at risk species and habitats in Colorado rates MIGE G1/S1, classifications that indicate a plant is critically imperiled and vulnerable to extinction (CNHP, 1997+, CO Rare Plant Guide, www.cnhp.colostate.edu. Latest update: November, 2015). Functionally a vegetative annual reproducing by bulbils, less commonly referred to as gemmae (Weber, 1972, Moody et al., 1999, Beardsley, 1997, Olmstead, 2001), MIGE rarely if ever reproduces sexually from flowers with viable seed. It has an annually reproducing clonal life strategy involving vegetative reproduction by unique petiole encased bulbils. MIGE has traditionally been classified as a member of the *Scrophulariaceae* family. A revised taxonomic classification based on molecular-phylogenetic and morpho-taxonomic studies would place MIGE in the *Phyrmaceae* family in the genus *Erythranthe* from a Clade with American-east Asian lineage. This framework describes new conscriptions and combinations affecting the genus *Erythranthe* (Barker et al., 2012). The phylogenetic position of MIGE is still debated. There is currently no evidence to indicate the otherwise undocumented petiole encased bulbils are a remnant of a previous, more widespread form of reproduction. This structure and strategy could be a novel development. Conservation of this species would enable further study that could illuminate incompletely understood pathways of evolution.

[1]www.fs.usda.gov/Internet/FSE_DOCUMENTS/stelprd3848211.pdf

MIGE demographics may put individual patches of populations, rarely larger than 1 m^2, at an elevated risk of loss due to stochastic events (Beardsley and Steingraeber, 2013). Active measures such as assisted establishment, the introduction of new plant patches within historic range and close proximity to existing patches, may mitigate an elevated risk of loss (Beardsley, 2014). Ex situ greenhouse propagation of MIGE plants and propagules can support assisted establishment and other active conservation efforts. General horticultural practices for propagation of seeds have been successfully applied to MIGE bulbils. While plants in the wild rarely produce > 100 propagules per plant (Moody et al., 1999, Beatty et al., 2003), greenhouse grown plants manipulated to allocate resources to growth structures from which bulbils are derived have high fecundity, with bulbil production of > 2,000 bulbils per plant (Chu, 2016). Storage of propagules as an element of an ex situ plant management plan allows for propagation of multiple crops, scheduling of plant growth to accommodate project schedules, and a layer of protection to mitigate potential losses of native, annually reproducing patches . There is a lack of published information regarding MIGE bulbil longevity or optimal storage conditions to retain viability and maximize length of storage.

Vegetative, asexually produced bulbils are not true seed embryos, but fully formed plantlets (Beardsley, 1997), that multiply from modular units similar to detachable branches with adventitious roots (Steingraeber). The storage characteristics of true seeds and general rules of thumb for storage and longevity were used as starting points for investigation of MIGE bulbil storage, as bulbil morphology and function appear analogous to that of dichotomous seeds (Beardsley, 1997). In general, lower temperatures slow metabolic activity, which in turn reduces production of harmful products of respiration, and increases longevity. Interactions between temperature, moisture, propagule desiccation tolerance, and different phases of water determine the ideal storage conditions to maximize viability over the longest time frame.

For many seeds lower temperature storage prolongs viability at some critical moisture content level. Finding low moisture content thresholds of propagules can indicate desiccation tolerance and suggest traits of storage behavior (Walters, 2015).High viscosity in drying systems with increased hydrogen bonding slows the rate of metabolic reactions allowing a change in state called the glass transition (T_g). Increased viscosity in a viable seed may slow deterioration associated with the loss of cellular matrix integrity and cellular function during desiccation (Walters and Koster, 2007). Seeds that remain viable at low moisture contents can be stored at freezing temperatures and retain viability for decades or longer. Seeds with higher low moisture content thresholds are more fluid systems with less stable cellular matrices that are prone to damage from intercellular ice formation at freezing temperatures. Differences in desiccation tolerance between populations could evidence potential for differences in response in native populations to changes in microclimate and habitat.

Characteristics such as empirically observed low moisture content thresholds and tolerance to cold storage temperatures can be used to guide determination of optimal MIGE bulbil storage parameters using orthodox, recalcitrant, and intermediate seed storage characteristics as a framework. The terms for orthodox and recalcitrant seeds that describe storage characteristics were coined by E.H Roberts in 1973. Orthodox seeds, which include many important crop species such as corn and peas and many economically important northern temperate tree species, are characterized by low moisture content thresholds < .10 gH_20g^{-1}DW and can be stored at -20^oC retaining viability for decades. Recalcitrant seeds have low moisture content levels > .2 gH_20g^{-1}DW, do not generally retain viability in cold storage depending on chilling sensitivity of individual species, and display longevity generally < 1 year (Bonner, 2008). Intermediate seeds are a more recently described category with storage characteristics intermediate between those of orthodox and recalcitrant seeds. Intermediate seeds such as coffee may retain viability for 2-5 years stored at 3^o-5^{o}C (Ellis, 1990). Additional storage behavior categories sub-orthodox, temperate recalcitrant, tropical recalcitrant, and intermediate are now also recognized.

Propagation and storage can be guided by knowledge of a plant's native habitat and natural history. Understanding of local and microhabitat scale climate conditions of extant populations along with measures of individual growth and patch demographics can help define baseline growth responses to measureable moisture, light, and temperature regimes. The natural dispersal unit is the petiole enclosed bulbil, which abscises from the stem with leaf or leaf remnant attached, or more rarely "free bulbils" that lack a coat of dried petiole (Beardsley, 1997). In extant wild populations propagules shatter from parent plants in late summer to fall, July to October and survive cold, potentially dry conditions at 2,560 m to 3,390 m for 6 to 9 months or longer before germinating in the spring (Beatty et al., 2003, personal observation East Inlet Trail population, 2016). Populations of MIGE inhabit varying elevational ranges, in different water basins, and on opposite sides of the Continental Divide.

Temperature and relative humidity are covarying factors to consider when determining optimal storage conditions. Moisture content of seeds and propagules will change as they equilibrate to the RH of a storage environment. Seeds equilibrated for several weeks at 5^{o}C and 24% RH result in moisture content levels of 5-10%, ideal moisture content for storage of orthodox seeds, but lethal levels of desiccation for recalcitrant seeds. In some cases, RH of storage environment may be a better parameter to use for storage conditions than moisture content of propagules. At high moisture contents, there can be significant decreases in moisture content values without inducement of water stress. Moisture contents at which processes such as respiration occur vary among species and correlate with lipid contents, however changes in physiological activities and phases of water occur at specific RH levels common across species (Vertucci, 1990). Properties of water change discretely with RH levels and physical phases of water occur at specific RH levels (Leopold, Vertucci, 1989). It is more common and less expensive to control the temperature of a storage environment than the RH. Propagules at a given moisture content level can be held in air proof packaging to prevent equilibration with ambient air (Bonner, 2008).

The questions addressed in this empirical study were whether or not different treatment levels of temperature factors -20°, -3° to 0°, or 3 ° to 5°C with confounding variable of relative humidity RH controlled resulted in statistically significant differences in post exposure viability, whether bulbil storage behavior was more akin to that of recalcitrant, intermediate, or orthodox seeds, and whether there were any differences in cold exposure or desiccation tolerance between populations. The objective of storage studies was to identify preferred moisture content and temperature conditions to maximize viability and longevity of stored bulbils. This experiment expected to find significant differences in post exposure viability between freezing and non-freezing storage temperatures in high moisture content bulbils, but fewer significant differences in post exposure viability between freezing and non-freezing storage temperature in low moisture content bulbils, and could reveal significant differences in low moisture content thresholds and post exposure viability between populations. The low moisture content threshold was defined as the mean moisture content on a dry weight basis (MCDWB, $gH_2Og^{-1}DW$) where≤ 10% germination wasobserved over 12 weeks. Low moisture content bulbils were difined as those with mean MCDWB < 0.2 gH2Og-1DW High moisture content bulbils were defined as those with mean MCDWB > 0.2 gH20g-1DW.

H_o: low moisture content threshold populations 1,2,3,4; $\mu_1=\mu_2=\mu_3=\mu_4$

H_a: low moisture content threshold of populations not all equal

H_o: post treatment Temp1, Temp2, Temp3, Control; germination $\mu_1=\mu_2=\mu_3=\mu_4$, at 4 time intervals

H_a: post treatment Temp1, Temp2, Temp3, Control; germination means not all equal, at 4 time intervals

H_o: low moisture content bulbils post treatment Temp1, Temp2, Temp3, Control; germination

$\mu_1=\mu_2=\mu_3=\mu_4$

H_a: low moisture content bulbils post treatment Temp1, Temp2, Temp3, Control; germination means not all equal

H_o: high moisture bulbils post treatment Temp1, Temp2, Temp3, Control; germination $\mu_1=\mu_2=\mu_3=\mu_4$

H_a: high moisture content bulbils post treatment viability Temp1, Temp2, Temp3, Control; germination means not all equal

3.2 Literature Review

3.2.1 In Situ Populations, Rarity, and Population Demographics

MIGE was first described in 1972 (Weber, 1972). At least eight geographically distinct populations defined as patches of plants within 30 m of each other are known to exist. These populations are in separate water basins, divided by major highways, occurring at various elevations from montane to upper subalpine, and on opposite sides of the Continental Divide. As of early 2016, eight populations had been documented as Black Mountain Creek (BM) and North Elk Creek (EC) from Staunton State Park, CO at approximately 3,000 m in elevation; Hankins Gulch Population (HG) at 2,560 m; Guanella Pass Population (GP) at 3,390 m from Pike National Forest**;** Saint Vrain (SV) from Arapaho National Forest at 3,085-3,127 m; and populations in Rocky Mountain National Park that occur between approximately 2,400 and 2,700 m in elevation. These include a Fall River Road population which is assumed extinct and a Horseshoe Park Alluvial Fan population with questionable status due to a lack of confirmed locations during recent surveys on the east side of the Continental Divide, and an East Inlet population negatively impacted by trail work and North Inlet population on the west side of the Continental Divide (Beatty et al., 2003).

HG in situ habitat was described by M. Beasley in 1997. He described plants growing in several patches under an overhang between the base of a south facing granite cliff and a trail in an aspen conifer upper montane forest approximately 2 km west of the Hankins Gulch Trailhead. Until new GP plants were discovered in 2016, HG was the largest known grouping of plants, occupying an area approximately 15 m^2. From 2005 to 2015 survey estimates of plant numbers in the 3 patches of the HG population declined from over 102, 000 to 16,750 while estimated number of propagules fell below 500,000 in 2015 (Beardsley, 2015).

The Saint Vrain population occurs at 3,085-3,127 m in Arapaho National Forest southeast of Allenspark, CO near Pleasant Valley. It consists of many small patches that grow along a steep tiered rock face. Dispersal of propagules appears to be vertical descending possibly with flowing water as a dispersal mechanism into available crags that serve as suitable microhabitats. This mechanism is not apparent in the other populations inhabiting different conditions. SV appears to support stable metapopulation dynamics (Beardsley and Steingraeber, 2013).

Distribution of MIGE is discontinuous on a regional scale, but densely clustered on a local scale (Beatty et al., 2003). Extant MIGE populations are spatially discrete patches within 30 m of one another grouped into 1 or more subpopulations (Beardsley, 2015). The patch as a demographic unit is a tightly associated group of clonal plants that behave on the level of an individual plant (Beardsley and Steingraeber, 2013). Patches can be dense with > 100 plants/dm^2 in the wild and grown at high density in greenhouse conditions can be made to form dense mat like clumps of tiny plants.

A narrow Colorado endemic, MIGE is a USFS species of concern and a plant recognized as critically imperiled and vulnerable to extinction (CNHP, 1997+, CO Rare Plant Guide, www.cnhp.colostate.edu. Latest update: November, 2015). The difficulty inherent in the monitoring of a small inconspicuous plant with an apparently ephemeral nature may contribute to an incomplete accounting of all occurrences at a given time. New patches of GP and SV plants were found in the latter half of 2016 that may alter future descriptions of rarity, populations, and population dynamics.

3.2.2 Reproduction and Life Strategy

The bulbils of MIGE are the main mode of reproduction. Sexual reproduction has never been observed in the wild, and has been observed only once in ex situ propagation (Beardsley, 1997). Theories for the loss of sexual reproduction in clonal plants consider ecological factors evidenced by reduced seed production in clonal plants near geographic range limits (Erickson, 1996) or possible genetic factors such as a change in ploidy as seen in triploids and other sterile polyploids found in aquatic clonal plants that could impair meiosis (Les and Philbrick 1993, Preston and Croft 1997). Polyploids have been found distributed at higher latitudes and altitudes than closely related diploids (Bierzychudek, 1987) and it has been suggested that greater tolerance of extreme environments can be associated with an increase in chromosome number (Levin, 1983).

MIGE propagules are classified as a unique form of brood bulbil encased in saccate petioles (Beardsley, 1997, Moody et al., 1999). Propagules shatter from typically 10 -20 cm tall parent plants with dispersal distances of only centimeters unless a vector such as water, wind, or animal moves the bulbil farther. MIGE has strict micro habitat requirements. The habitat parameters site size, overhang protection, cliff association, aspect, hydrology and micro-topography, substrate type, moss/herb presence, mean light intensity, direct sun frequency, mean temperature, high temperature frequency,and soil depth have been identified for EC and BM MIGE populations (Beardsley, 2014). It is unknown how changes in climate might affect the water seeps that help create MIGE micro habitats or how depletion of ground water and changes in hydrology could affect the frequency and duration of suitably moist micro habitats. If bulbils have limited dispersion potential, migration path distances between suitable microhabitats may prevent new establishment and migration.

Bulbils are analogous to seeds with the enclosing petiole serving as a thin seed coat, and two thickened semi hypogeal leaves that act like the storage leaves of cotyledonous seeds, and homologous to vegetative shoots (Beardsley, 1997),similar to the adventitious roots that emerge from Salix sp. Thin seed coats are found in some seeds that do not tolerate cold storage temperatures as readily as other seeds within the same category of seed behavior (Bonner, 2008).

3.2.3 Seed Storage

Seed storage characteristics can be classified along a spectrum of storage behaviors related to temperature exposure and desiccation responses as orthodox, sub orthodox, temperate recalcitrant, tropical recalcitrant, and intermediate (Roberts, 1973, Bonner, 1990, Ellis and Hong, 1990). In general, dryer seeds can be stored at colder temperatures, thus remain viable longer. Attempts to create general rules of thumb for storage of seeds have been proposed such as that for each 1% reduction in moisture content of propagules, or for each 5.6^{o}C decrease in storage temperature seed longevity is doubled (Harrington, 1972, Young and Young, 1986). These general guidelines can often be applied to orthodox seeds, but may not be applicable to recalcitrant and intermediate seeds.

Orthodox seeds include pioneering tropical and subtropical species, annual and biennial crops, many horticultural species, and temperate fruit and forest trees and shrubs (Walters and Towill, 2004). Grains, pulse crops, and vegetable seeds from corn, onion, pea, and tomato exhibit orthodox seed behavior. Many economically important northern temperate tree species such as *Abies, Alnus, Betula, Fraxinus, Larix, Picea, Pinus, Platanus, Prunus,* and *Pseudotsuga* are classified as having orthodox seeds, as are the seeds of valuable tropic and subtropic genera such as *Acacia, Eucalyptus,* mesquite, teak, and many *Fabaceae* (Bonner, 2008).

Orthodox seeds exhibit increased longevity with increased desiccation up to a threshold water content level and can survive desiccation to levels below 0.1 $gH_2Og^{-1}DW$. The moisture content threshold at which point longevity no longer increases or in fact decreases in orthodox seeds has been found between 0.03 and 0.07 $gH_2Og^{-1}DW$ (Vertucci and Leopold, 1987, Ellis et al., 1989, Vertucci and Roos, 1990, Walters, 1998, Ellis and Hong 2006). Dry orthodox seeds can be stored at subfreezing temperatures and remain viable for decades. The maximum longevity of orthodox seeds is unknown, although a Judean date palm seed found in a dry crypt and estimated to be 2,000 years old was able to germinate and grow into a mature fruit producing tree. Sub orthodox seeds have reduced longevity, usually less than 10 years. There is indirect evidence that high lipid contents in sub orthodox genera such as *Juglans, Fagus,* and *Carya* and thin seed coats in sub orthodox genera *Salix, Populus,* and *Acer* could play a role in this reduction of longevity (Bonner, 1990).

Recalcitrant seeds are found in herbacious hydrophytes, tropical perennials, some temperate deciduous trees, non tropical hardwoods and climax species such as *Aesculus hippocastanum, Quercus, Castenae, Camellia sinensis, Zizania palustris*, wildrice, avocado, mango, and cocoa (Bonner, 2008, Walters and Towhill, 2004). They exhibit low water thresholds at which point longevity is no longer extended at moisture contents > 0.2 g H_2O g^{-1} DW, and generally retain longevity for less than I year. Storage temperature for recalcitrant seeds depends on chilling sensitivity. Tropical recalcitrant seeds such as seen in mango, avocado, and cocoa exhibit high chilling sensitivity and must be stored above 15^oC and may remain viable for only 2 weeks to 3 months. Temperate species such as buckeye stored at 2^o-5^oC can remain viable for 6 months to 2 years.

Intermediate seeds are found in tropical and subtropical perennials such as coffee, papaya, and citrus, and some nut trees such as hickory. Intermediate seeds exhibit storage behaviors intermediate between those of orthodox and recalcitrant seeds. Maximum longevity attained under storage at 40-60% RH or 0.10 to 0.13 gH2O g -1 DW seed moisture content has been observed as 1-6 months survival at 25^oC, and 2-5 years at 5^oC (Walters and Towill, 2004).

Percent germination can be used as a metric for desiccation tolerance (Koster and Leopold, 1988). Critical water contents can be defined as the water content below which longevity cannot be prolonged (Ellis et al., Vertucci and Leopold, 1986). Results of germination tests plotted against seed moisture content levels that indicate desiccation tolerance can help determine storage guidelines by revealing characteristics common to orthodox, recalcitrant, or intermediate seeds. If seeds germinate at a high percentage when desiccated below 0.1 $gH_2Og^{-1}DW$ or approximately 5% on a fresh weight basis, they are exhibiting orthodox behavior. If seeds remain viable when desiccated to levels roughly between 0.1 - 0.2 $gH_2Og^{-1}DW$ or 10 – 12.5% on a fresh weight basis, they are closer to the spectrum of intermediate storage behavior. Seeds that lose viability when desiccated below 0.3 $gH_2Og^{-1}DW$ or 15-20% on a fresh weight basis exhibit the desiccation tolerance characteristics of recalcitrant seeds (Hong and Ellis, 1996).

Storage behavior cannot be concluded exclusively from desiccation tolerance. Exposure to storage temperatures is the next step for an empirical exploration of ideal storage conditions. Survival curves of seeds stored under controlled conditions are sigmoidal, conforming to a negative cumulative normal distribution (Ellis, 1991). Recalcitrant seeds originating from temperate climes can be stored at colder temperatures and remain viable for longer than those originating from tropical environments, and greater difficulty in lab manipulation of tropical recalcitrant seeds compared to temperate origin recalcitrant seeds has been found (Bonner, 2009, Walter and Koster, 2007). Commonly practiced storage temperature protocols call for -1^oC or 3^o to 5^oC. The bulbs of *Allium sativum* which typically deteriorate 6 months after harvest, retained qualities of firmness and taste when stored 9 months at -3^oC (Volke and Rotindo, 2004). Conventional storage practiced for a wide variety of agricultural and horticultural crops calls for -20^oC.

3.2.4 Temperature Water Interactions

The mechanisms behind storage behavior involve complex interactions between water, temperature, phase changes, and desiccation tolerance of propagules. Properties of water change discreetly with moisture content of proteins and physical phases of water change at specific RH values. (Leopold, Vertucci, 1989). Moisture sorption isotherms determined by experimental test graph the relationship between moisture content and equilibrium RH. For each value of RH there is a measureable corresponding water content at a specific constant temperature. For example, at 20^oC and 40-50% RH seeds reach equilibrium moisture contents between 1-2 $gH_2Og^{-1}DW$ (Hong and Ellis, 1996). Equilibration of seed moisture content at 24 percent RH and 5^oC for a minimum duration of 2 weeks results in 5-10% moisture contents across a wide variety of seeds (FOA, 2014).

Common ice molecules arranged in a hexagonal lattice pattern form ice crystals. Intercellular ice formation that may occur during subfreezing storage can damage propagules. Very low temperatures or high pressure can cause a phase change in water called a glass transition (T_g) that ice lacks this long-range order in molecular arrangement,and is less damaging to tissues. As material desicates, changes in volume occur, creating the pressure that can lead to this change in the state of water. Cytoplasm enters T_g at moisture content levels below 0.1 gH_2Og^1DW, so propagules that can survive this level of desiccation can be stored at freezing temperatures while avoiding intercellular ice formation (Walters and Koster, 2007).

3.2.5 Previous Studies and Inferences into Native Populations

A critical lethal temperature was found between -25^o and -30^oC for non desiccated, non acclimated ex situ propagated Hankins Gulch (HG) population MIGE bulbils at room temperature cooled in a methanol bath at a rate of temperature change of -10^oC per hour. The ex situ propagated HG population MIGE bulbils in enclosing petioles had moisture contents from 0.3887 $gH_20g^{-1}FW$ to 0.5781

$gH_20g^{-1}FW$ (grams of water per gram of fresh weight), equivalent to approximately 0.5 to 1.3 $gH_20g^{-1}DW$ (grams of water per gram of dry weight) desiccated 15-25% over 35 days germinated over 8 weeks at a mean of 18%. It was concluded that these bulbils were tolerant of this minimal level of desiccation (Beardsley, 1997).

Inferences about native population life strategies can be made from observations of ex situ bulbil storage behavior. Plants grown ex situ display a wide range of variation in characteristics and growth habits. Phenotypic expression within a clonal plant can be expressed on the subindividual level by modular units. Plants that display a greater degree of phenotypic plasticity may be better adapted to resist changes in climate (Richer, 2012). *Pinus sylvestris* seedlings from a variable environment in the Central Alps were compared to *P. sylvestris* seedlings originating from a temperate, less variable Mediterranean environment. When each was tested for response to drought, the *P. sylvestris* from the highly variable environment responded with greater root biomass plasticity than those from the less variable environment (Richter, 2012). Greater sensitivity to desiccation would be expected in species from nearly constant environments (Jenks and Wood, 2007). Phenology and morphology of extant MIGE plants vary in different spatial patches and presumably as a response to different environmental conditions (Beardsley, 1997).

MIGE bulbils originating from high elevation Guanella Pass plants are subject to greater extremes in temperature than those from lower elevation, such as Hankins Gulch and Elk Creek. Temperature and light in lumens were recorded by a previous researcher in 2012-2013 at Staunton State Park, elevation roughly 3,000 m, and habitat for 2 MIGE populations, Elk Creek and Black Mountain. Measurements were recorded every 30 minutes in 24 hour intervals on a daily basis for 11 months from August 2012 until June 2013. Mean temperature over that period was 13.11^{o} C. For the four months with average temperatures below freezing, December through March, the lowest temperature was -16.9°C. (Beardsley, M., shared data, 2013). Temperatures at elevations over 3,000 m where Guanella Pass plants grow can reach -30°C and remain below freezing for 6 months of the year.

3.3 Methods and Materials

Populations of greenhouse propagated plants were grown from August 2015 field collections of bulbils from native populations Guanella Pass (GP), Hankins Gulch (HG), and Saint Vrain (SV). A population of 4th generation (4G) propagated plants were grown from a tray of live Elk Creek (EC) 3rd generation (3G) parent plants obtained in September, 2015. Elk Creek plant material consisted of one flat of live plant material, numbering 20 individual plants at various stages of development from flowering to senescing. Bulbils were sown on September 1, 2015 at a density of 2 bulbils per 10 cm^2 in moistened Fafard 4P potting mix.

Plants were grown at CSU University Greenhouse on a south facing bench adjacent to a pad and fan cooling system (Figure 2.5). Temperatures were set at 17°-22°C day and 16°-21°C night. Plants had 16 hours of light each day supplemented with high intensity sodium discharge lamps in the early morning and late afternoons. Photosynthetically active radiation (PAR) was measured at morning light of 290 – 320 watts per m^2, 600-700 watts per m^2 in the late afternoon. Pots were bottom watered in the mixing tubs 2-3 times per week.

After 8-12 weeks of growth and development, plants were hardened off in preparation for harvest by gradual reduction in watering, from 2-3 times per week to 1 time per week. One to two weeks before harvest when plants showed signs of senescence with browning of vegetation and bulbils red to brown in color, watering was completely stopped. Unwatered, potted plants were placed upright in open paper bags to capture shattering bulbils.

At harvest, plants were pulled from soil and assigned a unique identification number within each population. Bulbils were separated from inert material and packaged in paper bags and envelopes labeled with population, plant identification number, and harvest date. 100 bulbil sample weights were used as basis to estimate total number of bulbils produced per plant. Bulbils were stored at 20C and 38-42% RH, and utilized for moisture content, viability, and cold storage tests.

Viability by germination test was determined for samples 5 to 13 days post harvest, pre and post cold storage experiment exposure, and over time as bulbils dried at ambient conditions, 20C and 38-42%RH at Days Post Harvest (DPH) time intervals 1: 0 to 30 DPH, 2: 60-90 DPH, 3: 120-180 DPH, and 4: 270+ DPH . 50 petiole encased bulbils from 8 to 30 uniquely numbered plants within each of the 4 populations, EC, GP, HG, and SV were placed on moistened germination blotter paper covered with moistened translucent paper towel, and labeled with plant ID, exposure temperature level and time interval. Prepared samples were placed in clear plastic cups with lids in a growth chamber set at $20^{o}/25^{o}C$ with 16 hours of light from fluorescent and incandescent bulbs (Moody et al., 1999). Viability was determined by root and shoot emergence and elongation (Beardsley, 1997). The number of newly emergent bulbils was read weekly for 12 weeks to determine mean percentage germination.

Samples of EC and GP were also tested for viability by Tetrazolium chloride staining. Solutions made from concentrated 2,3,5 triphenyl tetrazolium chloride (TTC), with buffering compounds KH_2PO_4 and Na_2HPO_4 , or the test itself, is commonly referred to as TZ. Testing was performed at the National Center for Genetic Resources Preservation (NCGRP) and the Colorado Seed Lab (CSL) in Fort Collins, Colorado. Petiole enclosed bulbils were placed on moistened blotter paper to imbibe water for 12 to 24 hours, then placed in a glass beaker containing 1% 2,3,5 triphenal tetrazolium chloride. Bulbils in TZ solution were heated in an oven at $30^{o}C$ for 24 hours, then removed for examination. Enclosing petioles were removed and bulbils were cut longitudinally. Presence of red to brownish red staining of the apical meristem, shoot, and root axes were read as positive for viability. Absence of staining was read as non-viable (Figures 3.1., 3.2).

Moisture contents of bulbils were determined using 120 bulbil weight samples measured on an O'haus Adventurer Pro and a Mettler AT201 analytical scale from plants randomly selected using random number generating functions in Excel. Samples of petiole encased bulbils harvested from individual, uniquely numbered plants within each population were randomly selected by halving method; all remaining harvested bulbils from a single plant piled and halved repeatedly then sample drawn from front of pile (AOSA, 2012). Bulbils were rejected only if signs of insect or mechanical damage were present. Bulbils with remnant leaves removed were weighed in 0.0000 g ± 0.0003g, dried at 103^{o}C for a minimum of 24 hours, and reweighed to obtain dry weight basis moisture content levels, (MCDWB), Fresh Weight (FW) –Dry Weight (DW) /DW, in $gH_20g^{-1}DW$ (0,∞). Replicates for moisture content tests ranged from n=10 to n=30 for each population with tests performed at DPH time intervals 1: 0 to 30 DPH, 2: 60-90 DPH, 3: 120-180 DPH, and 4:270+ DPH. Moisture contents were recorded at the start of select viability tests described above, and over time as summary statistics.

A cold storage experiment used a randomized block design with 10 replicates from each of the 4 populations EC, GP, HG, and SV ex situ propagated *Mimulus gemmiparus* plants. Populations were greenhouse propagated plants, N=50 for GP, HG, SV, and N=125 EC (Fig 3.3.). The replicate and block were the individual harvested plant selected with random number generator functions in Excel and SAS. The experimental unit is the plant with >1,000 bulbil fecundity. The measurement units were multiple random collections of 50 or 120 bulbils from each plant. The plant was measured at the level of 50 bulbils for germination test concurrent with a 120 bulbil moisture content test at start of experiment, and at level of 50 bulbil germination test alone for measurement of post exposure viability response. Control storage conditions were 20^{o}C with RH controlled from material equilibrated at 20^{o}C

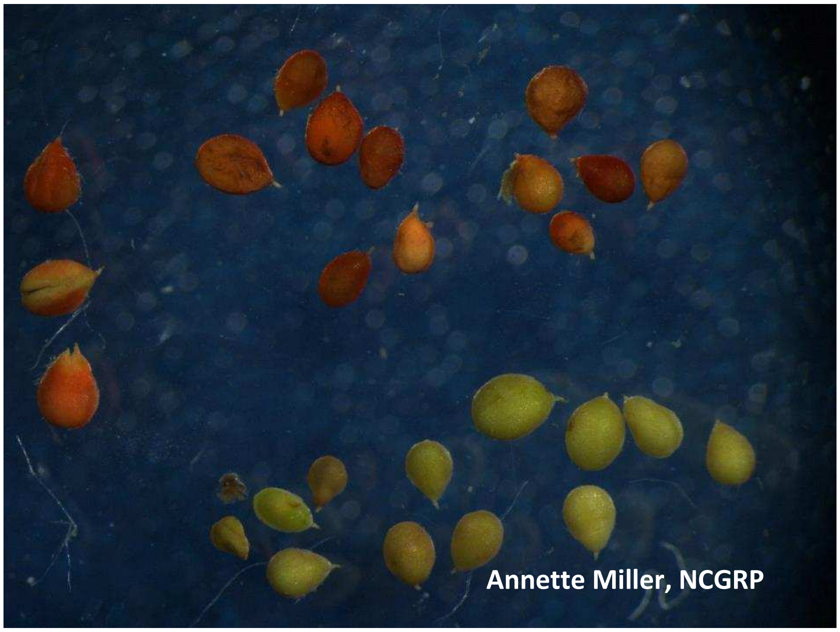


Figure 3.1. Viable red stained and non viable green Elk Creek population *Mimulus gemmiparus* bulbils with enclosing petioles removed.

Figure 3.2. Longitudinal bisection of *Mimulus gemmiparus* bulbil with Tetrazolium chloride (T*Z)* staining of shoot axis and apical meristem.

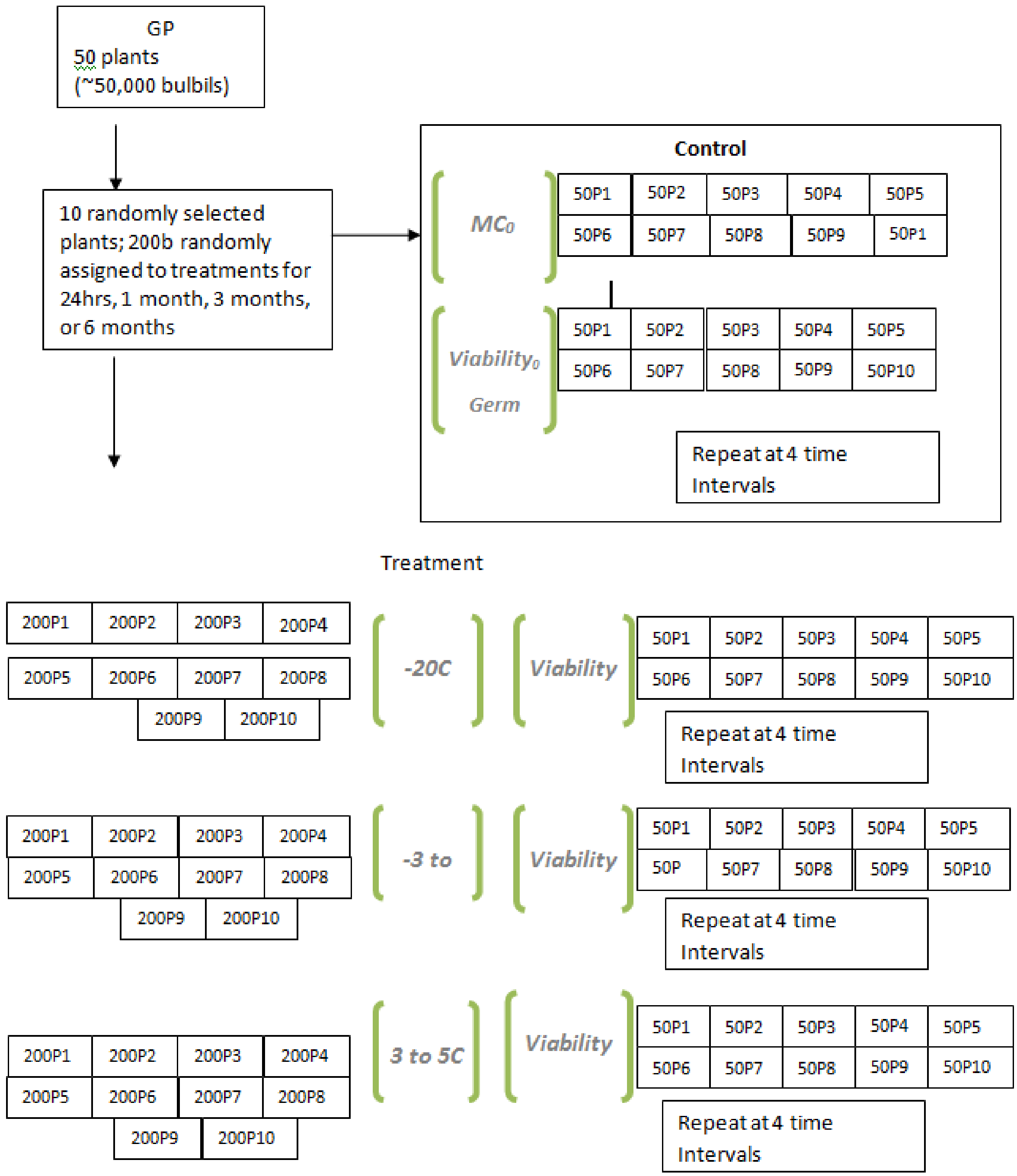


Figure 3.3. Cold storage experiment: randomized block design with 10 replicates of population Guanella Pass (GP) ex situ propagated *Mimulus gemmiparus* plants. The replicate and block were the individual randomly selected harvested plant. Moisture content of 120 bulbil sample weights were measured from each of 10 plants at initiation of experiment. 50 bulbil samples were removed at 24 hours, 1 month, 3 months, and 6 months exposure for post exposure viability germination tests.

and 38-42%RH. Bulbils were double wrapped in foil to prevent ambient air exposure and equilibration, then submitted to 1 of 3 cold storage temperatures -20^{o},-3^{o} to 0^{o}, or 3^{o} to 5^{o}C with 4 duration levels, 24 hours, 1 month, 3 month, and 6 month exposure. Each of the 4 populations received 12 treatments. The response variable was post exposure viability determined by 12 week germination test in a growth chamber at 20^{o}/25^{o}C with 16 hours of incandescent and fluorescent lighting. 6-month treatments were tested for viability by side by side germination test and TZ staining.

SAS and linear mixed modeling were used to analyze cold storage experiment data to compare traits of low moisture content thresholds, and post cold exposure viability between populations. Univariate procedures in SAS created box plots and tests for normality for moisture content results. T-tests in SAS generated results for statistically significant differences in germination rates and post exposure viability.

3.4 Results and Discussion

Timing of emergence in greenhouse propagated plants was variable between 5 and 84 days. The highest measures of viability determined by germination test observed over 12 weeks from EC, GP, and HG populations were found in freshly harvested bulbils tested 5-13 days post harvest which germinated at 92%, 96%, and 93%, respectively (Figure 3.4). The highest percentage germination for the SV population was found 57 days post harvest at 95%. (Table 3.1). There were no significant differences in emergence of propagules at fresh harvest between GP and HG when germination was observed over 12 weeks, however, at 8 weeks of observation, GP had a significantly higher percentage of germinated bulbils than HG ($p<.0002$) (Figure 3.5).

Table 3.1. Mean percent germination of bulbils observed from 4 ex situ propagated *Mimulus gemmiparus* populations, Saint Vrain (SV), Guanella Pass (GP), Hankins Gulch (HG) and Elk Creek (EC). Germination determined over 12 weeks of observation under 20/25C and 16 hours of light growth chamber conditions with bulbils placed on germination blotter paper tested 5 to 57 days post harvest.

Population	n=	Days Post Harvest	Range MCDWB	Mean MCDWB	Mean %Germ
EC	10	5	0.4865 - 0.7619	0.6909	92
GP	8	13	0.4933 - 0.93611	0.70152	96
HG	8	10	0.4988 - 0.6234	0.552175	93
SV	8	41	0.5592 - 0.8397	0.6896	79
SV	9	48	0.3980 - 0.5887	0.5063	87
SV	7	57	0.4861 - 0.5709	0.520429	95

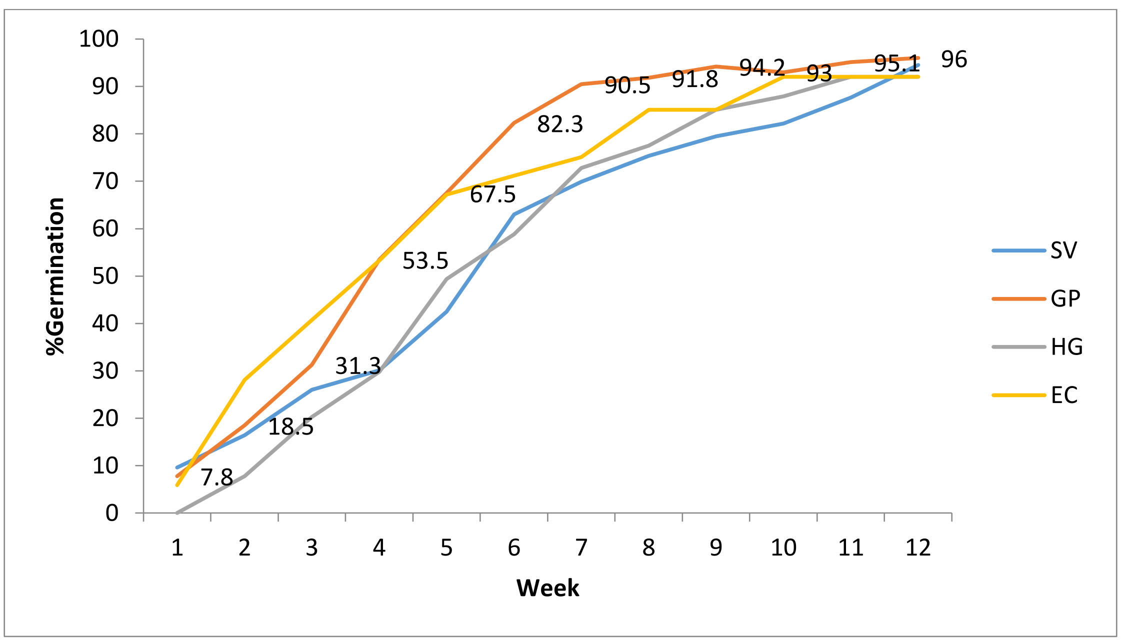


Figure 3.4.Mean percent weekly germination Saint Vrain (SV), Guanella Pass (GP), Hankins Gulch (HG), and Elk Creek (EC) *Mimulus gemmiparus* bulbils.

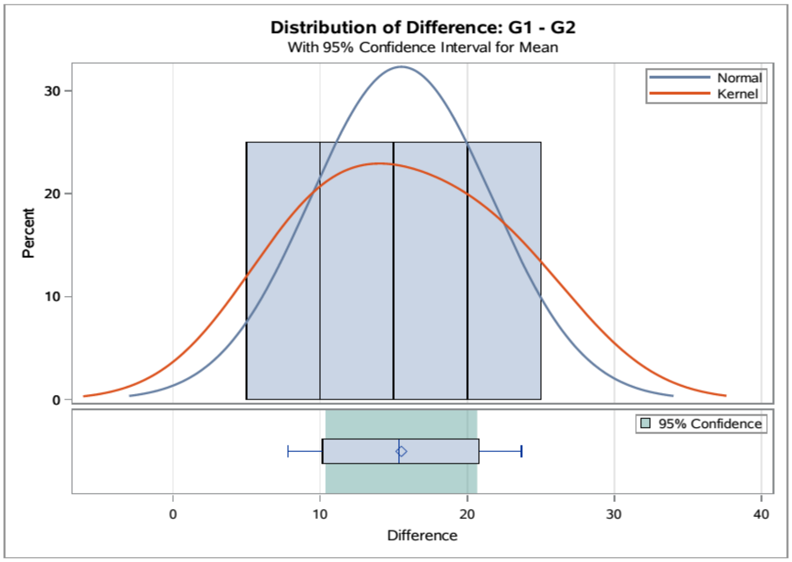
The TTEST Procedure

Difference: G1 - G2

N	Mean	Std Dev	Std Err	Minimum	Maximum
8	15.5125	6.1671	2.1804	7.8000	23.7000

Mean	95% CL Mean		Std Dev	95% CL Std Dev	
15.5125	10.3567	20.6683	6.1671	4.0775	12.5517

DF	t Value	Pr > |t|
7	7.11	0.0002



The TTEST Procedure

Difference: G1 - G2

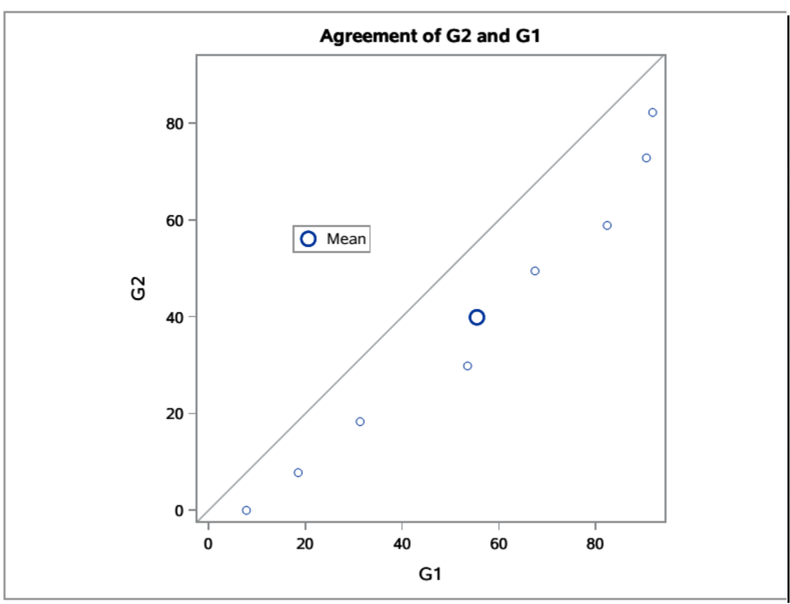


Figure 3.5. Guanella Pass and Hankins Gulch 10-13 days post harvest difference in mean percent germination of bulbils observed over 8 weeks.

HG population tested 10, 61, and 74 DPH at ambient storage ranged from 75-92% germination with a significant difference in viability observed over 12 weeks between 10 and 74 DPH bulbils ($p<.0055$) (Figure 3.6). GP viability for bulbils 30, 50, and 70 days post harvest ranged from 89-96% germination with no significant decline in germination observed over 12 weeks between 13 and 70 DPH bulbils stored at ambient conditions 20^{o}C, 38-42%RH.

The significantly higher rate of germination of the GP population at 8 weeks observation compared to the HG population ($p<.0002$) indicates faster germination response in GP population. GP had a mean moisture content of 0.7015 $gH_2Og^{-1}DW$, while HG had a mean moisture content of 0.5522 $gH_2Og^{-1}DW$. At 12 weeks of observation, the difference in germination response was no longer significant. 74 DPH HG bulbil germination observed over 12 weeks was significantly lower than HG 10 DPH germination observed over 12 weeks ($p<.0055$). MCDWB of 74 DPH HG bulbils was also significantly lower than MCDWB of 10 DPH HG bulbils. There were no significant differences in germination response observed over 12 weeks, nor MCDWB in GP bulbils 13 and 70 DPH.

HG native plants grow at an elevation of 2,560m, while GP native plants grow at an elevation of 3,390m. Growing seasons at high elevations are shorter than those at low elevation. There is evidence based on germination observations over 10 months (Walters, personal communication, 2016) that low moisture content bulbils may remain viable, but with delayed onset of germination. While it may be acceptable to achieve maximum emergence of plants over a 12 week period of propagation in a greenhouse, annual plants that fail to emerge at the start of a short growing season in the wild may have lower chances for success. Plants that emerge within 4 to 8 weeks would seem to stand a better chance to achieve full maturity in the wild before the onset of colder conditions at high elevations. However, if bulbils can remain viable while quiescent over longer or multiple growing seasons, delayed onset of germination in lower moisture content bulbils could serve as a bet hedging strategy that

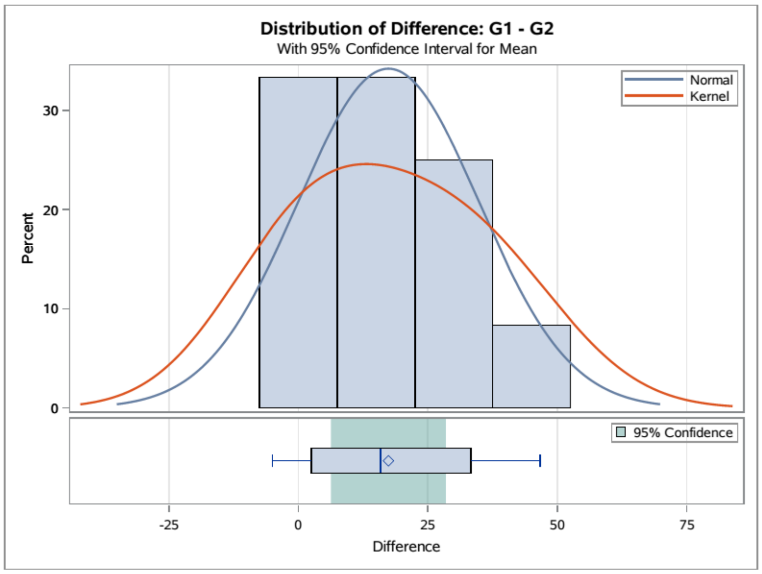
The TTEST Procedure

Difference: G1 - G2

N	Mean	Std Dev	Std Err	Minimum	Maximum
12	17.3617	17.4871	5.0481	-5.0000	46.6700

Mean	95% CL Mean		Std Dev	95% CL Std Dev	
17.3617	6.2509	28.4724	17.4871	12.3878	29.6909

DF	t Value	Pr > |t|
11	3.44	0.0055



The TTEST Procedure

Difference: G1 - G2

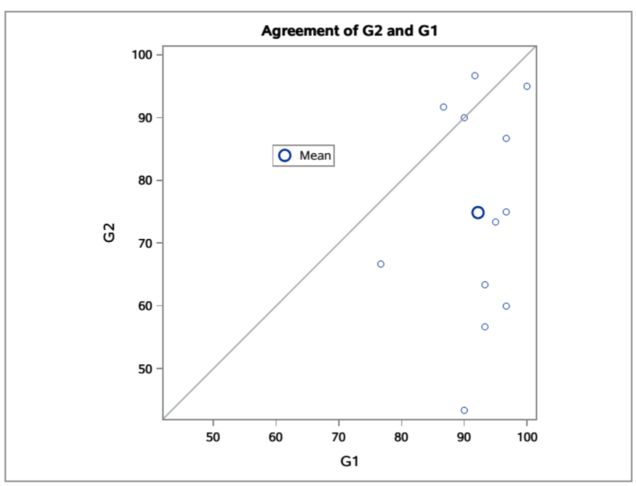


Figure 3.6. Hankins Gulch difference in mean percent germination observed over 12 weeks in 10 and 74 days post harvest bulbils.

increases a plant's long term fitness. For purposes of propagation to multiply bulbils, slow germinating, low moisture content bulbils would not be the best material for use due to time, material, and budget constraints.

Delays in reproduction can reduce maximum rate of increase in a predictable environment, but can increase the mean fitness of a genotype over generations in an unpredictable environment (citation). Ex situ GP propagated MIGE bulbils displayed apparently high risk high germination rates, as also seen in many desert annuals which have a short window of time with suitable conditions for germination and growth (citation). MIGE dispersal of propagules close to the mother plant, as MIGE displays, is often seen as a lower risk strategy with higher rates of dormancy. Field studies of experimental plots planted with bulbils at high or low moisture contents and observed over multiple years could lead to a better understanding of the combination of high and low risk life strategies utilized by MIGE in the wild.

A side by side TZ/germination test of 4G EC propagules grown in pots or tubs was conducted in collaboration with Colorado Seed Lab (CSL). Colorado Seed Lab TZ results for viability underestimated 12 week germination test results in both tub and pot grown samples. Mean viability of tub grown plants determined by TZ was 77% compared to 92% mean viability determined by germination test. There was weak correlation between percent germination and moisture content of bulbils measured on a dry weight basis (MCDWB) (Table 3.2).

The Association of Official Seed Analysts (AOSA) seed testing rules state the standards for determination of seed viability by a range of tests. It is more common that germination tests underestimate seed viability compared to TZ, which can detect living, dormant embryos. CSL lower estimate of actual germination could be due to the atypical staining pattern and colors observed in

Table 3.2. Side by side germination/Tetrazolium chloride (TZ) test of Elk Creek population *Mimulus gemmiparus* bulbils grown from pots or tubs. Independent TZ performed at Colorado Seed Lab, 2015. Moisture content measured on a dry weight basis (MCDWB) at initiation of viability testing. n=10 plants

Final%GermPot	CSLTZ%	MCDWB
100	60	1.2
90	70	0.4688
80	100	0.5
60	80	0.5652
100	80	0.5357
80	50	0.4706
40	60	0.7407
91	70	0.6333
90	40	0.7391
91	60	0.7143

Correlation Germ/MCDWB 0.120636
Mean %Germ Pot 83.1
Mean CSLTZ Pot% 67
Mean MCDWB 0.63265
Variance MCDWB 0.047441
SD MCDWB 0.217809

Final%GermTub	CSLTZ%	MCDB
90	60	0.6296
100	80	0.7407
80	60	0.4865
90	90	0.6957
100	80	0.6522
100	80	0.7391
100	90	0.75
80	70	0.7619
90	80	0.7391
91	80	0.7143

Correlation Germ/MCDWB 0.428119
Mean %Germ Tub 92.1
Mean CSLTZ Tub% 77
Mean MCDWB 0.69091
Variance MCDWB 0.00703

MIGE bulbils. Bulbils turned a dark leathery brown when soaked in water for > 12 hours before introduction into the TZ solution. This color indicates mortality in AOSA rules for some seed species There was variation in pattern of staining and plant part that took up the stain that are also not defined as rules for reading viable versus nonviable results. Dark leather brown or atypically TZ stained bulbils may have been preferentially rejected as nonviable, when in fact they were viable. There is no published protocol in the AOSA Seed Handbook that describes rules for reading viable versus nonviable MIGE propagules. AOSA is dedicated to commercially important seed crops. A similar document that codifies standards of viability testing specific to seed from species of concern could be of benefit.

There is a lack of uniformity in TZ test readings between labs and between testing technicians due to the subjective nature of determining viability by color and staining patterns. TZ testing may be an adequate measure to achieve fast, rough, baseline estimates of viability to guide ex situ plant management decisions. When faced with a choice of multiple collections for use in propagation, TZ testing could be used to quickly determine which collections appear to have the highest measure of viability and best likelihood for successful propagation. TZ testing can also be used to periodically measure viability of stored bulbils in order to guide decisions to continue storage, propagate, or out plant stocks of material.

To obtain a range of low and high moisture bulbils to subject to cold storage exposure, harvested bulbils were allowed to dry over time in storage conditions of 20^oC and 38-40% RH. Moisture content on a dry weight basis (MCDWB)measurements of these bulbils were conducted at 4 time intervals, 1: 0-30 days post harvest (DPH), 2: 60-90 DPH, 3: 120-180 DPH, and 4: 270 or greater DPH by the methods previously described. A selection of these measurements were paired with 12 week germination tests.

If correlations between moisture content and viability are established, and there is a measure of moisture content changes over time under controlled storage conditions, then it may be possible to estimate rates of germination by days post harvest as well as by MCDWB. A summary of measurements displayed in a scatter plot indicated a decline in moisture content to 0.1350 g H_2Og ^1DW or lower within a year under these storage conditions and created a pattern that resembles the sigmoidal shape typical of longevity curves (Figure 3.7).

There was weak correlation between percent germination and moisture content of bulbils measured on a dry weight basis at fresh harvest, likely because at the high moisture contents of recently harvested bulbils germination rates were uniformly high. Strong correlation between moisture content and germination was present, however, when a wider range of moisture contents were examined

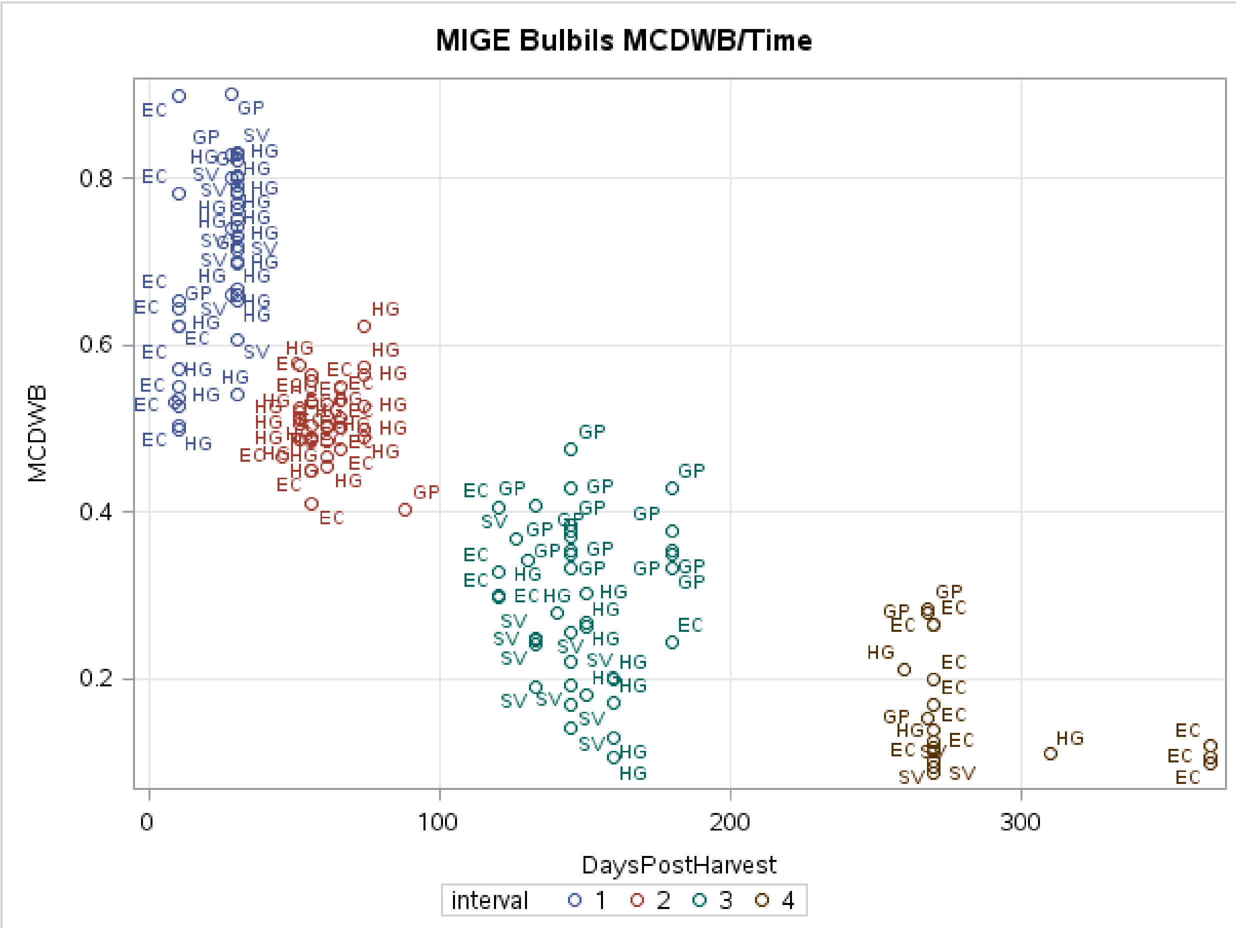


Figure 3.7. Moisture content on a dry weight basis (MCDWB) of Elk Creek (EC), Guanella Pass (GP), Hankins Gulch (HG), and Saint Vrain (SV) ex situ propagated *Mimulus gemmiparus* bulbils tested in 4 time intervals post harvest; interval 1: 0-30 days, 2: 60-90 days, 3: 120-180 days, 4: 270 and greater days.

(Figures 3.8-3.11). GP germinated more quickly than HG as evidenced by a higher mean percent germination at 8 weeks. Higher moisture content bulbils from GP and EC exposed to cold storage temperatures germinated at higher rates than those of the lower moisture content SV and HG samples when germination was observed for 12 weeks.

SV bulbils from n=10 plants with moisture contents from 0.14 to 2.493 $gH_20g^{-1}DW$ germinated at 2 – 10 percent with a mean of 5.4 percent viability determined by 12 week germination test. EC

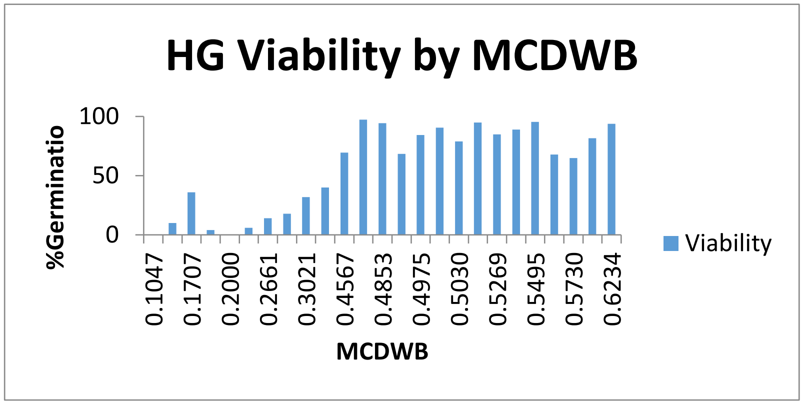


Figure 3.8. Hankins Gulch (HG) population *Mimulus gemmiparus* bulbils tested for for viability by 12 week germination test and moisture content on dry weight basis (MCDWB).

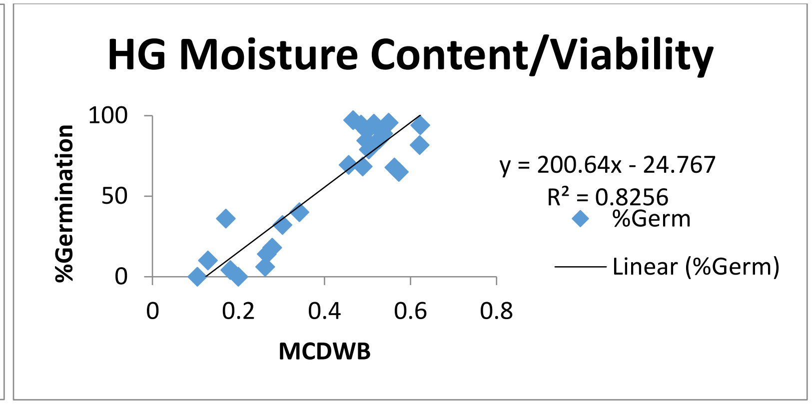


Figure 3.9. Correlation between moisture content on dry weight basis (MCDWB) and percent germination for Hankins Gulch (HG) population *Mimulus gemmiparaus.*

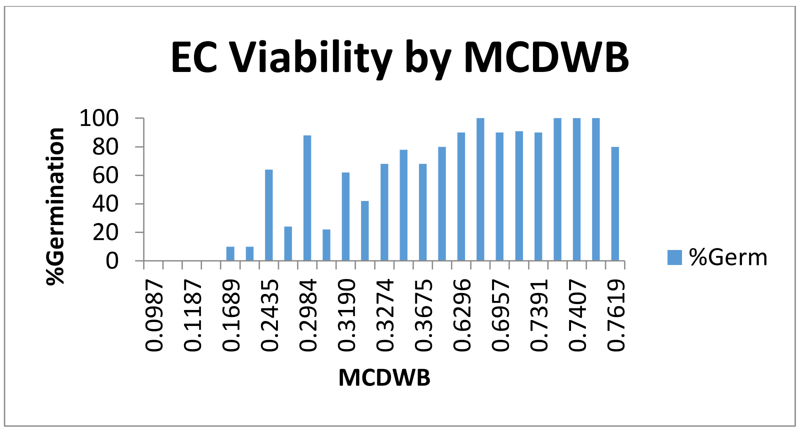


Figure 3.10. Elk Creek (EC) population of *Mimulus gemmiparus* bulbils tested for viability by germination test and moisture content on a dry weight basis (MCDWB).

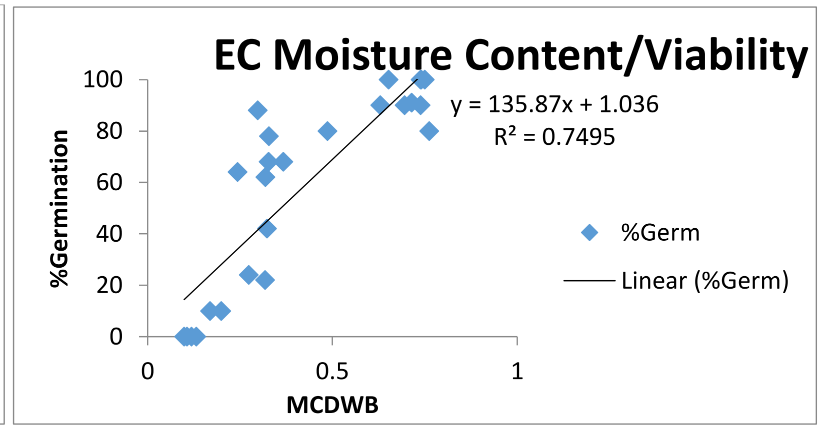


Figure 3.11. Correlation between moisture content on a dry weight basis (MCDWB) and percent germination for Elk Creek (EC) population *Mimulus gemmiparus* bulbils.

bulbils from n=10 plants had moisture contents from 0.1321 to 0.3675 MCDWB with a mean viability of 51.6 percent, and a low moisture content with 0 viability at 0.1321 gH_2Og^{-1} DW. HG bulbils from n=10 plants had mean viability 16.4 percent at a mean moisture content of 0.236918 gH_2Og^{-1}DW and <10% viability at moisture content values between 0.1290322-0.2784 gH_20g^{-1}DW. GP bulbils from n=10 plants had germination rates between 68 and 82 percent with a mean of 73.4 percent at a range of moisture contents between 0.33195-0.4760 gH_2Og^{-1}DW. One GP outlier with moisture content of 0.3689 gH_20g^{-1}DW germinated below 50 percent.

It is not possible from this data to reject or fail to reject the null hypothesis that the means of low moisture content thresholds are equal among the four populations. Sample sizes from individual populations that displayed low moisture content thresholds defined as the moisture content at which point 12 week germination response was ≤ 10 percent were not large enough to evidence differences in low moisture content thresholds between populations. However, when all samples that germinated at ≤ 10 percent from 3 populations, EC, HG, and SV were combined, low moisture thresholds were found between 0.1290 and 0.2493 gH_2Og^{-1}DW. The mean moisture content at which point germination was ≤ 10 percent was measured at 0.194513 gH_2Og^{-1}DW. A 95 percent confidence interval placed the true mean low moisture content threshold between 0.1350 and 0.1817 gH_20g^{-1}DW (Figure 3.12). Previous research (Beardsley, 1997) found HG bulbils to be tolerant to levels of 15-25 percent desiccation. He based this conclusion on 18 percent mean germination observed over 8 weeks in bulbils desiccated in a methanol bath over a period of 35 days. This research indicated the EC population succeeded in germinating at 23 percent observed over 12 weeks after desiccating 55 percent over a period of 120 -180 days. These different methods used to achieve desiccation and measure rates of germination may not be directly comparable, however, the more current research presented here evidences the potential for some MIGE population bulbils to be tolerant of higher levels of desiccation than previously described.

The TTEST Procedure

Variable: MCDWB

N	Mean	Std Dev	Std Err	Minimum	Maximum
20	0.1584	0.0499	0.0112	0.0987	0.2550

Mean	95% CL Mean		Std Dev	95% CL Std Dev	
0.1584	0.1350	0.1817	0.0499	0.0379	0.0729

DF	t Value	Pr > |t|
19	14.20	<.0001

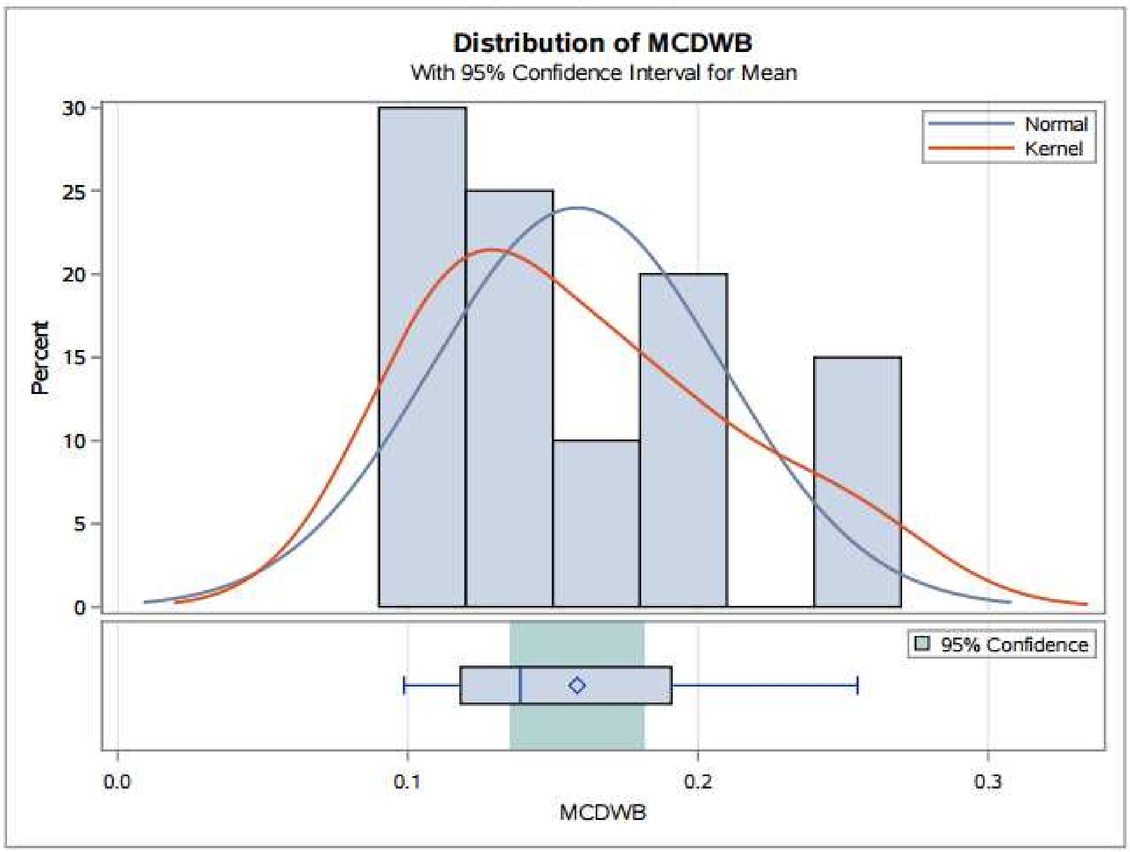
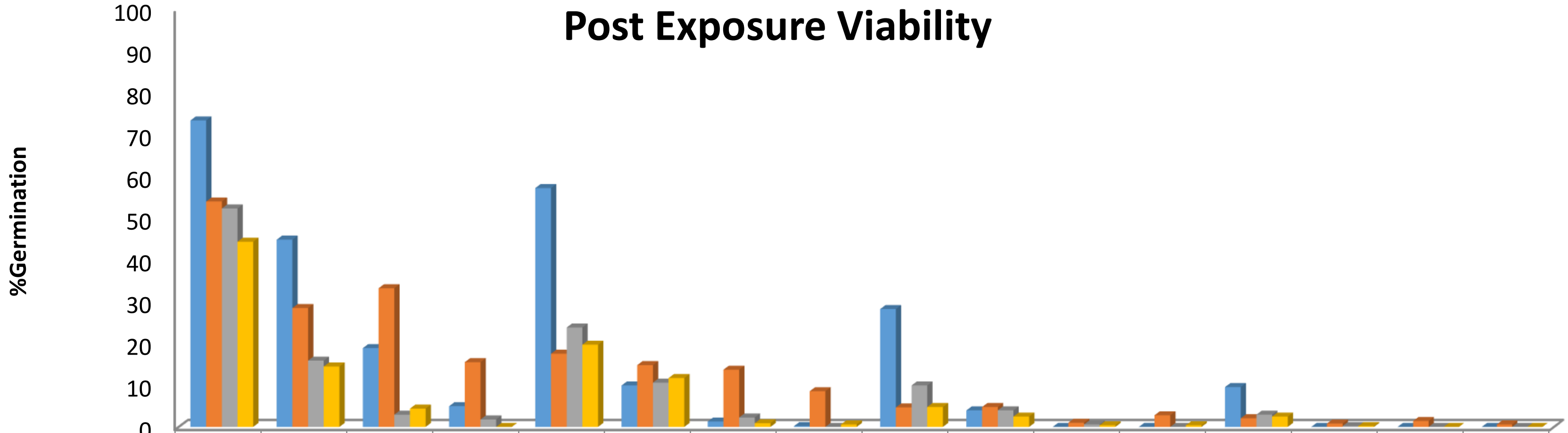


Figure 3.12. 95% Confidence Interval for the true mean low moisture content on a dry weight basis (MCDBW) of Elk Creek, Hankins Gulch, and Saint Vrain populations of *Mimulus gemmiparus* bulbils. Low moisture content threshold determined by MCDWB of bulbils with ≤ 10% germination observed over 12 weeks.

germination may not be directly comparable, however, the more current research evidences the potential for some MIGE population bulbils to be tolerant of higher levels of desiccation than previously described.

MIGE bulbils do not appear to be tolerant of desiccation below 0.1350 – 0.1817 $gH_2Og^{-1}DW$. At these low moisture contents, survivorship as measured by 12 week germination response decreased or approached zero as water content approached zero. This response does not conform to orthodox seed behavior, which in some cases demonstrates viability at moisture contents < 0.07 $gH_2Og^{-1}DW$ (Vertucci and Leopold, 1987). MIGE bulbils demonstrated a tolerance for desiccation greater than that which would be expected in recalcitrant seed storage behavior. Freshly harvested 5 DPH EC bulbils with a mean moisture content of 0.0609 $gH_2Og^{-1}DW$ tolerated desiccation of 73.3 percent before reaching the upper limit of the low threshold. Intermediate seeds can be desiccated to approximately 0.1 – 0.2 $gH_2Og^{-1}DW$, which is consistent with observations of MIGE behavior.

Bulbils from time interval 3 subjected to cold storage exposure temperatures showed differences in post exposure germination response (Figure 3.13). GP bulbils with moisture content range 0.33195-0.4760 $gH_2Og^{-1}DW$ and mean 0.3877 $gH_2Og^{-1}DW$ showed a significantly higher post exposure germination response in the control compared to the $3^o$to 5^o, -3^o to 0^o, and -20^oC temperature exposures at 24 hours (p=0.0057, p=0.0013, p=0.0001, respectively). At 1 month there was no significant difference between the control and 3^o to 5^oC temperature exposure response, but the control group continued to show significantly higher germination response than the -3^o to 0^o and -20^oC exposures (p=0.0013 and p=0.0016, respectively). At 1 month GP 3^o to 5^oC response was significantly higher than -3-0 and -20C response (p=0.0126 and p=0.0071, respectively). At 3 months of exposure GP 3^o to 5^oC exposure response began to exceed control response, but control response was still significantly higher only compared to the -3^o to 0^oC exposure (p=0.0016). -3^o-0^o and -20^oC GP exposure responses at 3 months were significantly lower than 3^o to 5^oC GP 3 month post exposure response (p=0.0006 and



	GP 24hr	GP 1mnth	GP 3mnth	GP 6mnth	EC 24hr	EC 1mnth	EC 3mnth	EC 6mnth	HG 24hr	HG 1mnth	HG 3mnth	HG 6mnth	SV 24hr	SV 1mnth	SV 3mnth	SV 6mnth
control	73.421	45	19	5	57.3	10	1.29	0.2	28.4	4	0	0	9.6	0	0	0
3-5C	54.092	28.65	33.4	15.6	17.65	14.89	13.78	8.6	4.7	4.8	1	2.8	2.1	0.81	1.43	0.6
-3 - 0C	52.424	15.97	2.95	1.8	23.95	10.67	2.27	0	10	4	0.6	0	2.96	0.2	0	0
-20C	44.498	14.57	4.42	0	19.81	11.78	0.89	0.6	4.8	2.5	0.4	0.4	2.49	0.21	0	0

Figue 3.13. Post treatment viability determined by percent germination over 12 weeks with temperature exposure 3^{o}-5^{o}, -3^{o} to 0^{o}, -20^{o}C or control at 20^{o}C at 24 hours, 1 month, 3 months , and 6 months. Guanella Pass (GP), Elk Creek (EC), Hankins Gulch (HG), and Saint Vrain (SV) populations N=50, n=10 plants from each population.

p=0.0021, respectively). At 6 months of exposure 3^{o}-5^{o}C response was significantly higher than the two freezing temperature exposures, -3^{o} to 0^{o}C (p=0.00390) and -20^{o}C (p=0.0014) (Figure 3.14).

EC bulbils with moisture content range 0.1321 to 0.3675 gH_2Og^{-1}DW and mean of 0.2931 gH_2Og^{-1}DW showed a somewhat different pattern in post exposure germination response. At 24 hours the EC control group stored at 20^{o}C had significantly higher post exposure germination response than the 3^{o} to 5^{o}C ($p<0.0001$), -3^{o} to 0^{o}C (p=0.001), and -20^{o}C ($p<0.0001$) storage temperature exposures. At 1 month there were no significant differences between temperature exposure responses. At 3 months the 3^{o} to 5^{o}C exposure response was significantly higher than that of the control (p=0.0029), -3-0C (p=0.0018), and -20^{o}C (p=0.0022). There were no significant differences in post exposure response at 6 months (Figure 3.15).

HG bulbils had a mean moisture content of 0.236918 gH_2Og^{-1}DW and with values between 0.1290322-0.2784 gH_20g^{-1}DW. The control at 24 hours had significantly higher germination response than the 3^{o} to 5^{o}C (p=0.0098) and the -3^{o} to 0^{o}C (p=0.0066) exposures. Germination response of bulbils was low at the start of the experiment at 16.4 percent, losses of germination response quickly approached zero, and no significant findings were measureable past 1 month of exposure (Table 3.3).

SV bulbils had a moisture content range of 0.14 to 2.493 gH_20g^{-1}DW with a mean of 0.2270 gH_20g^{-1}DW. At 24 hours of exposure the control germination response was significantly higher than the 3^{o} to 5^{o}C (p=0.0063), -3^{o} to 0^{o}C (p=0.001), and the -20^{o}C (p=0.0081) post exposure germination response. Mean germination response of bulbils was < 10 percent at the start of the experiment, losses of germination response quickly approached zero, and no significant findings were measureable past 24 hours of exposure (Table 3.3).

Lower moisture content bulbils from the HG and SV populations did not show higher post exposure survivorship at freezing storage temperatures compared to the control and above freezing storage temperature as hypothesized. The low viability determined by 12 week germination test of the

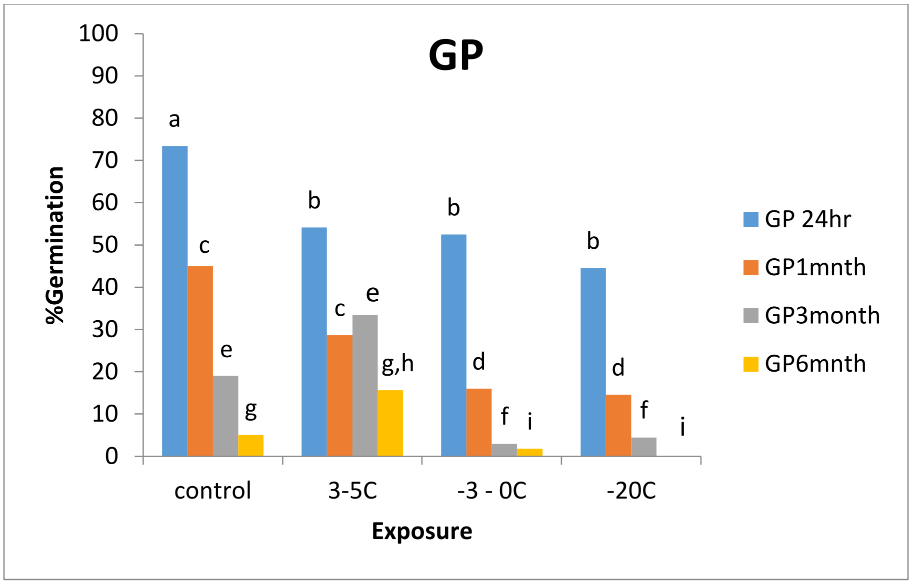


Figure 3.14. Guanella Pass (GP) population ex situ propagated *Mimulus gemmiparus* bulbils treated to 3 temperature exposures -20^{o}, -3^{o} to 0^{o}, or 3^{o}-5^{o}C for 24 hours, 1 month, 3 months, or 6 months and a control maintained at 20^{o}C.

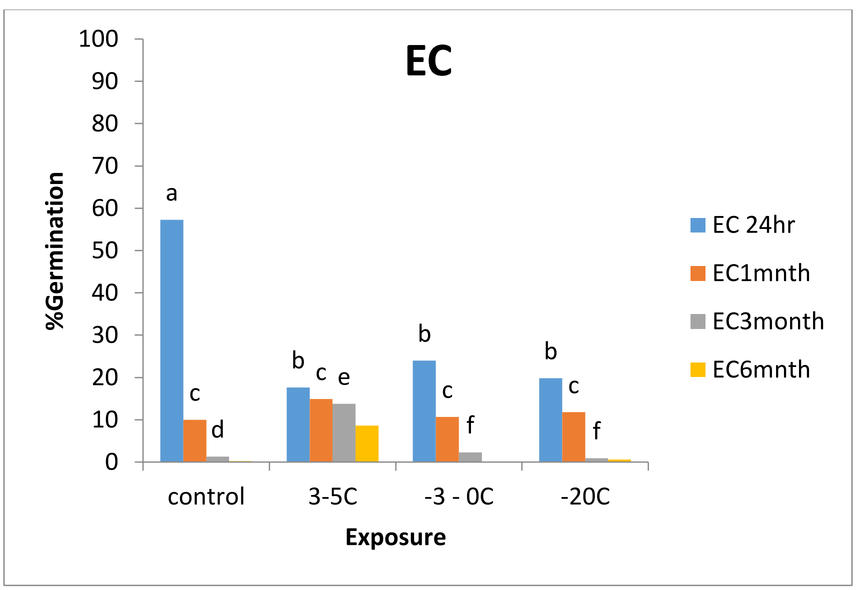


Figure 3.15. Elk Creek (EC) population ex situ propagated *Mimulus gemmiparus* bulbils treated to 3 temperature exposures -20^{o}, -3^{o} to 0^{o}, or 3^{o}-5^{o}C for 24 hours, 1 month, 3 months, or 6 months and a control maintained at 20^{o}C.

Table 3.3. P-values of differences in post storage temperature exposure mean germination response of bulbils from Guanella Pass (GP), Elk Creek (EC), Saint Vrain (SV), and Hankins Gulch (HG) populations of ex situ propagated *Mimulus gemmiparus*.

	Control					3-5C					-3 - 0C				
Population	Treatment	Interval	df	t	P	Treatment	Interval	df	t	P	Treatment	Interval	df	t	P
GP	3-5C	24hr	9,9	3.6	**0.0057**		24hr	9,9				24hr	9,9		
	-3 - 0C	24hr	9,9	4.58	**0.0013**	-3 - 0C	24hr	9,9	0.23	0.8237		24hr	9,9		
	-20C	24hr	9,9	7.85	**0.0001**	-20C	24hr	9,9	2.26	0.0506	-20C	24hr	9,9	1.25	0.2423
GP	3-5C	1mnth	9,9	1.93	0.0852		1mnth	9,9				1mnth	9,9		
	-3 - 0C	1mnth	9,9	4.58	**0.0013**	-3 - 0C	1mnth	9,9	3.1	**0.0126**		1mnth	9,9		
	-20C	1mnth	9,9	4.45	**0.0016**	-20C	1mnth	9,9	3.46	**0.0071**	-20C	1mnth	9,9	0.55	0.5967
GP	3-5C	3mnth	9,9	-2.65	0.0264		3mnth	9,9				3mnth	9,9		
	-3 - 0C	3mnth	9,9	4.43	**0.0016**	-3 - 0C	3mnth	9,9	5.13	**0.0006**		3mnth	9,9		
	-20C	3mnth	9,9	2.73	0.0231	-20C	3mnth	9,9	4.27	**0.0021**	-20C	3mnth	9,9	-1.22	0.2545
GP	3-5C	6mnth	9,9	-3.02	0.0145		6mnth	9,9				6mnth	9,9		
	-3 - 0C	6mnth	9,9	1.85	0.0954	-3 - 0C	6mnth	9,9	3.85	**0.0039**		6mnth	9,9		
	-20C	6mnth	9,9	2.91	0.0174	-20C	6mnth	9,9	4.52	**0.0014**	-20C	6mnth	9,9	2.86	0.0187
EC	3-5C	24hr	9,9	7.36	**<.0001**		24hr	9,9				24hr	9,9		
	-3 - 0C	24hr	9,9	5.07	**0.001**	-3 - 0C	24hr	9,9	-1.62	0.1448		24hr	9,9		
	-20C	24hr	9,9	8.58	**<.0001**	-20C	24hr	9,9	-0.74	0.4797	-20C	24hr	9,9	1.2	0.2646
EC	3-5C	1mnth	9,9	-1.01	0.3412		1mnth	9,9				1mnth	9,9		
	-3 - 0C	1mnth	9,9	-0.16	0.8756	-3 - 0C	1mnth	9,9	1.23	0.2544		1mnth	9,9		
	-20C	1mnth	9,9	-0.82	0.4367	-20C	1mnth	9,9	0.89	0.4018	-20C	1mnth	9,9	0.47	0.6503
EC	3-5C	3mnth	9,9	-4.22	**0.0029**		3mnth	9,9				3mnth	9,9		
	-3 - 0C	3mnth	9,9	-1.62	0.1449	-3 - 0C	3mnth	9,9	4.59	**0.0018**		3mnth	9,9		
	-20C	3mnth	9,9	0.43	0.6768	-20C	3mnth	9,9	4.43	**0.0022**	-20C	3mnth	9,9	1.16	0.2786
EC	3-5C	6mnth	9,9	-2.96	0.016		6mnth	9,9				6mnth	9,9		
	-3 - 0C	6mnth	9,9	1.00	0.3434	-3 - 0C	6mnth	9,9	3.03	0.0164		6mnth	9,9		
	-20C	6mnth	9,9	-0.8	0.4433	-20C	6mnth	9,9	2.75	0.0224	-20C	6mnth	9,9	-1.41	0.1934
SV	3-5C	24hr	9,9	3.55	**0.0063**		24hr	9,9				24hr	9,9		
	-3 - 0C	24hr	9,9	4.80	**0.001**	-3 - 0C	24hr	9,9	-0.52	0.6152		24hr	9,9		
	-20C	24hr	9,9	3.38	**0.0081**	-20C	24hr	9,9	-0.67	0.5271		24hr	9,9	0.29	0.7755
SV	3-5C	1mnth	9,9	-1.81	0.1039		1mnth	9,9				1mnth	9,9		
	-3 - 0C	1mnth	9,9	-1.00	0.3434		1mnth	9,9	1.15	0.2789		1mnth	9,9		
	-20C	1mnth	9,9	-1.00	0.3434		1mnth	9,9	1.41	0.1934		1mnth	9,9	0	1.0000
HG	3-5C	24hr	9,9	3.26	**0.0098**		24hr	9,9				24hr	9,9		
	-3 - 0C	24hr	9,9	3.52	**0.0066**		24hr	9,9	-1.21	0.2553		24hr	9,9		
	-20C	24hr	9,9	3.14	0.0118		24hr	9,9	-0.08	0.9362		24hr	9,9	1.43	0.1864
HG	3-5C	1mnth	9,9	-1.65	0.1341		1mnth	9,9				1mnth	9,9		
	-3 - 0C	1mnth	9,9	-2.23	0.0528		1mnth	9,9	0.83	0.4269		1mnth	9,9		
	-20C	1mnth	9,9	-2.25	0.0510		1mnth	9,9	0.78	0.4543		1mnth	9,9	0.39	0.7052

low moisture content control HG and SV samples at 24 hours, 28.4 percent and 9.6 percent respectively, did not allow for analysis of survivorship beyond the 1 to 3 month interval because viability by that time was <10% in the controls, and approached 0 in samples exposed to storage temperatures. More controlled methods of desiccation, larger sample sizes, or observations of germination over longer time periods could be undertaken, and might yet evidence greater survivorship of low moisture content bulbils in freezing temperatures.

Higher moisture content bulbils were represented by the GP and EC populations. At 3 and 6 months, GP retained the highest level of post exposure germination response at 3^{o} to $5^{o}C$ storage. EC retained the highest level of post exposure germination response at 3^{o} to $5^{o}C$ storage at 3 months. That pattern did not persist as a significant finding for the 6 month duration as germination response in the 3^{o} to $5^{o}C$ storage continued to decline.

Study bulbils did not exhibit orthodox seed behavior. At desiccation levels below .10 $gH_2Og^{-1}DW$ EC, HG, and SV had very low or absent germination observed over 12 weeks. GP samples never reached desiccation levels below .10 $gH_2Og^{-1}DW$. Bulbils exposed to the conventional storage temperature for orthodox seeds, $-20^{o}C$, lost viability within the 6 month interval of testing. Bulbils retained viability at low moisture contents well below those that would be fatal to recalcitrant seeds and did not immediately succumb to freezing temperature exposures as would be predicted by recalcitrant seed behavior. MIGE bulbils appeared to exhibit intermediate storage behaviors. Long term study could confirm the 3 to 5 year at 0.13 $gH_2Og^{-1}DW$ storage potential of intermediate seeds.

The family wise error rate for t-tests when doing multple comparsons of the control and 3 temperature factors at alpha=0.01 is 0.056, indicating a 5.6 percent chance of falsely rejecting the null hypothesis. Analysis of covariance and a general linear mixed model analysis (GLM) was used to address the disadvantages of multiple t= tests. A GLM analysis of all four MIGE populations combined showed a significant difference between the 3^{o} to 5^{o} temperature exposure, the control, and the two subfreezing temperature exposures ($p<0.0001$). There were no significant differences found in combined populations 6 month response between the control, -3^{o} to 0^{o}C, and -20^{o}C treatments at 6 months (Figure 3.16). Analyzed separately from the other 3 populations, the high moisture content bulbils from GP had significantly higher post 3^{o} to 5^{o}C storage temperature germination response compared to the freezing temperature exposures ($p<0.0001$) (Figures 3.17, 3.18). Analyzed separately from the other 3 populations, the low moisture content bulbils from HG showed no significant differences between the control or any cold storage temperature treatments (Figure 3.19).

3.4 Conclusion

Optimal storage parameters consider temperature and moisture content interactions that lengthen storage time by maintaining viability of bulbils over short, medium, or long term time frames. Recommendations for storage depend upon the manager’s objective for material: short term storage for immediate use, medium term storage for establishment of ex situ plant banks, or long term storage for establishment of a gene bank (Bonner, 2008).

Recommendations for storage of recently harvested bulbils intended for assisted establishment and direct out planting or immediate propagation differ from recommendations for the manager who wants to preserve at least a minimum level of viability for the longest possible time. There was an immediate decline in germination observed in bulbils subjected to any of the 3 cold storage temperatures examined. This coupled with observations of high rates of germination in freshly

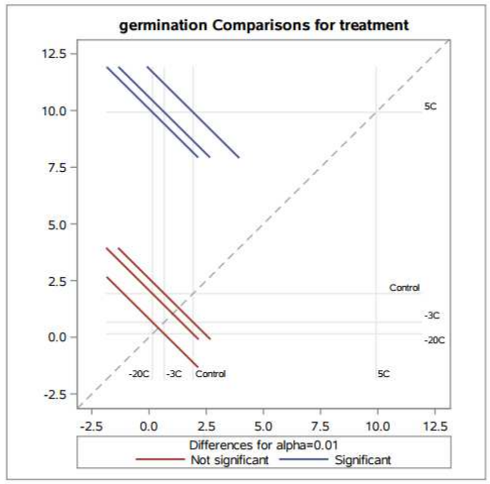
Ancova Treatment Response 6 Months

The GLM Procedure

Class Level Information		
Class	Levels	Values
treatment	4	-20C -3C 5C Control

Number of Observations Read	107
Number of Observations Used	107

The GLM Procedure
Least Squares Means



Ancova Treatment Response 6 Months

The GLM Procedure

Dependent Variable: germination

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	1688.520755	562.840252	17.94	<.0001
Error	103	3231.105413	31.369955		
Corrected Total	106	4919.626168			

R-Square	Coeff Var	Root MSE	germination Mean
0.343221	176.2633	5.600889	3.177570

Source	DF	Type I SS	Mean Square	F Value	Pr > F
treatment	3	1688.520755	562.840252	17.94	<.0001

Source	DF	Type III SS	Mean Square	F Value	Pr > F
treatment	3	1688.520755	562.840252	17.94	<.0001

Parameter	Estimate		Standard Error	t Value	Pr > |t|
Intercept	1.923076923	B	1.09842467	1.75	0.0830
treatment -20C	-1.774928775	B	1.53895645	-1.15	0.2514
treatment -3C	-1.256410256	B	1.53895645	-0.82	0.4162
treatment 5C	8.002849003	B	1.53895645	5.20	<.0001
treatment Control	0.000000000	B	.	.	.

Figure 3.16. SAS generated LS means; comparison of 6 month exposure germination response of bulbils from all 4 *Mimulus gemmiparus* populations combined treated at -20⁰, -3⁰ to 0⁰, and 3⁰ to 5⁰C, with control maintained at 20⁰C. Treatment response was measured by percent germination observed over 12 weeks at -20⁰, -3⁰-0⁰, 3⁰-5⁰C, or Control 20⁰C exposure tested at 6 months duration.

Dependent Variable: Germination

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	1458.307692	486.102564	12.49	<.0001
Error	35	1362.000000	38.914286		
Corrected Total	38	2820.307692			

R-Square	Coeff Var	Root MSE	Germination Mean
0.517074	109.5888	6.238132	5.692308

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Treatment	3	1458.307692	486.102564	12.49	<.0001

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Treatment	3	1458.307692	486.102564	12.49	<.0001

The GLM Procedure

Dependent Variable: germination

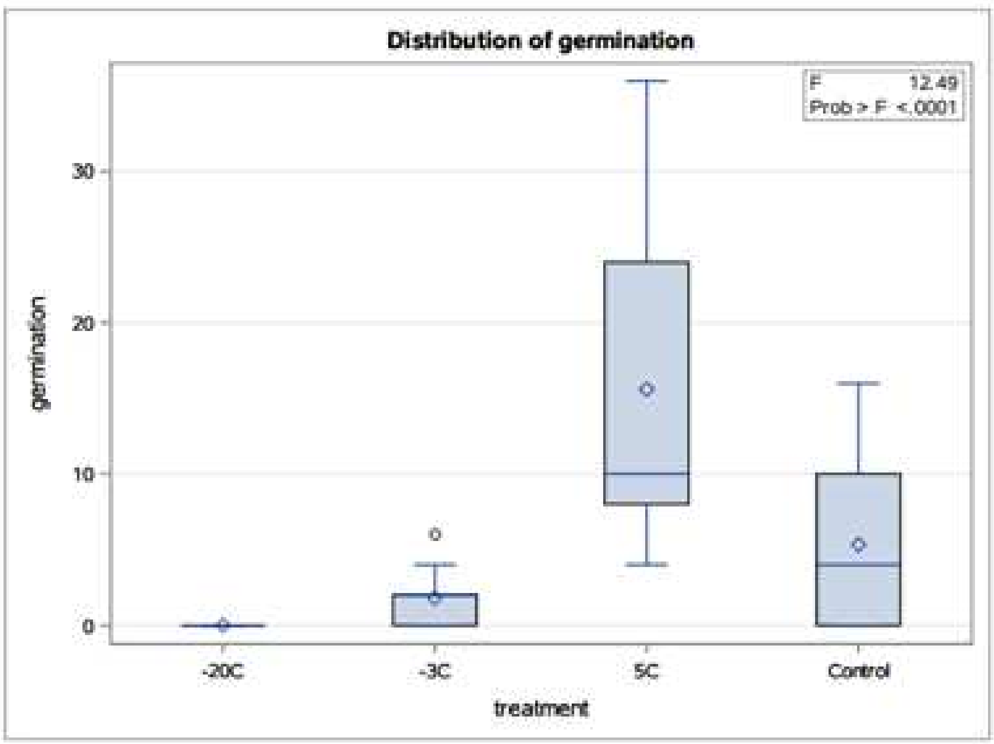


Figure 3.17. SAS generated box plots of the distribution of 6 month exposure germination response of bulbils from Guanella Pass population of *Mimulus gemmiparus* treated at -20^{o}, -3^{o} to 0^{o}, and 3^{o} to 5^{o}C, with control maintained at 20^{o}C.

The GLM Procedure
Least Squares Means

Treatment	Germination LSMEAN	Standard Error	Pr > |t|	LSMEAN Number
-20C	0.0000000	1.9726704	1.0000	1
-3C	1.8000000	1.9726704	0.3678	2
5C	15.6000000	1.9726704	<.0001	3
Control	5.3333333	2.0793772	0.0148	4

Least Squares Means for effect Treatment
Pr > |t| for H0: LSMean(i)=LSMean(j)

Dependent Variable: Germination

i/j	1	2	3	4
1		0.5230	<.0001	0.0712
2	0.5230		<.0001	0.2259
3	<.0001	<.0001		0.0010
4	0.0712	0.2259	0.0010	

The GLM Procedure
Least Squares Means

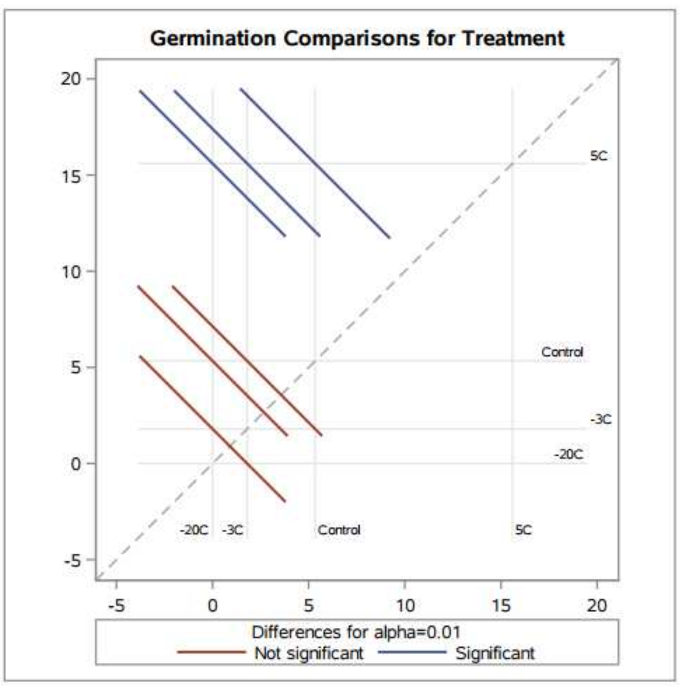
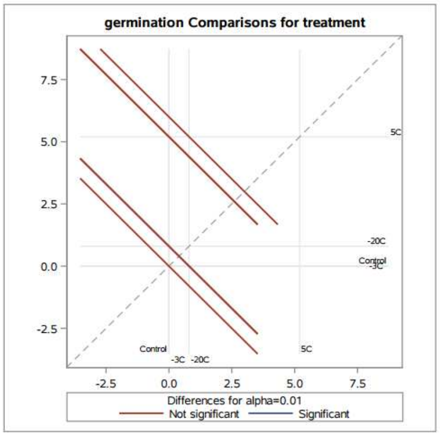


Figure 3.18. SAS generated diagram of LS means; comparison of high moisture content bulbils of Guanella Pass population of *Mimulus gemmiparus* post treatment response as measured by percent germination observed over 12 weeks at -20^o, -3^o-0^o, 3^o-5^{o}C, or Control 20^{o}C exposure for 6 months.

HG Ancova 6 Month Treatment Response



The GLM Procedure

Class Level Information		
Class	Levels	Values
treatment	4	-20C -3C 5C Control

Number of Observations Read	20
Number of Observations Used	20

The GLM Procedure
Least Squares Means

treatment	germination LSMEAN	Standard Error	Pr > |t|	LSMEAN Number
-20C	0.80000000	1.70880075	0.6460	1
-3C	0.00000000	1.70880075	1.0000	2
5C	5.20000000	1.70880075	0.0078	3
Control	0.00000000	1.70880075	1.0000	4

Figure 3.19**.** SAS generated diagram of LS means; comparison of low moisture content bulbils of Hankins Gulch population of *Mimulus gemmiparus* post treatment response as measured by mean percent germination observed over 12 weeks at -20^{o}, -3^{o}-0^{o}, 3^{o}-5^{o}C, or Control 20^{o}C exposure for 6 months.No significant differences in post exposure viability were found between 3 cold temperature exposures and control in low moisture content bulbils.

harvested, high moisture content bulbils indicates that material intended for use within 2 months of harvest should not be put into cold storage. Rather, bulbils should be maintained at a constant temperature, 20^{o}C in the case of this study. It is recommended that bulbils be maintained at 20^{o}C and 38-40% RH for less than 2 months to retain the highest level of germination potential within 12 weeks of sowing.

Longer term storage greater than 2 months yielded a decline in germination potential within 12 weeks of sowing. The slowest rate of decline was observed in material equilibrated at 20^{o}C, 38-40% RH, then stored at moisture content levels of 0.33195-0.4760 $gH_2Og^{-1}DW$ in packaging to prevent exposure to ambient air at 3^{o} to 5^{o}C. There did not appear to be an advantage to desiccating bulbils below this level to achieve the highest rates of germination within 12 weeks. More long term study is needed to determine if desiccation below 0.33195-0.4760 $gH_2Og^{-1}DW$ is advantageous to retain viability at subfreezing temperature to achieve the highest rates of germination within greater than 12 weeks post sowing. Subfreezing temperature storage is not recommended using the methods outlined in this study. However, more controlled methods of desiccation could potentially lead to options for subfreezing storage that could potentially extend the longevity of bulbils. Preservation of bulbils or meristematic tissue in liquid N has not yet been fully investigated, but may hold promise for long term, multi decadal preservation of germplasm. Propagule storage may contribute to conservation efforts, but is not a substitute for in situ conservation.

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APPENDIX I

Decision Matrix Design Summary

A summary of the parameters and scoring system used in a decision matrix to identify priority Colorado rare plant species and species of concern to consider for ex situ propagation follows:

- Federal Status: Legally listed by Endangered Species Act (ESA) as Threatened or Endangered, or previous to elimination of category C , designated as Candidate: **0 or 1**

- USFS Region 2 (Rocky Mountain Region) – National Forests: Arapaho, Bighorn, Black Hills, Grand Mesa, Gunnison, Medicine Bow, Nebraska, Pike, Rio Grande, Roosevelt, Routt, Samuel R. McKelvie, San Isabel, San Juan, Shoshone, Uncompagre, White River
  National Grasslands: Buffalo Gap, Cimarron, Comanche, Fort Pierre, Oglala, Pawnee, and Thunder Basin States: Colorado, Kansas, Nebraska, South Dakota, Eastern Wyoming: **0 or 1**

- USFS Region 4 (Intermountain Region) - National Forests: Ashley, Boise, Bridger-Teton, Caribou-Targhee, Dixie, Fishlake, Humboldt-Toiyabe, Manti-LaSal, Payette, Salmon-Challis, Sawtooth, Uinta, Wasatch-Cache States: Southern Idaho, Nevada, Utah, Western Wyoming: **0 or 1**

- BLM – not by field office, according to CNHP tracking list: **0 or 1**

- Colorado NPS Unit; National Park (NP) or National Monument (NM) – B/C/D/F/G/M/R: Black Canyon of the Gunnison/Colorado NM /Dinosaur NM/Florissant Fossil Beds NM/Great Sand Dunes NP/Mesa Verde NP/Rocky Mountain NP, 1pt each, all 4 CO National Parks, 3 National Monuments: **0 to 1**

- Multiple Agencies – no additional score, convenience of use
- CNHP Global Ranking – see CNHP Natural Heritage Ranks (NaturReserve Conservation Status Ranks) www.natureserve.org/explorer/rankining.htm 0=not ranked, 5=least rare, 1=most rare. These scores are used for conversion only and not counted in the raw form in the final matrix Score. Additional alpha codes addressed in Comments of decision matrix:

  not ranked or 0 to 5
- CNHP State Ranking - see CNHP Natural Heritage Ranks (NaturReserve Conservation Status Ranks) www.natureserve.org/explorer/rankining.htm 0=not ranked, 5=least rare, 1=most rare. These scores are used for conversion only and not counted in the raw form in the final matrix Score. Additional alpha codes addressed in Comments of decision matrix:

  not ranked or 0 to 5
- Matrix Rarity Ranking - Strategic Precaution Priority Matrix (SPPM) score reverses rarity scoring of CNHP Ranking for SPPM Global and State Raw Rankings: nr=not ranked, 1=least rare, 5=most rare: **not rated or 0 to 5**
- Matrix Rarity Ranking – SPPM state rarity score is expressed as a fraction of the CNHP state rarity score of 0 to 5 divided by the highest possible SPPM global rarity score of 5 for converted state rarity values ranging from 0.2 lowest priority to 1 for highest priority (Table 1):

  0, 0.2, 0.4,.0.6, 0.8. or 1

  Any questionable, conflicting, or missing rarity rankings constrains recommendation. Ambiguous reportings noted as AR in comments. **not recommended NR**

- Horticultural Feasibility - Biological/Horticultural Constraints: eg. Lack of propagule source, too rare for collections from native populations, unlikely to propagate successfully, difficult to propagate or no known propagation protocol for related species, questionable taxonomic or rarity status, possible extirpations, extinctions negates recommendation, also not tracked by CNHP not considered for propagation. (NR) designation over rides numerical score:

recommended (R) or not recommended (NR)

- Alpine/At Risk Habitat – incorporates elements that increase urgency of potential loss, At Rick Habitat includes potential loss due to changing climate conditions or land use practices such as oil and gas development. Criteria for documentation of at risk habitat or increased risk due to population dynamics is published research specific to local occurences: **0 or 1**

- Endemic – Colorado endemism is additional factor accounting for urgency of potential loss. Not weighted higher than 0/1 because element should already be partially accounted for by CNHP Global rarity ranking: **0 or 1**

Colorado Rare Species and Species of Concern Recommended for Consideration for Ex Situ Propagation

Key

- USFS Region 2
- USFS Region 4
- CNHP Tracked

Species	Federal Status	USFS Region 4	USFS Region 2	BLM	CO NPS Unit	Multiple Agencies	CNHP Global Ranking State Ranking	Strategic Precaution Rarity Score	Hort Feasibility	Alpine/At Risk Habitat	Endemic	Score	Comments
Aquilegia chrysantha var. rydbergii	0	0	1	1	0	FS, BLM	G4T1 S1	GT5 S1	R	1	1	10	indentified risks to habitat[1]
Aquilegia chrysantha var. rydbergii	0	0	1	1	0	FS, BLM	G4T1 S1	GT5 S1	R	1	1	10	indentified risks to habitat[1]
Astragalus microcymbus	C (1)	0	0	1	0		G1 S1	G5 S1	R	1	1	10	Identified risks to habitat[2]
Astragalus tortipes	C (1)	0	0	1	0		G1 S1	G5 S1	R	1	1	10	Identified risks to habitat[2]
Mimulus gemmiparus	0	0	1	0	R(1)	FS, NPS	G1 S1	G5 S1	R-vegprop	1	1	10	Identified risks to habitat[3]
Mimulus gemmiparus	0	0	1	0	R(1)	FS, NPS	G1 S1	G5 S1	R-vegprop	1	1	10	Identified risks to habitat[3]
Gutierrezia elegans	0	0	1	1	0	FS,BLM	G1 S1	G5 S1	R	1	1	10	Identified risks to habitat[4]
Gutierrezia elegans	0	0	1	1	0	FS,BLM	G1 S1	G5 S1	R	1	1	10	Identified risks to habitat[4]
Draba weberi	0	1	0	0	0		G1 S1	G5 S1	R	1	1	9	Identified risks to habitat[5]
Draba weberi	0	1	0	0	0		G1 S1	G5 S1	R	1	1	9	Identified risks to habitat[5]
Oreoxis humilis	0	0	1	0	0		G1 S1	G5 S1	R	1	1	9	roads, erosion[2], some alpine
Oreoxis humilis	0	0	1	0	0		G1 S1	G5 S1	R	1	1	9	
Physaria pulvinata	0	0	1	1	0	FS,BLM	G1 S1	G5 S1	R	0	1	9	
Physaria pulvinata	0	0	1	1	0	FS,BLM	G1 S1	G5 S1	R	0	1	9	
Draba grayana	0	0	1	0	R (1)	FS,NPS	G2 S2	G4 S0.8	R	1	1	8.8	alpine
Draba grayana	0	0	1	0	R (1)	FS,NPS	G2 S2	G4 S0.8	R	1	1	8.8	alpine
Penstemon degeneri	0	0	1	1	0	FS,BLM	G2 S2	G4 S0.8	R	1	1	8.8	Identified risk to habitat[2]
Penstemon degeneri	0	0	1	1	0	FS,BLM	G2 S2	G4 S0.8	R	1	1	8.8	Identified risk to habitat[2]
Aliciella sedifolia	0	0	1	1	0	FS, BLM	G1 S1	G5 S1	R	0	0	8	
Aliciella sedifolia	0	0	1	1	0	FS, BLM	G1 S1	G5 S1	R	0	0	8	
Astragalus schmolliae	C (1)	0	0	0	0		G1 S1	G5 S1	R	0	1	8	
Astragalus osterhoutii	LE (1)	0	0	0	0		G1 S1	G5 S1	R	0	1	8	
Descurainia kenheilii	0	0	0	0	0		G1 S1	G5 S1	R	1	1	8	

[1] Ladyman, 2005, [2]Rondeau et al., 2011, [3]Beardsley and Steingraeber, 2013, [4]Panjabi et al., [5]Decker, 2006, 2011, [6]Anderson, 2004, [7]Reveal 2003, [8]Anderson 2006, [9]Coles 2003, [10]O'Kane 1988, [11]Jennings et al. 1997, [12]CNHP, 2013

Key

- USFS Region 2
- USFS Region 4
- CNHP Tracked

	Federal Status	USFS Region 4	USFS Region 2	BLM	CO NPS Unit	Multiple Agnecies	CNHP Global Ranking State Ranking	Strategic Precaution Rarity Score	Hort Feasibility	Alpine/At Risk Habitat	Endemic	Score	Commments
Erigeron wilkenii	0	0	0	0	D(1)		G1 S1	G5 S1	R	0	1	8	
Ipomopsis polyantha	LE (1)	0	0	0	0		G1 S1	G5 S1	R	0	1	8	
Packera mancosana	0	0	0	0	0		G1 S1	G5 S1	R	1	1	8	hunting access, grazing[12]
Penstemon debilis	LT (1)	0	0	0	0		G1 S1	G5 S1	R	0	1	8	
Penstemon penlandii	LE (1)	0	0	0	0		G1 S1	G5 S1	R	0	1	8	
Phacelia formosula	LE (1)	0	0	0	0		G1 S1	G5 S1	R	0	1	8	
Physaria congesta	LT (1)	0	0	0	0		G1 S1	G5 S1	R	0	1	8	
Physaria scrotiformis	0	0	1	0	0		G1 S1	G5 S1	R	0	1	8	
Physaria scrotiformis	0	0	1	0	0		G1 S1	G5 S1	R	0	1	8	
Draba exunguiculata	0	0	1	0	0		G2 S2	G4 S0.8	R	1	1	7.8	
Draba exunguiculata	0	0	1	0	0		G2 S2	G4 S0.8	R	1	1	7.8	
Eriogonum coloradense	0	0	0	1	0		G2 S2	G4 S0.8	R	1	1	7.8	Identified risk to habitat[6], some alpine
Eriogonum pelinophilum	LE (1)	0	0	0	0		G2 S2	G4 S0.8	R	1	1	7.8	Identified risk to habitat[7]
Lupinus crassus	0	0	0	1	0		G2 S2	G4 S0.8	R	1	1	7.8	Identified risk to habitat[2]
Nuttallia chrysantha	0	0	0	1	0		G2 S2	G4 S0.8	R	1	1	7.8	Identified risk to habitat[8]
Nuttallia densa	0	0	0	1	0		G2 S2	G4 S0.8	R	1	1	7.8	Identified risk to habitat[2]
Oreocarya revealii	0	0	0	1	0		G2 S2	G4 S0.8	R	1	1	7.8	Identified risks to habitat [5]
Phacelia submutica	LT (1)	0	0	0	0		G2 S2	G4 S0.8	R	1	1	7.8	Identified risk to habitat[2], oil and gas
Physaria vicina	0	0	0	1	0		G2 S2	G4 S0.8	R	1	1	7.8	Identified risk to habitat[2]
Astragalus humillimus	LE (1)	0	0	0	0		G1 S1	G5 S1	R	0	0	7	
Astragalus missouriensis var. humistratus	0	0	1	0	0		G5T1 S1	GT5 S1	R	0	0	7	T=Intraspecific taxon, potential hybrid
Ipomopsis ramosa	0	0	0	0	0		G1 S1	G5 S1	R	0	1	7	
Oenothera coloradensis ssp. coloradensis	LT (1)	0	0	0	0		G3T2 S1	GT4 S1	R	1	0	7	Indentfied risks to habitat[11]
Penstemon grahamii	0	0	0	1	0		G2 S1	G4 S1	R	1	0	7	Identified risk to habitat[2], oil and gas
Penstemon scariosus var. albifluvis	C (1)	0	0	0	0		G4T1 S1	GT5 S1	R	0	0	7	AR, T=Intraspecific taxon
Physaria rollinsii	0	0	0	0	0		G1 S1	G5 S1	R	0	1	7	
Astragalus debequaeus	0	0	0	0	0		G2 S2	G4 S0.8	R	1	1	6.8	Identified risk to habitat[2]
Boechera crandallii	0	0	1	1	0	FS, BLM	G2 S2	G4 S0.8	R	0	0	6.8	Imminent risks specific unconfirmed
Draba graminea	0	0	0	0	0		G2 S2	G4 S0.8	R	1	1	6.8	some alpine, identified risk to habitat[2]

[1] Ladyman, 2005, [2]Rondeau et al., 2011, [3]Beardsley and Steingraeber, 2013, [4]Panjabi et al., [5]Decker, 2006, 2011, [6]Anderson, 2004, [7]Reveal 2003, [8]Anderson 2006, [9]Coles 2003, [10]O'Kane 1988, [11]Jennings et al. 1997, [12]CNHP, 2013

Key

- USFS Region 2
- USFS Region 4
- CNHP Tracked

Species	Federal Status	USFS Region 4	USFS Region 2	BLM	CO NPS Unit	Multiple Agencies	CNHP Global Ranking State Ranking	Strategic Precaution Rarity Score	Hort Feasibility	Alpine/At Risk Habitat	Endemic	Score	Commments
Draba smithii	0	0	1	0	0		G2 S2	G4 S0.8	R	0	1	6.8	
Draba smithii	0	0	1	0	0		G2 S2	G4 S0.8	R	0	1	6.8	
Ipomopsis globularis	0	0	0	0	0		G2 S2	G4 S0.8	R	1	1	6.8	some alpine, identified risk to habitat[2]
Nuttallia rhizomata	0	0	0	1	0		G2 S2	G4 S0.8	R	0	1	6.8	
Potentilla rupincola	0	0	1	0	0		G2 S2	G4 S0.8	R	0	1	6.8	
Potentilla rupincola	0	0	1	0	0		G2 S2	G4 S0.8	R	0	1	6.8	
Physaria alpina	0	0	0	0	0		G2 S2	G4 S0.8	R	1	1	6.8	
Physaria parviflora	0	0	0	1	0		G2 S2	G4 S0.8	R	0	1	6.8	
Physaria pruinosa	0	0	0	1	0	FS,BLM	G2 S2	G4 S0.8	R	1	0	6.8	Identified risk to habitat[2,8]
Ptilagrostis porteri	0	0	1	0	0		G2 S2	G4 S0.8	R	0	1	6.8	
Ptilagrostis porteri	0	0	1	0	0		G2 S2	G4 S0.8	R	0	1	6.8	
Sclerocactus mesae - verdae	LT (1)	0	0	0	0		G2 S2	G4 S0.8	R	1	0	6.8	Identified risk to habitat[2,9,10]
Telesonix jamesii	0	0	0	0	0		G2 S2	G4 S0.8	R	1	1	6.8	some alpine
Thalictrum heliophilum	0	0	1	1	0	FS,BLM	G2 S2	G4 S0.8	R	0	0	6.8	
Thalictrum heliophilum	0	0	1	1	0	FS,BLM	G2 S2	G4 S0.8	R	0	0	6.8	
Townsendia glabella	0	0	0	0	0		G2 S2	G4 S0.8	R	1	1	6.8	Identified risk to habitat[2]
Aquilegia saximontana	0	0	0	0	R (1)		G3 S3	G3 S0.6	R	1	1	6.6	existing prop protocols, some alpine
Penstemon harringtonii	0	0	1	1	0	FS,BLM	G3 S3	G3 S0.6	R	0	1	6.6	
Penstemon harringtonii	0	0	1	1	0	FS,BLM	G3 S3	G3 S0.6	R	0	1	6.6	
Astragalus missouriensis var. humistratus	0	0	0	0	0	0	G1T1 S1	GT5 S1	R	0	0	6	T=Intraspecific taxon
Boechera glareosa	0	0	0	0	0		G1 S1	G5 S1	R	0	0	6	
Camissonia eastwoodiae	0	0	0	1	0		G2 S1	G4 S1	R	0	0	6	
Descurainia ramosissima	0	0	0	0	0		G1 S1	G5 S1	R	0	0	6	
Draba globosa	0	1	0	0	0		G3 S1	G3 S1	R	1	0	6	alpine
Draba globosa	0	1	0	0	0		G3 S1	G3 S1	R	1	0	6	alpine
Eriogonum clavellatum	0	0	0	1	0		G2 S1	G4 S1	R	0	0	6	
Sphaeromeria capitata	0	1	0	1	0	FS,BLM	G3 S1	G3 S1	R	0	0	6	oil and gas potential risk, not confirmed
Sphaeromeria capitata	0	1	0	1	0	FS,BLM	G3 S1	G3 S1	R	0	0	6	oil and gas potential risk, not confirmed
Anticlea vaginatus	0	0	0	0	D		G2 S2	G4 S0.8	R	0	0	5.8	unconfirmed risk to hydrologic alterations
Astragalus molybdenus	0	0	0	0	0		G3 S2	G3 S0.8	R	1	1	5.8	

[1] Ladyman, 2005, [2]Rondeau et al., 2011, [3]Beardsley and Steingraeber, 2013, [4]Panjabi et al., [5]Decker, 2006, 2011, [6]Anderson, 2004, [7]Reveal 2003, [8]Anderson 2006, [9]Coles 2003, [10]O'Kane 1988, [11]Jennings et al. 1997, [12]CNHP, 2013

Key

USFS Region 2

USFS Region 4

CNHP Tracked

Species	Federal Status	USFS Region 4	USFS Region 2	BLM	CO NPS Unit	Multiple Agencies	CNHP Global Ranking State Ranking	Strategic Precaution Rarity Score	Hort Feasibility	Alpine/At Risk Habitat	Endemic	Score	Commments
Astragalus ripleyi	0	0	1	1	0	FS,BLM	G3 S2	G3 S0.8	R	0	0	5.8	
Astragalus ripleyi	0	0	1	1	0	FS,BLM	G3 S2	G3 S0.8	R	0	0	5.8	
Erigeron kachinensis	0	1	0	1	0	FS, BLM	G2 S1	G4 S0.8	R	0	0	5.8	unconfirmed risk to hydrologic alterations
Erigeron kachinensis	0	1	0	1	0	FS,BLM	G2 S1	G4 S0.8	R	0	0	5.8	unconfirmed risk to hydrologic alerations
Ipomopsis aggregata ssp. weberi	0	0	1	0	0		G5T2 S2	GT4 S.08	R	0	0	5.8	T=Intraspecific taxon
Ipomopsis aggregata ssp. weberi	0	0	1	0	0		G5T2 S2	GT4 S.08	R	0	0	5.8	T=Intraspecific taxon
Oenothera acutissima	0	0	0	1	0		G2 S2	G4 S0.8	R	0	0	5.8	unconfirmed risk to hydrologic alterations
Oonopsis sp. 1 (Oönopsis puebloensis)	0	0	0	0	0		G2 S2	G4 S0.8	R	0	1	5.8	
Oxytropis besseyi var. obnapiformis	0	0	0	0	0		G5T2 S2	GT4 S0.8	R	1	0	5.8	Identified risk to habitat[2,12]
Oxybaphus rotundifolius	0	0	0	0	0		G2 S2	G4 S0.8	R	0	1	5.8	
Penstemon mensarum	0	0	0	0	0		G2 S2	G4 S0.8	R	0	1	5.8	
Penstemon scariosus var. cyanomontanus	0	0	0	0	D(1)		G4T2 S2	GT4 S0.8	R	0	0	5.8	AR, T=Intraspecific taxon
Aletes lithophilus	0	0	0	1	0	FS, BLM	G3 S3	G3 S0.6	R	1	0	5.6	FS Region 3 Carson NF, habitat risks[2]
Machaeranthera coloradoensis	0	0	1	0	0		G3 S3	G3 S0.6	R	1	0	5.6	some alpine
Machaeranthera coloradoensis	0	0	1	0	0		G3 S3	G3 S0.6	R	1	0	5.6	some alpine
Astragalus iodopetalus	0	0	0	0	0		G2 S1	G4 S1	R	0	0	5	unconfirmed recreation risks to habitat
Caesalpinia repens	0	0	0	0	0		G2 S1	G4 S1	R	0	0	5	
Calochortus ciscoensis	0	0	0	0	0		G2 S1	G4 S1	R	0	0	5	
Cymopterus duchesnensis	0	0	0	1	0		G3 S1	G3 S1	R	0	0	5	
Eriogonum acaule	0	0	0	1	0		G3 S1	G3 S1	R	0	0	5	
Eriogonum ephedroides	0	0	0	1	0	FS, BLM	G3 S1	G3 S1	R	0	0	5	not listed Sensitive with FS
Fastuca hallii	0	0	1	0	0		G4 S1	G2 S1	R	1	0	5	subalpine and alpine
Festuca hallii	0	0	1	0	0		G4 S1	G2 S1	R	1	0	5	subalpine and alpine
Gilia stenothyrsa	0	0	0	1	0		G3 S1	G3 S1	R	0	0	5	
Limnorchis zothecina	0	0	0	0	0		G2 S1	G4 S1	R	0	0	5	
Mertensia humilis	0	0	0	0	0	FS, BLM	G2 S1	G4 S1	R	0	0	5	present but not listed Sensitive FS, BLM
Salix serissima	0	0	1	0	R (1)		G4 S1	G2 S1	R	0	0	5	
Salix serissima	0	0	1	0	R (1)		G4 S1	G2 S1	R	0	0	5	
Astragalus cronquistii	0	0	0	0	0		G2 S2	G4 S0.8	R	0	0	4.8	
Astragalus sparsiflorus	0	0	0	0	0		G2 S2	G4 S0.8	R	0	0	4.8	
Bolophyta ligulata	0	0	0	1	0		G3 S2	G3 S0.8	R	0	0	4.8	
Carex stenoptila	0	0	0	0	0		G2 S2	G4 S0.8	R	0	0	4.8	

[1] Ladyman, 2005, [2]Rondeau et al., 2011, [3]Beardsley and Steingraeber, 2013, [4]Panjabi et al., [5]Decker, 2006, 2011, [6]Anderson, 2004, [7]Reveal 2003, [8]Anderson 2006, [9]Coles 2003, [10]O'Kane 1988, [11]Jennings et al. 1997, [12]CNHP, 2013

Key

- USFS Region 2
- USFS Region 4
- CNHP Tracked

	Federal Status	USFS Region 4	USFS Region 2	BLM	CO NPS Unit	Multiple Agencies	CNHP Global Ranking State Ranking	Strategic Precaution Rarity Score	Hort Feasibility	Alpine/At Risk Habitat	Endemic	Score	Commments
Eriogonum contortum	0	0	0	1	0		G3 S2	G3 S0.8	R	0	0	4.8	
Eriogonum exilifolium	0	0	1	0	0		G3 S2	G3 S0.8	R	0	0	4.8	
Eriogonum exilifolium	0	0	1	0	0		G3 S2	G3 S0.8	R	0	0	4.8	
Lepidium crenatum	0	0	0	0	0		G2 S2	G4 S0.8	R	0	0	4.8	
Oreocarya rollinsii	0	0	0	1	0		G3 S2	G3 S0.8	R	0	0	4.8	
Pediomelum aromaticum	0	0	0	1	0		G3 S2	G3 S0.8	R	0	0	4.8	
Penstemon acaulis var. yampaensis	0	0	0	0	0		G3T2 S2	GT4 S0.8	R	0	0	4.8	T=Intraspecific taxon
Potentilla ambigens	0	0	0	0	0		G3 S2	G3 S0.8	R	1	0	4.8	Identified risks[7]stochastic events
Primula egaliksensis	0	1	1	0	0		G4 S2	G2 S0.8	R	0	0	4.8	unconfirmed risk hydrologic alterations
Primula egaliksensis	0	1	1	0	0		G4 S2	G2 S0.8	R	0	0	4.8	unconfirmed risk hydrologic alterations
Primula egaliksensis	0	1	1	0	0		G4 S2	G2 S0.8	R	0	0	4.8	unconfirmed risk hydrologic alterations
Sisyrinchium pallidum	0	0	0	1	0		G3 S2	G3 S0.8	R	0	0	4.8	unconfirmed risk hydrologic alterations
Thelypodiopsis juniperorum	0	0	0	0	0		G2 S2	G4 S0.8	R	0	0	4.8	
Townsendia fendleri	0	0	0	0	0		G2 S2	G4 S0.8	R	0	0	4.8	general habitat risks not specific
Gilia penstemonoides	0	0	0	0	0		G3 S3	G3 S0.6	R	0	1	4.6	
Penstemon idahoensis	0	1	0	0	0		G3 S3	G3 S0.6	R	0	0	4.6	
Nuttallia multicaulis	0	0	0	0	0		G3 S3	G3 S0.6	R	1	0	4.6	Identified risks habitat[12]
Nuttallia speciosa	0	0	0	0	0		G3 S3	G3 S0.6	R	1	0	4.6	Identified risks habitat[12]
Oenothera harringtonii	0	0	0	0	0		G3 S3	G3 S0.6	R	0	1	4.6	
Oreocarya longiflora	0	0	0	0	0		G3 S3	G3 S0.6	R	1	0	4.6	Identified risks habitat[12]
Penstemon retrorsus	0	0	0	0	0		G3 S3	G3 S0.6	R	0	1	4.6	
Sullivantia hapemanii var. purpusii	0	0	0	0	0		G3T3 S3	GT3 S0.6	R	0	1	4.6	T=Intraspecific taxon
Astragalus nelsonianus	0	0	0	0	0		G3 S1	G3 S1	R	0	0	4	
Aletes nuttallii	0	0	0	0	0		G3 S1	G3 S1	R	0	0	4	
Aletes sessiliflorus	0	0	0	0	0		G3 S1	G3 S1	R	0	0	4	
Astragalus jejunus	0	0	0	0	0		G3 S1	G3 S1	R	0	0	4	
Astragalus musiniensis	0	0	0	0	0		G3 S1	G3 S1	R	0	0	4	
Draba ventosa	0	0	0	0	0		G3 S1	G3 S1	R	0	0	4	
Eriogonum saurinum	0	0	0	0	0		G4T3 S1	GT3 S1	R	0	0	4	
Eriogonum scabrellum	0	0	0	0	0		G3 S1	G3 S1	R	0	0	4	
Frasera paniculata	0	0	0	1	0		G4 S1	G2 S1	R	0	0	4	
Mentzelia sivinskii	0	0	0	0	0		G3 S1	G3 S1	R	0	0	4	

[1] Ladyman, 2005, [2]Rondeau et al., 2011, [3]Beardsley and Steingraeber, 2013, [4]Panjabi et al., [5]Decker, 2006, 2011, [6]Anderson, 2004, [7]Reveal 2003, [8]Anderson 2006, [9]Coles 2003, [10]O'Kane 1988, [11]Jennings et al. 1997, [12]CNHP, 2013

Key

- USFS Region 2
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	Federal Status	USFS Region 4	USFS Region 2	BLM	CO NPS Unit	Multiple Agencies	CNHP Global Ranking State Ranking	Strategic Precaution Rarity Score	Hort Feasibility	Alpine/At Risk Habitat	Endemic	Score	Commments
Oenothera coloradensis ssp. neomexican	0	0	0	0	0		G3T3 S1	GT3 S1	R	0	0	4	
Oonopsis wardii	0	0	0	0	0		G3 S1	G3 S1	R	0	0	4	
Oreocarya mensana	0	0	0	0	0		G3 S1	G3 S1	R	0	0	4	
Penstemon angustifolius var. vernalensis	0	0	0	0	0		G5T3 S1	GT3 S1	R	0	0	4	T=Intraspecific taxon
Proatriplex pleiantha	0	0	0	0	0		G3 S1	G3 S1	R	0	0	4	
Townsendia strigosa	0	0	0	1	0		G4 S1	G2 S1	R	0	0	4	
Trifolium andinum	0	0	0	0	0		G3 S1	G3 S1	R	0	0	4	
Astragalus detritalis	0	0	0	0	0		G3 S2	G3 S0.8	R	0	0	3.8	
Astragalus leptaleus	0	0	1	0	0		G4 S2	G2 S0.8	R	0	0	3.8	
Astragalus leptaleus	0	0	1	0	0		G4 S2	G2 S0.8	R	0	0	3.8	
Astragalus proximus	0	0	1	0	0		G4 S2	G2 S0.8	R	0	0	3.8	
Astragalus proximus	0	0	1	0	0		G4 S2	G2 S0.8	R	0	0	3.8	
Calochortus flexuosus	0	0	1	0	0		G4 S2	G2 S0.8	R	0	0	3.8	
Calochortus flexuosus	0	0	1	0	0		G4 S2	G2 S0.8	R	0	0	3.8	
Carex oreocharis	0	0	0	0	0		G3 S2	G3 S0.8	R	0	0	3.8	
Cirsium ownbeyi	0	0	0	0	0		G3 S2	G3 S0.8	R	0	0	3.8	
Erigeron nematophyllus	0	0	0	0	0		G3 S2	G3 S0.8	R	0	0	3.8	
Gilia haydenii	0	0	0	0	0		G3 S2	G3 S0.8	R	0	0	3.8	
Kobresia simpliciuscula	0	0	1	0	0		G5 S2	G1 S0.8	R	1	0	3.8	
Kobresia simpliciuscula	0	0	1	0	0		G5 S2	G1 S0.8	R	1	0	3.8	
Oreocarya caespitosa	0	0	0	1	0		G4 S2	G2 S0.8	R	0	0	3.8	
Oreocarya elata	0	0	0	0	0		G3 S2	G3 S0.8	R	0	0	3.8	
Oreocarya stricta	0	0	0	0	0		G3 S2	G3 S0.8	R	0	0	3.8	
Physaria subumbellata	0	0	0	0	0		G3 S2	G3 S.08	R	0	0	3.8	
Stanleya albescens	0	0	0	0	0		G3 S2	G3 S0.8	R	0	0	3.8	
Alsinanthe macrantha	0	0	0	0	0		G3 S3	G3 S0.6	R	0	0	3.6	
Ambrosia linearis	0	0	0	0	0		G3 S3	G3 S0.6	R	0	0	3.6	
Argillochloa dasyclada	0	0	0	0	0		G3 S3	G3 S0.6	R	0	0	3.6	
Argyrochosma fendleri	0	0	0	0	0		G3 S3	G3 S0.6	R	0	0	3.6	

[1] Ladyman, 2005, [2]Rondeau et al., 2011, [3]Beardsley and Steingraeber, 2013, [4]Panjabi et al., [5]Decker, 2006, 2011, [6]Anderson, 2004, [7]Reveal 2003, [8]Anderson 2006, [9]Coles 2003, [10]O'Kane 1988, [11]Jennings et al. 1997, [12]CNHP, 2013

Key

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Species	Federal Status	USFS Region 4	USFS Region 2	BLM	CO NPS Unit	Multiple Agnecies	CNHP Global Ranking State Ranking	Strategic Precaution Rarity Score	Hort Feasibility	Alpine/At Risk Habitat	Endemic	Score	Commments
Artemisia parryi	0	0	0	0	0		G3 S3	G3 S0.6	R	0	0	3.6	
Asclepias hallii	0	0	0	0	0		G3 S3	G3 S0.6	R	0	0	3.6	
Astragalus wetherillii	0	0	0	0	0		G3 S3	G3 S0.6	R	0	0	3.6	
Boechera gunnisoniana	0	0	0	0	0		G3 S3	G3 S0.6	R	0	0	3.6	
Boechera oxylobula	0	0	0	0	0		G3 S3	G3 S0.6	R	0	0	3.6	
Bolophyta alpina	0	0	0	0	0		G3 S3	G3 S0.6	R	0	0	3.6	
Bolophyta tetraneuris	0	0	0	0	0		G3 S3	G3 S0.6	R	0	0	3.6	
Draba streptobrachia	0	0	0	0	0		G3 S3	G3 S0.6	R	0	0	3.6	
Luzula subcapitata	0	0	0	0	0		G3 S3	G3 S0.6	R	0	0	3.6	
Nuttallia sinuata	0	0	0	0	0		G3 S3	G3 S0.6	R	0	0	3.6	
Oreocarya weberi	0	0	0	0	0		G3 S3	G3 S0.6	R	0	0	3.6	
Phacelia splendens	0	0	0	0	0		G3 S3	G3 S0.6	R	0	0	3.6	
Physaria calcicola	0	0	0	0	0		G3 S3	G3 S0.6	R	0	0	3.6	
Physaria vitulifera	0	0	0	0	0		G3 S3	G3 S.06	R	0	0	3.6	
Abronia nana	0	0	0	0	0		G4 S1	G2 S1	R	0	0	3	
Arnica alpina var. tomentosa	0	0	0	0	0		G5T5 S1	GT1 S1	R	1	0	3	T=Intraspecific taxon
Asclepias macrosperma	0	0	0	0	0		G4 S1	G2 S1	R	0	0	3	
Asplenium trichomanes - ramosum	0	0	0	0	0		G4 S1	G2 S1	R	0	0	3	
Aster alpinus var. vierhapperi	0	0	0	0	0		G5T5 S1	GT1 S1	R	1	0	3	T=Intraspecific taxon
Astragalus cibarius	0	0	0	0	0		G4 S1	G2 S1	R	0	0	3	
Camissonia andina	0	0	0	0	0		G4 S1	G2 S1	R	0	0	3	
Carex diandra	0	0	1	0	0		G5 S1	G1 S1	R	0	0	3	
Carex diandra	0	0	1	0	0		G5 S1	G1 S1	R	0	0	3	
Carex livida	0	0	1	0	0		G5 S1	G1 S1	R	0	0	3	
Carex livida	0	0	1	0	0		G5 S1	G1 S1	R	0	0	3	
Carex molesta	0	0	0	0	0		G4 S1	G2 S1	R	0	0	3	
Carex sychnocephala	0	0	0	0	0		G4 S1	G2 S1	R	0	0	3	

[1] Ladyman, 2005, [2]Rondeau et al., 2011, [3]Beardsley and Steingraeber, 2013, [4]Panjabi et al., [5]Decker, 2006, 2011, [6]Anderson, 2004, [7]Reveal 2003, [8]Anderson 2006, [9]Coles 2003, [10]O'Kane 1988, [11]Jennings et al. 1997, [12]CNHP, 2013

Key

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	Federal Status	USFS Region 4	USFS Region 2	BLM	CO NPS Unit	Multiple Agencies	CNHP Global Ranking State Ranking	Strategic Precaution Rarity Score	Hort Feasibility	Alpine/At Risk Habitat	Endemic	Score	Commments
Carex torreyi	0	0	0	0	0		G4 S1	G2 S1	R	0	0	3	
Ceanothus martinii	0	0	0	0	0		G4 S1	G2 S1	R	0	0	3	
Cheilanthes standleyi	0	0	0	0	0		G4 S1	G2 S1	R	0	0	3	
Drosera anglica	0	0	1	0	0		G5 S1	G1 S1	R	0	0	3	
Drosera anglica	0	0	1	0	0		G5 S1	G1 S1	R	0	0	3	
Drymaria effusa var. depressa	0	0	0	0	0		G4T4 S1	GT2 S1	R	0	0	3	
Eriogonum palmerianum	0	0	0	0	0		G4 S1	G2 S1	R	0	0	3	
Eriophorum chamissonis	0	0	1	0	0		G5 S1	G1 S1	R	0	0	3	
Eriophorum chamissonis	0	0	1	0	0		G5 S1	G1 S1	R	0	0	3	
Halimolobos virgata	0	0	0	0	0		G4 S1	G2 S1	R	0	0	3	
Hermidium alipes var. pallidum	0	0	0	0	0		G4 S1	G2 S1	R	0	0	3	
Juncus bryoides	0	0	0	0	0		G4 S1	G2 S1	R	0	0	3	
Liatris lancifolia	0	0	0	0	0		G4 S1	G2 S1	R	0	0	3	
Nama dichotomum	0	0	0	0	0		G4 S1	G2 S1	R	0	0	3	
Nama hispidum	0	0	0	0	0		G4 S1	G2 S1	R	0	0	3	
Oenothera engelmannii	0	0	0	0	0		G4 S1	G2 S1	R	0	0	3	
Oenothera longissima	0	0	0	0	0		G4 S1	G2 S1	R	0	0	3	
Oreocarya breviflora	0	0	0	0	0		G4 S1	G2 S1	R	0	0	3	
Orophaca aretioides	0	0	0	0	0		G4 S1	G2 S1	R	0	0	3	
Packera debilis	0	0	0	0	0		G4 S1	G2 S1	R	0	0	3	
Pediomelum cuspidatum	0	0	0	0	0		G4 S1	G2 S1	R	0	0	3	
Penstemon eriantherus	0	0	0	0	0		G4 S1	G2 S1	R	0	0	3	
Penstemon jamesii	0	0	0	0	0		G4 S1	G2 S1	R	0	0	3	
Phacelia constancei	0	0	0	0	0		G4 S1	G2 S1	R	0	0	3	
Phacelia integrifolia	0	0	0	0	0		G4 S1	G2 S1	R	0	0	3	
Physaria arenosa ssp. argillosa	0	0	0	0	0		G5T4 S1	GT2 S1	R	0	0	3	T=Intraspecific taxon
Polystichum scopulinum	0	0	0	0	0		G4 S1	G2 S1	R	0	0	3	
Salix myrtillifolia	0	0	1	0	0		G5 S1	G1 S1	R	0	0	3	
Salix myrtillifolia	0	0	1	0	0		G5 S1	G1 S1	R	0	0	3	

[1] Ladyman, 2005, [2]Rondeau et al., 2011, [3]Beardsley and Steingraeber, 2013, [4]Panjabi et al., [5]Decker, 2006, 2011, [6]Anderson, 2004, [7]Reveal 2003, [8]Anderson 2006, [9]Coles 2003, [10]O'Kane 1988, [11]Jennings et al. 1997, [12]CNHP, 2013

Key

- USFS Region 2 (light green)
- USFS Region 4 (dark green)
- CNHP Tracked (light blue)

Species	Federal Status	USFS Region 4	USFS Region 2	BLM	CO NPS Unit	Multiple Agencies	CNHP Global Ranking State Ranking	Strategic Precaution Rarity Score	Hort Feasibility	Alpine/At Risk Habitat	Endemic	Score	Commments
Seriphidium pygmaeum	0	0	0	0	0		G4 S1	G2 S1	R	0	0	3	
Spatularia foliolosa	0	0	0	0	0		G4 S1	G2 S1	R	0	0	3	
Thelypodium sagittatum	0	0	0	0	0		G4 S1	G2 S1	R	0	0	3	
Abronia carletonii	0	0	0	0	0		G4 S2	G2 S0.8	R	0	0	2.8	
Aletes tenuifolius	0	0	0	0	0		G4 S2	G2 S0.8	R	0	0	2.8	
Allium nevadense	0	0	0	0	0		G4 S2	G2 S0.8	R	0	0	2.8	
Amsonia jonesii	0	0	0	0	0		G4 S2	G2 S0.8	R	0	0	2.8	
Asclepias macrotis	0	0	0	0	0		G4 S2	G2 S0.8	R	0	0	2.8	
Astragalus bodinii	0	0	0	0	0		G4 S2	G2 S0.8	R	0	0	2.8	
Boechera fernaldiana	0	0	0	0	0		G4 S2	G2 S0.8	R	0	0	2.8	
Cryptogramma stelleri	0	0	0	1	0		G5 S2	G1 S0.8	R	0	0	2.8	
Drosera rotundifolia	0	0	1	0	0		G5 S2	G1 S0.8	R	0	0	2.8	
Drosera rotundifolia	0	0	1	0	0		G5 S2	G1 S0.8	R	0	0	2.8	
Forsellesia planitierum	0	0	0	0	0		G4 S2	G2 S0.8	R	0	0	2.8	
Grindelia arizonica	0	0	0	0	0		G4 S2	G2 S0.8	R	0	0	2.8	
Listera borealis	0	0	0	0	0		G4 S2	G2 S0.8	R	0	0	2.8	
Nuttallia pterosperma	0	0	0	0	0		G4 S2	G2 S0.8	R	0	0	2.8	
Parnassia kotzebuei	0	0	1	0	0		G5 S2	G1 S0.8	R	0	0	2.8	
Parnassia kotzebuei	0	0	1	0	0		G5 S2	G1 S0.8	R	0	0	2.8	
Penstemon utahensis	0	0	0	0	0		G4 S2	G2 S0.8	R	0	0	2.8	
Salix candida	0	0	1	0	0		G5 S2	G1 S0.8	R	0	0	2.8	
Salix candida	0	0	1	0	0		G5 S2	G1 S0.8	R	0	0	2.8	
Trichophorum pumilum	0	0	0	1	0		G5 S2	G1 S0.8	R	0	0	2.8	up to 11,000 ft
Utricularia minor	0	0	1	0	0		G5 S2	G1 S0.8	R	0	0	2.8	
Utricularia minor	0	0	1	0	0		G5 S2	G1 S0.8	R	0	0	2.8	
Astragalus oocalycis	0	0	0	0	0		G4 S3	G2 S0.6	R	0	0	2.6	Watchlist
Draba fladnizensis	0	0	0	0	0		G4 S3	G2 S0.6	R	0	0	2.6	Watchlist
Adiantum aleuticum	0	0	0	0	0		G5? S1	G1S1	R	0	0	2	
Allium schoenoprasum var. sibiricum	0	0	0	0	0		GT5 S1	GT1 S1	R	0	0	2	listed as Threatened, MI, MN, NH

[1] Ladyman, 2005, [2]Rondeau et al., 2011, [3]Beardsley and Steingraeber, 2013, [4]Panjabi et al., [5]Decker, 2006, 2011, [6]Anderson, 2004, [7]Reveal 2003, [8]Anderson 2006, [9]Coles 2003, [10]O'Kane 1988, [11]Jennings et al. 1997, [12]CNHP, 2013

Key

- USFS Region 2
- USFS Region 4
- CNHP Tracked

Species	Federal Status	USFS Region 4	USFS Region 2	BLM	CO NPS Unit	Multiple Agencies	CNHP Global Ranking State Ranking	Strategic Precaution Rarity Score	Hort Feasibility	Alpine/At Risk Habitat	Endemic	Score	Commments
Alsinanthe stricta	0	0	0	0	0		G5 S1	G1 S1	R	0	0	2	
Anagallis minima	0	0	0	0	0		G5 S1	G1 S1	R	0	0	2	
Apios americana	0	0	0	0	0		G5 S1	G1 S1	R	0	0	2	
Aristida basiramea	0	0	0	0	0		G5 S1	G1 S1	R	0	0	2	
Armeria scabra ssp. sibirica	0	0	0	0	0		G5T5 S1	GT1 S1	R	0	0	2	T=Intraspecific taxon
Asclepias involucrata	0	0	0	0	0		G5 S1	G1 S1	R	0	0	2	
Asplenium adiantum - nigrum	0	0	0	0	0		G5 S1	G1 S1	R	0	0	2	
Asplenium platyneuron	0	0	0	0	0		G5 S1	G1 S1	R	0	0	2	
Asplenium resiliens	0	0	0	0	0		G5 S1	G1 S1	R	0	0	2	
Astragalus newberryi	0	0	0	0	0		G5 S1	G1 S1	R	0	0	2	
Astragalus plattensis	0	0	0	0	0		G5 S1	G1 S1	R	0	0	2	
Besseya wyomingensis	0	0	0	0	0		G5 S1	G1 S1	R	0	0	2	
Betula papyrifera	0	0	0	0	0		G5 S1	G1 S1	R	0	0	2	
Bothriochloa springfieldii	0	0	0	0	0		G5 S1	G1 S1	R	0	0	2	
Botrypus virginianus ssp. europaeus	0	0	0	0	0		G5 S1	G1 S1	R	0	0	2	
Bupleurum triradiatum ssp. arcticum	0	0	0	0	0		G5 S1	G1 S1	R	0	0	2	
Callitriche heterophylla	0	0	0	0	0		G5 S1	G1 S1	R	0	0	2	
Carex concinna	0	0	0	0	0		G5 S1	G1 S1	R	0	0	2	
Carex conoidea	0	0	0	0	0		G5 S1	G1 S1	R	0	0	2	
Carex crawei	0	0	0	0	0		G5 S1	G1 S1	R	0	0	2	
Carex lasiocarpa	0	0	0	0	0		G5 S1	G1 S1	R	0	0	2	
Carex peckii	0	0	0	0	0		G5 S1	G1 S1	R	0	0	2	
Carex retrorsa	0	0	0	0	0		G5 S1	G1 S1	R	0	0	2	
Carex saximontana	0	0	0	0	0		G5 S1	G1 S1	R	0	0	2	
Carex tenuiflora	0	0	0	0	0		G5 S1	G1 S1	R	0	0	2	
Carex viridula	0	0	0	0	0		G5 S1	G1 S1	R	0	0	2	
Centaurium exaltatum	0	0	0	0	0		G5 S1	G1 S1	R	0	0	2	
Chamaesyce parryi	0	0	0	0	0		G5 S1	G1 S1	R	0	0	2	
Cheilanthes wootonii	0	0	0	0	0		G5 S1	G1 S1	R	0	0	2	

[1] Ladyman, 2005, [2]Rondeau et al., 2011, [3]Beardsley and Steingraeber, 2013, [4]Panjabi et al., [5]Decker, 2006, 2011, [6]Anderson, 2004, [7]Reveal 2003, [8]Anderson 2006, [9]Coles 2003, [10]O'Kane 1988, [11]Jennings et al. 1997, [12]CNHP, 2013

Key

- USFS Region 2
- USFS Region 4
- CNHP Tracked

	Federal Status	USFS Region 4	USFS Region 2	BLM	CO NPS Unit	Multiple Agencies	CNHP Global Ranking State Ranking	Strategic Precaution Rarity Score	Hort Feasibility	Alpine/At Risk Habitat	Endemic	Score	Commments
Claytonia rubra	0	0	0	0	0		G5 S1	G1 S1	R	0	0	2	
Cleomella angustifolia	0	0	0	0	0		G5 S1	G1 S1	R	0	0	2	
Collomia grandiflora	0	0	0	0	0		G5 S1	G1 S1	R	0	0	2	
Commelina dianthifolia	0	0	0	0	0		G5 S1	G1 S1	R	0	0	2	
Conopholis alpina var. mexicana	0	0	0	0	0		G5T5 S1	G1 S1	R	0	0	2	T=Intraspecific taxon
Crataegus chrysocarpa	0	0	0	0	0		G5 S1	G1 S1	R	0	0	2	
Crocanthemum bicknellii	0	0	0	0	0		G5 S1	G1 S1	R	0	0	2	
Cylactis arctica ssp. acaulis	0	Unknown	Unknown	0	0		G5T5 S1	GT1 S1	R	0	0	2	T=Intrspecific taxon
Cystopteris montana	0	0	0	0	0		G5 S1	G1 S1	R	0	0	2	
Draba incerta	0	0	0	0	0		G5 S1	G1 S1	R	0	0	2	
Dryopteris expansa	0	0	0	0	0		G5 S1	G1 S1	R	0	0	2	
Enceliopsis nudicaulis	0	0	0	0	0		G5 S1	G1 S1	R	0	0	2	
Enneapogon desvauxii	0	0	0	0	0		G5 S1	G1 S1	R	0	0	2	
Erigeron humilis	0	0	0	0	0		G5 S1	G1 S1	R	0	0	2	
Erigeron philadelphicus	0	0	0	0	0		G5 S1	G1 S1	R	0	0	2	
Eriogonum leptocladon var. ramosissimur	0	0	0	0	0		G5T5 S1	GT1 S1	R	0	0	2	T=Intraspecific taxon
Eriogonum pauciflorum var. pauciflorum	0	0	0	0	0		G5T5 S1	G1 S1	R	0	0	2	T=Intraspecific taxon
Eucephalus perelegans	0	0	0	0	0		G5 S1	G1 S1	R	0	0	2	
Fritillaria pudica	0	0	0	0	0		G5 S1	G1 S1	R	0	0	2	
Grammica umbellata	0	0	0	0	0		G5 S1	G1 S1	R	0	0	2	
Guilleminea densa	0	0	0	0	0		G5 S1	G1 S1	R	0	0	2	
Hedyotis nigricans	0	0	0	0	0		G5 S1	G1 S1	R	0	0	2	
Heterotheca latifolia	0	0	0	0	0		G5T5 S1	GT1 S1	R	0	0	2	T=Intraspecific taxon
Heuchera richardsonii	0	0	0	0	0		G5 S1	G1 S1	R	0	0	2	
Heuchera rubescens	0	0	0	0	0		G5 S1	G1 S1	R	0	0	2	
Hippochaete variegata	0	0	0	0	0		G5 S1	G1 S1	R	0	0	2	
Hypoxis hirsuta	0	0	0	0	0		G5 S1	G1 S1	R	0	0	2	
Juncus brachycephalus	0	0	0	0	0		G5 S1	G1 S1	R	0	0	2	

[1] Ladyman, 2005, [2]Rondeau et al., 2011, [3]Beardsley and Steingraeber, 2013, [4]Panjabi et al., [5]Decker, 2006, 2011, [6]Anderson, 2004, [7]Reveal 2003, [8]Anderson 2006, [9]Coles 2003, [10]O'Kane 1988, [11]Jennings et al. 1997, [12]CNHP, 2013

Key

- USFS Region 2
- USFS Region 4
- CNHP Tracked

Species	Federal Status	USFS Region 4	USFS Region 2	BLM	CO NPS Unit	Multiple Agencies	CNHP Global Ranking State Ranking	Strategic Precaution Rarity Score	Hort Feasibility	Alpine/At Risk Habitat	Endemic	Score	Commments
Krigia biflora	0	0	0	0	0		G5 S1	G1 S1	R	0	0	2	
Liatris squarrosa var. glabrata	0	0	0	0	0		G5T5 S1	GT1 S1	R	0	0	2	T=Intraspecific taxon
Minuopsis nuttallii	0	0	0	0	0		G5 S1	G1 S1	R	0	0	2	
Muhlenbergia depauperata	0	0	0	0	0		G5 S1	G1 S1	R	0	0	2	
Myriophyllum verticillatum	0	0	0	0	0		G5 S1	G1 S1	R	0	0	2	
Nama densum	0	0	0	0	0		G5 S1	G1 S1	R	0	0	2	
Naumburgia thyrsiflora	0	0	0	0	0		G5 S1	G1 S1	R	0	0	2	
Nitrophila occidentalis	0	0	0	0	0		G5 S1	G1 S1	R	0	0	2	
Nolina texana	0	0	0	0	0		G5 S1	G1 S1	R	0	0	2	
Orophaca triphylla	0	0	0	0	0		G5 S1	G1 S1	R	0	0	2	
Oxybaphus decumbens	0	0	0	0	0		G5 S1	G1 S1	R	0	0	2	
Oxytropis parryi	0	0	0	0	0		G5 S1	G1 S1	R	0	0	2	
Packera paupercula	0	0	0	0	0		G5 S1	G1 S1	R	0	0	2	
Penstemon radicosus	0	0	0	0	0		G5 S1	G1 S1	R	0	0	2	
Physaria brassicoides	0	0	0	0	0		G5 S1	G1 S1	R	0	0	2	
Poliomintha incana	0	0	0	0	0		G5 S1	G1 S1	R	0	0	2	
Portulaca parvula	0	0	0	0	0		G5 S1	G1 S1	R	0	0	2	
Potamogeton diversifolius	0	0	0	0	0		G5 S1	G1 S1	R	0	0	2	
Prunus angustifolia	0	0	0	0	0		G5 S1	G1 S1	R	0	0	2	
Rhynchospora alba	0	0	0	0	0		G5 S1	G1 S1	R	0	0	2	
Rotala ramosior	0	0	0	0	0		G5 S1	G1 S1	R	0	0	2	
Sagittaria graminea	0	0	0	0	0		G5 S1	G1 S1	R	0	0	2	
Salix nigra	0	0	0	0	0		G5 S1	G1 S1	R	0	0	2	
Sapindus drummondii	0	0	0	0	0		G5 S1	G1 S1	R	0	0	2	
Schoenoplectus saximontanus	0	0	0	0	0		G5 S1	G1 S1	R	0	0	2	
Sporobolus flexuosus	0	0	0	0	0		G5 S1	G1 S1	R	0	0	2	
Sporobolus nealleyi	0	0	0	0	0		G5 S1	G1 S1	R	0	0	2	
Stillingia sylvatica	0	0	0	0	0		G5 S1	G1 S1	R	0	0	2	
Stipa richardsonii	0	0	0	0	0		G5 S1	G1 S1	R	0	0	2	

[1] Ladyman, 2005, [2]Rondeau et al., 2011, [3]Beardsley and Steingraeber, 2013, [4]Panjabi et al., [5]Decker, 2006, 2011, [6]Anderson, 2004, [7]Reveal 2003, [8]Anderson 2006, [9]Coles 2003, [10]O'Kane 1988, [11]Jennings et al. 1997, [12]CNHP, 2013

Key

- USFS Region 2
- USFS Region 4
- CNHP Tracked

Species	Federal Status	USFS Region 4	USFS Region 2	BLM	CO NPS Unit	Multiple Agencies	CNHP Global Ranking State Ranking	Strategic Precaution Rarity Score	Hort Feasibility	Alpine/At Risk Habitat	Endemic	Score	Comments
Subularia aquatica		0	0	0	0	0	G5 S1	G1 S1	R	0	0	2	
Trautvetteria caroliniensis		0	0	0	0	0	G5 S1	G1 S1	R	0	0	2	
Trifolium kingii		0	0	0	0	0	G5 S1	G1 S1	R	0	0	2	
Truellum sagittatum		0	0	0	0	0	G5 S1	G1 S1	R	0	0	2	
Unamia alba		0	0	0	0	0	G5 S1	G1 S1	R	0	0	2	
Utricularia intermedia		0	0	0	0	0	G5 S1	G1 S1	R	0	0	2	
Vernonia baldwinii ssp. interior		0	0	0	0	0	G5T5 S1	GT1 S1	R	0	0	2	
Virgulus novae - angliae		0	0	0	0	0	G5 S1	G1 S1	R	0	0	2	
Woodsia plummerae		0	0	0	0	0	G5 S1	G1 S1	R	0	0	2	
Zosterella dubia		0	0	0	0	0	G5 S1	G1 S1	R	0	0	2	
Adiantum capillus - veneris		0	0	0	0	D,M(1)	G5 S2	G1 S0.8	R	0	0	1.8	
Allionia incarnata		0	0	0	0	0	G5 S2	G1 S0.8	R	0	0	1.8	
Amorpha nana		0	0	0	0	0	G5 S2	G1 S0.8	R	0	0	1.8	
Azaleastrum albiflorum		0	0	0	0	0	G5 S2	G1 S0.8	R	0	0	1.8	
Bergia texana		0	0	0	0	0	G5 S2	G1 S0.8	R	0	0	1.8	
Braya humilis		0	0	0	0	0	G5 S2	G1 S0.8	R	0	0	1.8	
Carex leporinella		0	0	0	0	0	G5 S2	G1 S0.8	R	0	0	1.8	
Carex leptalea		0	0	0	0	0	G5 S1	G1 S0.8	R	0	0	1.8	
Carex limosa		0	0	0	0	0	G5 S2	G1 S0.8	R	0	0	1.8	
Carex scirpoidea		0	0	0	0	0	G5 S2	G1 S0.8	R	0	0	1.8	
Carex sprengelii		0	0	0	0	0	G5 S2	G1 S0.8	R	0	0	1.8	
Draba oligosperma		0	0	0	0	0	G5 S2	G1 S0.8	R	0	0	1.8	
Elatine rubella		0	0	0	0	0	G5 S2	G1 S0.8	R	0	0	1.8	
Elatine triandra		0	0	0	0	0	G5 S2	G1 S0.8	R	0	0	1.8	
Geranium bicknellii		0	0	0	0	0	G5 S2	G1 S0.8	R	0	0	1.8	
Lewisia rediviva		0	0	0	0	0	G5 S2	G1 S0.8	R	0	0	1.8	
Listera convallarioides		0	0	0	0	0	G5 S2	G1 S0.8	R	0	0	1.8	
Lobelia cardinalis		0	0	0	0	0	G5 S2	G1 S0.8	R	0	0	1.8	

[1] Ladyman, 2005, [2]Rondeau et al., 2011, [3]Beardsley and Steingraeber, 2013, [4]Panjabi et al., [5]Decker, 2006, 2011, [6]Anderson, 2004, [7]Reveal 2003, [8]Anderson 2006, [9]Coles 2003, [10]O'Kane 1988, [11]Jennings et al. 1997, [12]CNHP, 2013

Key

- USFS Region 2
- USFS Region 4
- CNHP Tracked

	Federal Status	USFS Region 4	USFS Region 2	BLM	CO NPS Unit	Multiple Agnecies	CNHP Global Ranking State Ranking	Strategic Precaution Rarity Score	Hort Feasibility	Alpine/At Risk Habitat	Endemic	Score	Commments
Lomatogonium rotatum	0	0	0	0	0		G5 S2	G1 S0.8	R	0	0	1.8	
Mimulus lewisii	0	0	0	0	0		G5 S2	G1 S0.8	R	0	0	1.8	
Muscaria monticola	0	0	0	0	0		G5T5 S2	GT1 S0.8	R	0	0	1.8	T=Intraspecific taxon
Oreocarya cana	0	0	0	0	0		G5 S2	G1 S0.8	R	0	0	1.8	
Pellaea breweri	0	0	0	0	0		G5 S2	G1 S0.8	R	0	0	1.8	
Pellaea wrightiana	0	0	0	0	0		G5 S2	G1 S0.8	R	0	0	1.8	
Phacelia demissa	0	0	0	0	0		G5 S2	G1 S.08	R	0	0	1.8	
Phippsia algida	0	0	0	0	0		G5 S2	G1 S0.8	R	0	0	1.8	
Ribes americanum	0	0	0	0	0		G5 S2	G1 S0.8	R	0	0	1.8	
Sisyrinchium demissum	0	0	0	0	0		G5 S2	G1 S0.8	R	0	0	1.8	
Sparganium eurycarpum	0	0	0	0	0		G5 S2	G1 S0.8	R	0	0	1.8	
Viola pedatifida	0	0	0	0	0		G5 S2	G1 S0.8	R	0	0	1.8	

[1] Ladyman, 2005, [2]Rondeau et al., 2011, [3]Beardsley and Steingraeber, 2013, [4]Panjabi et al., [5]Decker, 2006, 2011, [6]Anderson, 2004, [7]Reveal 2003, [8]Anderson 2006, [9]Coles 2003, [10]O'Kane 1988, [11]Jennings et al. 1997, [12]CNHP, 2013

Common Names Colorado Rare Species and Species of Concern

Scientific Name	Common Name:
Abronia carletonii	Carleton's sand verbena
Abronia nana	drawf sand verbena
Abutilon incanum	hoary mallow
Acer grandidentatum	bigtooth maple
Acorus calamus	sweet flag
Adiantum aleuticum	Aleutian maidenhair fern
Adiantum capillus - veneris	southern maidenhair
Agastache foeniculum	lavender hyssop
Agoseris lackschewitzii	Mill Creek agoseris
Aletes anisatus	Rocky Mountain aletes
Aletes humilis	Larimer aletes
Aletes latilobus	Canyonlands aletes, lomatium
Aletes lithophilus	Rock-loving neoparrya
Aletes macdougalii ssp. breviradiatus	Mesa Verde aletes
Aletes nuttallii	dog parsley
Aletes sessiliflorus	New Mexico false carrot
Aletes tenuifolius	slender parsley
Aliciella caespitosa	Rabbit Valley gilia
Aliciella sedifolia	Stonecrop gilia
Aliciella sedifolia	Stonecrop gilia
Allionia incarnata	trailing windmills
Allium geyeri var. chatterleyi	Geyer's onion
Allium madidum	swamp onion
Allium nevadense	Nevada onion
Allium schoenoprasum var. sibiricum	wild chives
Allium tolmiei var. persimile	sevendevils onion
Allotropa virgata	sugarstick

Alsinanthe macrantha	House's stichwort
Alsinanthe stricta	bog stichwort
Ambrosia linearis	plains ragweed
Amerorchis rotundifolia	roundleaf orchid
Amorpha nana	dwarf false indigo
Amsonia jonesii	Jones' bluestar
Anagallis minima	chaffweed
Androsace chamaejasme ssp. carinata	sweetflower rockjasmine
Anemone riparia	tall thimbleweed
Angelica scabrida	Charleston Mountain angelica
Angelica wheeleri	Utah angelica
Antennaria arcuata	box pussytoes
Antennaria soliceps	Charleston Mountain pussytoes
Anticlea vaginatus	Alcove death camus
Apios americana	groundnut
Aquilegia chrysantha var. rydbergii	golden columbine
Aquilegia flavescens var. rubicunda	yellow columbine
Aquilegia grahamii	Grahm's columbine
Aquilegia laramiensis	Larimie columbine
Aquilegia micrantha var. mancosana	Mancos columbine
Aquilegia saximontana	Rocky Mountain columbine
Aralia racemosa	American spikenard
Aralia racemosa ssp. bicrenata	American spikenard
Arenaria kingii ssp. Rosea	King's rosy sandwort
Argillochloa dasyclada	oil shale fescue
Argyrochosma fendleri	Fendler cloak-fern
Argythamnia humilis	low silverbush
Aristida basiramea	forked threeawn
Armeria maritima ssp. Sibirica	Siberian sea thrift
Armeria scabra ssp. sibirica	sea pink
Arnica alpina var. tomentosa	narrowleaf arnica
Artemisia campestris ssp. borealis var. petiolata	field sagewort
Artemisia parryi	Parry's wormwood

Artemisia pattersonii	Patterson's wormwood
Asclepias eastwoodiana	Eastwood milkvetch
Asclepias hallii	Hall's milkweed
Asclepias involucrata	dwarf milkweed
Asclepias macrosperma	dwarf milkweed
Asclepias macrotis	longhood milkweed
Asclepias oenotheroides	zizotes milkweed
Asclepias stenophylla	slimleaf milkweed
Ascelpias unicalis	dwarf milkweed
Asclepias uncialis ssp. uncialis	drawf milkweed
Askellia nana	drawf hawksbeard
Asplenium adiantum - nigrum	black spleenwort
Asplenium platyneuron	ebony spleenwort
Asplenium resiliens	blackstem spleenwort
Asplenium septentrionale	forked spleenwort
Asplenium trichomanes - ramosum	green spleenwort
Aster alpinus var. vierhapperi	Viehapper's aster
Astragalus aequalis	Clokey's milkvetch
Astragalus albulus	cibola milkvetch
Astragalus americanus	American milkvetch
Astragalus amnis-amissi	Custer milkvetch
Astragalus anisus	Gunnison milkvetch
Astragalus anserinus	Goose Creek milkvetch
Astragalus aquilonius	Lemhi milkvetch
Astragalus argophyllus var. martinii	silverleaf milkvetch
Astragalus barrii	Barr's milkvetch
Astragalus bodinii	Bodin's milkvetch
Astragalus brandegeei	Brandegee milkvetch
Astragalus calycosus var. scaposus	Torrey's milkvetch
Astragalus cerussatus	powdery milkvetch
Astragalus cibarius	browse milkvetch
Astragalus coltonii var. moabensis	Moab milkvetch
Astragalus consobrinus	Bicknell's milkvetch
Astragalus cronquistii	Cronquist's milkvetch
Astragalus debequaeus	DeBeque milkvetch

Astragalus desereticus	deseret milkvetch
Astragalus deterior	Cliff Palace milkvetch
Astragalus detritalis	debris milkvetch
Astragalus diversifolius var. diversifolius	
Astragalus duchesnensis	Duchesne milkvetch
Astragalus eastwoodiae	Eastwood's milkvetch
Astragalus equisolensis	horseshoe milkvetch
Astragalus henrimontanensis	silverleaf milkvetch
Astragalus humillimus	Mancos milkvetch
Astragalus iodopetalus	violet milkvetch
Astragalus iselyi	Isely's milkvetch
Astragalus jejunus	starveling milkvetch
Astragalus johannis-howellii	Long Valley milkvetch
Astragalus lentiginosus var. latus	freckled milkvetch
Astragalus leptaleus	park milkvetch
Astragalus limnocharis var. limnocharis	Cedar Breaks milkvetch
Astragalus limnocharis var. montii	Monti's milkvetch
Astragalus limnocharis var. tabulaeus	
Astragalus linifolius	Grand Junction milkvetch
Astragalus lutosus	dragon milkvetch
Astragalus microcymbus	skiff milkvetch
Astragalus missouriensis var. humistratus	Missouri milkvetch
Astragalus molybdenus	Leadville milkvetch
Astragalus monumentalis	Monument Valley milkvetch
Astragalus musiniensis	Ferron's milkvetch
Astragalus naturitensis	naturita milkvetch
Astragalus nelsonianus	Nelson milkvetch
Astragalus newberryi	Newberry's milkvetch
Astragalus oocalycis	Arboles milkvetch
Astragalus oophorus var. clokeyanus	egg milkvetch
Astragalus oophorus var. lavinii	Lavin's milkvetch
Astragalus osterhoutii	Kremmling Osterhout milkvetch
Astragalus paysonii	Payson's milkvetch

Astragalus piscator	Fisher Towers milkvetch
Astragalus plattensis	Platte River milkvetch
Astragalus proximus	Aztec milkvetch
Astragalus rafaelensis	San Rafael milkvetch
Astragalus remotus	Spring Mountain milkvetch
Astragalus ripleyi	Ripley milkvetch
Astragalus robbinsii var. occidentalis	western milkvetch
Astragalus saurinus	Dinosaur milkvetch
Astragalus schmolliae	Chapin Mesa milkvetch
Astragalus sesquiflorus	sandstone milkvetch
Astragalus sparsiflorus	Front Range milkvetch
Astragalus toquimanus	Toquima milkvetch
Astragalus tortipes	sleeping Ute milkvetch
Astragalus uncialis	currant milkvetch
Astragalus vexilliflexus var. nubilus	White Clouds milkvetch
Astragalus wetherillii	Wetherill's milkvetch
Astragalus wootonii var. wootonii	Wooton milkvetch
Astragalus zionis var. vigulus	Zion milkvetch
Astrolepis cochisensis	Cochise scaly cloakfern
Azaleastrum albiflorum	Cascade azalea
Bergia texana	Texas bergia
Besseya ritteriana	Ritter's coraldrops
Besseya wyomingensis	Wyoming besseya
Betula papyrifera	paper birch
Boechera (=Arabis) rigidissima var. demota	a rockcress
Boechera bodiensis	a rockcress
Boechera crandallii	Crandall's rockcress
Boechera falcatoria	a rockcress
Boechera fernaldiana	park rockcress
Boechera glareosa	Dorn's rockcress
Boechera gunnisoniana	a rockcress
Boechera nevadensis	a rockcress
Boechera ophira	a rockcress
Boechera oxylobula	a rockcress

Boechera rectissima var. simulans	a rockcress
Boechera tiehmii	a rockcress
Bolophyta alpina	Wyoming feverfew
Bolophyta ligulata	ligulate feverfew
Bolophyta tetraneuris	Barneby's feverfew
Bothriochloa springfieldii	Springfield bluestem
Botrychium ascendens	upward lobed moonwort
Botrychium campestre	praire moonwort
Botrychium crenulatum	crenulate moonwort
Botrychium echo	reflected moonwort
Botrychium hesperium	western moonwort
Botrychium lanceolatum var. lanceolatum	lance-leafed moonwort
Botrychium lineare	narrowleaf grapefern
Botrychium minganense	Mingan's moonwort
Botrychium multifidum	leathery grape fern
Botrychium pallidum	pale moonwort
Botrychium paradoxum	paradox moonwort
Botrychium pinnatum	northern moonwort
Botrychium simplex	least moonwort
Botrychium spathulatum	spatulate moonwort
Botrychium tunux	moosewort
Botrypus virginianus ssp. europaeus	rattlesnake fern
Braya glabella	arctic braya
Braya humilis	alpine braya
Bupleurum triradiatum ssp. arcticum	thoroughwax
Caesalpinia repens	creeping nicker
Calamagrostis tweedyi	Tweedy's reedgrass
Callitriche heterophylla	large water starwort
Calochortus ciscoensis	Cisco sego lilly
Calochortus flexuosus	weak stemmed mariposa lilly
Camassia cusickii	Cusick's camus
Camissonia andina	mountain camissonia
Camissonia eastwoodiae	Eastwood evening primrose

Campanula aparinoides	marsh bellflower
Carex alopecoidea	foxtail sedge
Carex capitata ssp. arctogena	round headed sedge
Carex concinna	low northern sedge
Carex conoidea	openfield sedge
Carex crawei	Crawe sedge
Carex diandra	lesser panicled sedge
Carex gravida var. lunelliana	Lunell's heavy fruited sedge
Carex incurviformis	coastal sand sedge
Carex lasiocarpa	slender sedge
Carex leporinella	Sierra hare sedge
Carex leptalea	bristle stalk sedge
Carex limosa	mud sedge
Carex livida	livid sedge
Carex luzulina var. atropurpurea	woodrush sedge
Carex molesta	troublesome sedge
Carex oreocharis	grassyslope sedge
Carex peckii	Peck sedge
Carex perglobosa	globe sedge
Carex retrorsa	retrorse sedge
Carex sartwellii	Sartwell's sedge
Carex saximontana	Rocky Mountain sedge
Carex scirpoidea	Canadian single spike sedge
Carex sprengelii	Sprengel's sedge
Carex stenoptila	small winged sedge
Carex sychnocephala	many headed sedge
Carex tenuiflora	slender flower sedge
Carex tiogana	Tioga Pass Sedge
Carex torreyi	Torrey sedge
Carex viridula	green sedge
Castilleja aquariensis	Aquarius Plateau Indian paintbrush
Castilleja christii	Christ's Indian paintbrush
Castilleja lineata	marsh meadow Indian paintbrush

Castilleja parvula var. parvula	
Castilleja parvula var. revealii	Bryce Canyon Indian paintbrush
Castilleja puberula	downy Indian paintbrush
Ceanothus martinii	Utah mountain lilac
Centaurium arizonicum	Arizona centaury
Centaurium exaltatum	Great Basin centaury
Chamaesyce parryi	Parry's sandmat
Cheilanthes eatonii	Eaton's lip fern
Cheilanthes standleyi	Standley's cloak fern
Cheilanthes wootonii	Wooton's lop fern
Chenopodium cycloides	sandhill goosefoot
Chionophila jamesii	Rocky Mountain snowlover
Chrysothamnus parryi ssp. Montanus	Parry's rabbitbrush
Cirsium barnebyi	Barneby's thistle
Cirsium clavatum var. osterhoutii	
Cirsium oreophilum	meadow thistle
Cirsium ownbeyi	Ownbey's thistle
Cirsium perplexans	Adobe Hills thistle
Claytonia rubra	redstem springbeauty
Cleomella angustifolia	northern rhombo-pod
Cleomella palmerana	Rocky Mountain rhombo-pod
Collomia debilis var. camporum	alpine collomia
Collomia grandiflora	showy collomia
Commelina dianthifolia	birdbill dayflower
Conimitella williamsii	Williams bishop's cap
Conopholis alpina var. mexicana	squaw root
Corispermum navicula	North Park bugseed
Corydalis caseana spp. Brachycarpa	
Corydalis caseana ssp. brandegeei	Sierra corydalis
Crassula aquatica	water pygmyweed
Crataegus chrysocarpa	yellow hawthorn
Crataegus saligna	willow hawthorn
Crocanthemum bicknellii	frostweed
Cryptantha creutzfeldtii	Creutzfeldt's cryptantha

Cryptantha ochroleuca	yellowwhite crytantha
Cryptogramma stelleri	slender rockbrake
Cuscuta plattensis	prairie dodder
Cusickiella quadricostata	Bodie Hills cusickiella
Cylactis arctica ssp. acaulis	nagoon berry
Cymopterus beckii	featherleaf spring parsley
Cymopterus davisii	Davis' spring parsley
Cymopterus douglassii	Douglass' spring parsley
Cymopterus duchesnensis	Uinta Basin spring parsley
Cymopterus goodrichii	Toiyabe spring parsley
Cymopterus minimus	Cedar Breaks spring parsley
Cymopterus planosus	Rocky Mountain spring parsley
Cypripedium calceolus ssp. parviflorum	American yellow lady's slipper
Cypripedium fasciculatum	clustered lady's slipper
Cypripedium montanum	monatin lady's slipper
Cypripedium parviflorum	lesser yellow lady's slipper
Cystopteris montana	mountain bladder fern
Cystopteris utahensis	Utah bladder fern
Delphinium ramosum var. alpestre	Colorado larkspur
Delphinium robustum	Wahatoya Creek larkspur
Descurainia kenheilii	Heil's tansy mustard
Descurainia ramosissima	Villa Grove tansy mustard
Descurainia torulosa	mountain tansymustard
Desmodium rigidum	rigid tick trefoil
Dichanthelium acuminatum var. sericeum	Pacific panicgrass
Dicoria wetherillii	Wetherill's dicoria
Dimorphocarpa wislizeni	spectacle pod
Diphasiastrum alpinum	alpine clubmoss
Dodecatheon utahense	
Douglasia idahoensis	Idaho dwarf primrose

Draba abajoensis (D. spectabilis in A Utah Flora)	
Draba arida	desert draba
Draba asterophora var. asterophora	Lake Tahoe draba
Draba borealis	northern rockcress
Draba brachystylis	shortstyle draba
Draba burkei	snowbasin draba
Draba crassa	thick leaf whitlow grass
Draba exunguiculata	clawless draba
Draba fladnizensis	arctic draba
Draba globosa	rockcress draba
Draba graminea	San Juan draba
Draba grayana	Gray's Peak whitlow grass
Draba incerta	Yellowstone whitlow grass
Draba jaegeri Jaeger	
Draba macounii	Macoun's whitlow grass
Draba maguirei	Maguire's draba
Draba malpighiacea	whitlow grass
Draba oligosperma	woods draba
Draba oreibata var. serpentina	serpentine draba
Draba paucifructa	Charleston Mountain draba
Draba pennellii	Schell Creek draba
Draba ramulosa	Tushar Mountain draba
Draba rectifructa	mountain draba
Draba santaquinensis	
Draba smithii	Smith whitlow grass
Draba sobolifera	stolen draba
Draba streptobrachia	alpine tundra draba
Draba trichocarpa	Stanley Creek draba
Draba ventosa	Wind River draba
Draba weberi	Weber's draba
Drosera anglica	English sundew
Drosera rotundifolia	roundleaf sundew
Drymaria effusa var. depressa	spreading drymaria
Dryopteris expansa	spreading wood fern

Elatine rubella	southwestern waterwort
Elatine triandra	longstem waterwort
Eleocharis elliptica	elliptic spikerush
Enceliopsis nudicaulis	nakedstem
Enneapogon desvauxii	spike pappusgrass
Epilobium nevadense	Nevada willowherb
Epipactis gigantea	giant helleborine
Ericameria compacta	Charleston Mountain goldenbush
Ericameria crispa	crisped goldenbush
Ericameria discoidea var. linearis	whitestem goldenbush
Erigeron abajoensis	Abajo fleabane
Erigeron carringtoniae	Indian Canyon fleabane
Erigeron cavernensis	lone fleabane
Erigeron cronquistii	Cronquist's fleabane
Erigeron garrettii	Garrett's fleabane
Erigeron humilis	low fleabane
Erigeron kachinensis	Kachina daisy
Erigeron lanatus	woolly fleabane
Erigeron maguirei	Maguire's fleabane
Erigeron mancus	depauperate fleabane
Erigeron nematophyllus	needleleaf fleabane
Erigeron philadelphicus	Philadelphia fleabane
Erigeron scopulinus	Winn Falls fleabane
Erigeron untermannii	Indian Canyon fleabane
Erigeron wilkenii	Wilken fleabane
Eriogonum acaule	sinlge stemmed wild buckwheat
Eriogonum aretioides	Red Canyon buckwheat
Eriogonum batemanii var. ostlundii	Elsinore buckwheat
Eriogonum bicolor	pretty buckwheat
Eriogonum brandegeei	Brandegee wild buckwheat
Eriogonum brevicaule var. desertorum	Great Basiin Desert buckwheat
Eriogonum capistratum var. welshii	Welsh's buckwheat
Eriogonum clavellatum	Comb Wash buckwheat
Eriogonum coloradense	Colorado wild buckwheat

Eriogonum contortum	Grand buckwheat
Eriogonum douglasii var. elkoense	
Eriogonum ephedroides	ephedra buckwheat
Eriogonum esmeraldense var. toiyabense	Toiyabe buckwheat
Eriogonum exilifolium	dropleaf buckwheat
Eriogonum heermannii var. clokeyi	Clokey's buckwheat
Eriogonum leptocladon var. leptocladon	sand buckwheat
Eriogonum leptocladon var. ramosissimum	sand buckwheat
Eriogonum leptophyllum	slenderleaf buckwheat
Eriogonum lewisii	Lewis' buckwheat
Eriogonum meledonum	bridle buckwheat
Eriogonum palmerianum	Palmer's buckwheat
Eriogonum pauciflorum var. pauciflorum	fewflower buckwheat
Eriogonum pelinophilum	clay-loving wild buckwheat
Eriogonum robustum	granite buckwheat
Eriogonum saurinum	spearleaf buckwheat
Eriogonum scabrellum	Westwater buckwheat
Eriogonum tumulosum	woodside buckwheat
Eriogonum viridulum	Clay Hill buckwheat
Eriogonum visheri	Visher's buckwheat
Eriophorum altaicum var. neogaeum	Altai cottongrass
Eriophorum chamissonis	Chamisso's cottongrass
Eriophorum gracile	slender cottongrass
Erocallis triphylla	threeleaf lewisia
Eucephalus perelegans	elegant aster
Eustoma grandiflorum	showy prairie gentian
Eutrema penlandii	Mosquito Range mustard
Festuca campestris	big rough fescue
Festuca hallii	plains rough fescue
Forsellesia planitierum	plains greasebush
Frasera coloradensis	Colorado green gentian
Frasera paniculata	tufted green gentian
Fritillaria pudica	yellow fritillary

Gastrolychnis kingii	King's campio
Gentianella tortuosa	Cathedral Bluff dwarf gentian
Geranium bicknellii	Bicknell's cranebill
Gilia haydenii	San Juan gilia
Gilia penstemonoides	Black Canyon gilia
Gilia sinistra	Alva Day's gilia
Gilia stenothyrsa	Unita Basin gilia
Glossopetalon clokeyi	Clokey's greasebush
Glossopetalon pungens var. glabra	
Goodyera repens	dwarf rattlesnake plantain
Grammica umbellata	flatglobe dodder
Grindelia arizonica	Arizona gumweed
Grindelia fastigiata	pointed gumweed
Guilleminea densa	small matweed
Gutierrezia elegans	Lone Mesa snakeweed
Hackelia besseyi	Bessey's stickseed
Hackelia gracilenta	Mesa Verde stickseed
Halimolobos perplexa var. perplexa	perplexed halimolobos
Halimolobos virgata	rod halimolobos
Hedyotis nigricans	diamondflowers
Hedysarum occidentale var. canone	western sweetvetch
Helenium microcephalum	smallhead sneezeweed
Hermidium alipes var. pallidum	
Herrickia horrida	Canadian River spiny aster
Hesperochiron pumilus	dwarf hesperochiron
Heterocodon rariflorum	rareflower heterocodon
Heterosperma pinnatum	wingpetal
Heterotheca jonesii	Jone's false goldenaster
Heterotheca latifolia	camphorweed
Heuchera richardsonii	Richardson's alumroot
Heuchera rubescens	pink alumroot
Hippochaete variegata	variegated scouringrush
Hypoxis hirsuta	common goldstar
Iliamna crandallii	Crandell's wild hollyhock

Iliamna grandiflora	largeflower wild hollyhock
Ipomopsis aggregata ssp. weberi	Rabbit Ears gilia
Ipomopsis congesta ssp. crebrifolia	ballhead ipomopsis
Ipomopsis globularis	globe gilia
Ipomopsis multiflora	manyflowered ipomopsis
Ipomopsis polyantha	Pagosa skyrocket
Ipomopsis ramosa	coral ipomopsis
Isoetes occidentalis	western quillwort
Isoetes setacea ssp. muricata	spiny-spore quillwort
Ivesia aperta var. aperta	Silver Valley mousetail
Ivesia aperta var. canina	Sierra Valley mousetail
Ivesia cryptocaulis	Charleston Peak mousetail
Ivesia jaegeri	Jaeger's mousetail
Ivesia sericoleuca	Plumas mousetail
Ivesia utahensis	Utah mousetail
Ivesia webberi	wire mousetail
Jamesia americana var. macrocalyx	fivepetal cliffbush
Jamesia americana var. zionis	Zion Cliffbush
Jamesia tetrapetala	fourpetal cliffbush
Juncus brachycephalus	smallhead rush
Juncus brevicaudatus	narrowpanicled rush
Juncus bryoides	minute rush
Juncus tweedyi	Tweedy's rush
Juncus vaseyi	Vasey bulrush
Kobresia simpliciuscula	simple kobresia
Krigia biflora	dwarf dandelion
Lathyrus grimesii	Grimes' pea
Lepidium crenatum	alkaline pepperweed
Lepidium huberi	Huber's pepperweed
Lepidium montanum var. alpinum	alpine pepperweed
Lepidium montanum var. neeseae	Elizabeth's pepperweed
Lepidium papilliferum	Idaho pepperweed
Leptodactylon pungens ssp. Hazeliae	granite prickly phlox
Leptodactylon watsonii	Watson's prickly phlox

Lesquerella alpina ssp. alpina	
Lesquerella fremontii	Freemont's bladderpod
Lesquerella garrettii	Garrett's bladderpod
Lesquerella hitchcockii var. hitchcockii	
Lesquerella paysonii	Payson's bladderpod
Lesquerella pruinosa	Pagosa Springs bladderpod
Lewisia maguirei	Maguire's bladderpod
Lewisia rediviva	bitterroot
Lewisia sacajaweana	Sacajawea bitter root
Liatris lancifolia	gayfeather
Liatris ligulistylis	gayfeather
Liatris squarrosa var. glabrata	scaly blazing star
Lilium philadelphicum	wood lily
Limnorchis ensifolia	canyon bog orchic
Limnorchis zothecina	alcove bog orchid
Liparis loeselii	yellow widelip orchid
Listera borealis	northern twayblade
Listera convallarioides	broadleaved twayblade
Lobelia cardinalis	cardinalflower
Lomatium bicolor var. bicolor	Wasatch biscuitroot
Lomatium bicolor var. leptocarpum	Oregon biscuitroog
Lomatium concinnum	Colorado desert parsley
Lomatium eastwoodiae	Eastwood desert parsley
Lomatium foeniculaceum ssp. macdougalii	desert parsley
Lomatium latilobum	Canyonlands biscuitroot
Lomatogonium rotatum	marsh felwort
Lupinus crassus	Payson lupine
Luzula subcapitata	Colorado wood rush
Lygodesmia doloresensis	Dolores River skeletonplant
Machaeranthera coloradoensis	Colorado tansy aster
Mahonia haematocarpa	Colorado mahonia
Malaxis brachypoda	white adder's mouth orchid
Malaxis monophyllos ssp. brachypoda	white adder's mouth
Mentzelia goodrichii	goodrich's blazingstar

Mentzelia paradoxensis	Paradox stickleaf
Mentzelia pumila var. lagarosa	drawf mentzelia
Mentzelia sivinskii	
Mertensia alpina	alpine bluebells
Mertensia humilis	Rocky Mountain bluebells
Mesyniopsis kingii	yellow flax
Mesynium aristatum	bristle flax
Mimulus clivicola	North Idaho monkeyflower
Mimulus eastwoodiae	Eastwood monkeyflower
Mimulus gemmiparus	budding monkeyflower
Mimulus lewisii	Lewis monkeyflower
Mimulus ringens	squarestem monkeyflower
Minuopsis nuttallii	Nuttall sandwort
Monardella odoratissima	mountain wild mint
Monolepis pusilla	red povertyweed
Muhlenbergia depauperata	sixweeks muhly
Muhlenbergia glomerata	marsh muhly
Muhlenbergia thurberi	Thurber's muhly
Muscaria monticola	tundra saxifrage
Myosurus cupulatus	western mousetail
Myosurus nitidus	western mousetail
Myriophyllum verticillatum	whorled watermilfoil
Najas caespitosa	nodding waternymph
Nama densum	matted fiddleleaf
Nama dichotomum	livemore fiddleleaf
Nama hispidum	rough fiddleleaf
Naumburgia thyrsiflora	tufted loosestrife
Navarretia saximontata	Rocky Mountain pincuchion plant
Neoparrya lithophila	Bill's neoparrya
Nitrophila occidentalis	western boraxweed
Noccaea idahoensis var. aileeniae	Idaho pennycress
Nolina texana	Texas beargrass
Nuttallia chrysantha	golden blazing star

Nuttallia cronquistii	Cronguist's stickleaf
Nuttallia densa	Arkansas Canyon stickleaf
Nuttallia multicaulis	many stem stickleaf
Nuttallia pterosperma	wingseed blazingstar
Nuttallia rhizomata	Roan Cliff's blazing star
Nuttallia sinuata	wavyleaf stickleaf
Nuttallia speciosa	jeweled blazing star
Oenothera acutissima	narrow-leaf evening primrose
Oenothera coloradensis ssp. coloradensis	Colorado butterfly plant
Oenothera coloradensis ssp. neomexicana	New Mexico butterfly weed
Oenothera engelmannii	Englemann's evening primrose
Oenothera grandis	showy evening primrose
Oenothera harringtonii	Arkansas Valley evening primrose
Oenothera kleinii	Wolf Creek evening primrose
Oenothera longissima	longstem evening primrose
Onoclea sensibilis	sensiive fern
Oonopsis engelmannii	Englemann goldenweed
Oonopsis foliosa var. monocephala	rayless goldenweed
Oonopsis sp. 1 (Oönopsis puebloensis)	Pueblo goldenweed
Oonopsis wardii	Ward's goldenweed
Opuntia heacockiae	Heacock's prickly pear
Oreocarya aperta	Grand Junction cat's eye
Oreocarya breviflora	shortflower cryptanth
Oreocarya caespitosa	caespitose cat's eye
Oreocarya cana	mountain cat's eye
Oreocarya elata	cliff dweller's candlestick cat's eye
Oreocarya longiflora	long-flower cat's eye
Oreocarya mensana	southwestern cat's eye
Oreocarya osterhoutii	Osterhout cat's eye
Oreocarya pustulosa	cat's eye
Oreocarya revealii	Gysum Valley cat's eye

Oreocarya rollinsii	Rollin's cat's eye
Oreocarya stricta	erect cryptanth
Oreocarya weberi	Weber's cat's eye
Oreoxis humilis	Pikes Peak spring parsley
Orophaca aretioides	cushion orophaca
Orophaca hyalina	summer orophaca
Orophaca triphylla	plains milkvetch
Oryzopsis contracta	contracted ricegrass
Oxybaphus decumbens	Great Plains four-o-clock
Oxybaphus rotundifolius	round-leaf four-o-clock
Oxytropis besseyi var. obnapiformis	Bessey locoweed
Oxytropis besseyi var. salmonensis	Salmon River locoweed
Oxytropis parryi	Parry's crazyweed
Packera castoreus	Beaver Mountain ragwort
Packera debilis	Rocky Mountain ragwort
Packera malmstenii	Podunk ragwort
Packera mancosana	Mancos shale packera
Packera pauciflora	few flowered ragwort
Packera paupercula	balsam ragweed
Papaver kluanensis	alpine poppy
Papaver radicatum var. pygmaeum	alpine poppy
Parnassia kotzebuei	Kotzebue's grass-of-parnassus
Parrya nudicaulis	nakedstem wallflower
Parryella filifolia	narrowleaf dunebroom
Pediocactus despainii	Despain's pincushion cactus
Pediocactus knowltonii	Knowlton's cactus
Pediocactus winkleri	Winkler's pincushion cactus
Pediomelum aromaticum	paradox breadroot
Pediomelum cuspidatum	largebract Indian breadroot
Pediomelum megalanthum	largeflowered breadroot
Pediomelum pariense	Paria River Indian breadroot
Pellaea atropurpurea	purple cliffbrake
Pellaea breweri	Brewer's cliffbrake
Pellaea glabella ssp. simplex	smooth cliffbrake

Pellaea wrightiana	Wright's cliffbrake
Penstemon absarokensis	Absaroka Range beardtongue
Penstemon acaulis var. acaulis	
Penstemon acaulis var. yampaensis	Yampa beardtongue
Penstemon angustifolius var. vernalensis	vernal narrowleaf penstemon
Penstemon arenarius	Nevada sanddune beardtongue
Penstemon arenicola	red desert beardtongue
Penstemon bracteatus	red canyon beardtongue
Penstemon breviculus	little penstemon
Penstemon caryi	Cary's beardtongue
Penstemon compactus	compact penstemon
Penstemon crandallii ssp. atratus	Crandall's beardtongue
Penstemon crandallii ssp. procumbens	Crandall's beardtongue
Penstemon cyathophorus	Middle Park penstemon
Penstemon debilis	Parachute penstemon
Penstemon degeneri	Degener beardtongue
Penstemon eriantherus	crested beardtongue
Penstemon fremontii var. glabrescens	Fremont's beardtongue
Penstemon gibbensii	Gibbens's beardtongue
Penstemon grahamii	Graham beardtongue
Penstemon grandiflorus	largeflower beardtongue
Penstemon harbourii	Harbour beardtongue
Penstemon harringtonii	Harrington beardtoungue
Penstemon idahoensis	Idaho beardtongue
Penstemon jamesii	James' beardtongue
Penstemon laricifolius ssp. exilifolius	larchleaf beardtongue
Penstemon leiophyllus var. keckii	Keck's beardtongue
Penstemon lemhiensis	Lemhi penstemon
Penstemon lentus	Abajo penstemon
Penstemon mensarum	Grand Mesa penstemon
Penstemon moriahensis	Mt. Moriah beardtongue
Penstemon parviflorus	small flower beardtongue
Penstemon parvus	Aquarius Plateau beardtongue

Penstemon penlandii	Kremmling beardtongue
Penstemon pinorum	Pine Valley penstemon
Penstemon pudicus	Kawitch Range beardtongue
Penstemon radicosus	matroot penstemon
Penstemon retrorsus	adobe beardtongue
Penstemon rhizomatosus	Scheel Creek beardtongue
Penstemon rubicundus	Wassuk Range beardtongue
Penstemon scariosus var. albifluvis	White River penstemon
Penstemon scariosus var. cyanomontanus	plateau penstemon
Penstemon teucrioides	germander beardtongue
Penstemon thompsoniae ssp. Jaegeri	Jaeger's beardtongue
Penstemon utahensis	Utah penstemon
Penstemon wardii	Ward's beardtongue
Peritoma multicaulis	slender spiderflower
Phacelia argillacea	Attwood's phacelia
Phacelia constancei	Constanc's phacelia
Phacelia demissa	intermountain phacelia
Phacelia denticulata	Rocky Mountain phacelia
Phacelia formosula	North Park phacelia
Phacelia incana	hoary phacelia
Phacelia inconspicua	hidden phacelia
Phacelia integrifolia	gyp phacelia
Phacelia minutissima	small phacelia
Phacelia monoensis	mono phacelia
Phacelia splendens	Eastwood phacelia
Phacelia submutica	DeBeque phacelia
Phippsia algida	snow grass
Phlox caryophylla	Pagosa phlox
Phlox kelseyi ssp. salina	marsh phlox
Physaria alpina	Avery Peak twinpod
Physaria arenosa ssp. argillosa	secund bladderpod
Physaria bellii	Bell's twinpod

Physaria brassicoides	Rydberg's double twinpot
Physaria calcicola	Rocky Mountain bladderpod
Physaria congesta	Dudley Bluff's bladderpod
Physaria didymocarpa var. lyrata	Idaho twinpod
Physaria didynicarpa var. lanata	
Physaria integrifolia var. monticola	Snake River Twinpod
Physaria obcordata	Piceance twinpod
Physaria osterhoutii	Colorado twinpod
Physaria parviflora	Piceance bladderpod
Physaria parvula	pygmy bladderpod
Physaria pruinosa	Pagosa bladderpod
Physaria pulvinata	cushion bladderpod
Physaria rollinsii	Rollin's twinpod
Physaria scrotiformis	west silver bladderpod
Physaria subumbellata	parasol bladderpod
Physaria vicina	good neighbor bladderpod
Physaria vitulifera	Rydberg twinpod
Pinus albicaulis	whitebark pine
Plagiobothrys glomeratus	clustered popcornflower
Platanthera orbiculata	lesser roundleaved orchid
Poa abbreviata ssp. Marshii	Marsh's bluegrass
Polemonium chartaceum	Mason's Jacob's-ladder
Poliomintha incana	purple sage
Polyctenium williamsii	
Polygala subspinosa	spiny milkwort
Polypodium hesperium	western polypody
Polypodium saximontanum	Rocky Mountain polypody
Polystichum scopulinum	crag holly fern
Portulaca parvula	dwarf purslane
Potamogeton diversifolius	waterthread pondweed
Potentilla ambigens	Southern Rocky Mountain cinquefoil
Potentilla angelliae	Boulder Mountain cinquefoil

Potentilla cottamii	Pilot Range cinquefoil
Potentilla johnstonii	
Potentilla rupincola	Rocky Mountain cinquefoil
Potentilla subviscosa	Navajo cinquefoil
Primula alcalina	bluedome primrose
Primula capillaris	Ruby Mountain primrose
Primula cusickiana var. maguirei	Maguire's primrose
Primula cusickiana var. nevadensis	Nevada primrose
Primula egaliksensis	Greenland primrose
Proatriplex pleiantha	Mancos saltbush
Prosopis glandulosa	honey mesquite
Prunus angustifolia	Chickasaw plum
Prunus gracilis	Oklahoma plum
Ptilagrostis porteri	Porter feathergrass
Puccinellia parishii	Parish's alkali grass
Pyrola picta	pictureleaf wintergreen
Pyrrocoma carthamoides var. subsquarrosa	largeflower goldenweed
Pyrrocoma clementis var. villosa	tranquil goldenweed
Pyrrocoma insecticruris	wholeleaf goldenweed
Pyrrocoma integrifolia	many-flowered goldenweed
Pyrrocoma radiata	ray goldenweed
Ranunculus gelidus	tundra buttercup
Ranunculus karelinii	ice cold buttercup
Reverchonia arenaria	sand reverchonia
Rhynchospora alba	white beakrush
Ribes americanum	American currant
Ribes niveum	snow gooseberry
Rorippa coloradensis	Colorado watercress
Rotala ramosior	toothcup
Rubus articus ssp. acaulis	
Rubus bartonianus	Barton's raspberry
Sagittaria graminea	grassy arrowhead
Sagittaria montevidensis ssp. calycina	long-lobe arrowhead
Salix arizonica	Arizona willow

Salix barrattiana	Barratt's willow
Salix calcicola	lime loving willow
Salix candida	hoary or silver willow
Salix myrtillifolia	low blueberry willow
Salix nigra	black willow
Salix serissima	autumn willow
Sanguinaria canadensis	bloodroot
Sapindus drummondii	soapberry
Sarcostemma crispum	twinevine
Saussurea weberi	Weber saussurea
Saxifraga bryophora var. tobiasiae	Tobias' saxifrage
Saxifraga tolmiei var. ledifolia (=Micranthes)	Tolmie's saxifrage
Schoenoplectus hallii	Hall's bulrush
Schoenoplectus saximontanus	mountain bulrush
Sclerocactus glaucus	Colorado hookless cactus
Sclerocactus mesae - verdae	Mesa Verde cactus
Selaginella weatherbiana	Weatherby's spikemoss
Senecio musiniensis	Musinea ragwort
Senecio pattersonensis	Mono ragwort
Senecio sphaerocephalus	roughhead groundsel
Seriphidium pygmaeum	pygmy sagebrush
Shoshonea pulvinata	Shoshone carrot
Silene clokeyi	Clokey's catchfly
Silene nachlingerae	Nevada catchfly
Silene petersonii	plateau catchfly
Silphium integrifolium	wholeleaf rosinwood
Silphium laciniatum	compassplant
Sisyrinchium demissum	blue-eyed grass
Sisyrinchium pallidum	pale blue-eyed grass
Smilax lasioneura	carrionflower
Sparganium eurycarpum	broadfruit burreed
Spatularia foliolosa	leafy saxifrage
Sphaeralcea caespitosa var. williamsiae	Railroad Valley globemallow

Sphaeromeria argentea	Nuttall's false sagebrush
Sphaeromeria capitata	rock tansy
Sphaeromeria compacta	compact chickensage
Spiranthes diluvialis	Ute ladies' tresses
Sporobolus flexuosus	mesa dropseed
Sporobolus nealleyi	Nealley's dropseed
Stanleya albescens	Arizona prince plume
Stellaria irrigua	Altai chickweed
Stillingia sylvatica	queen's-delight
Stipa richardsonii	Richardson needlegrass
Streptanthus oliganthus	Masonic Mountain jewelflower
Subularia aquatica	water awlwort
Sullivantia hapemanii var. purpusii	Hanging Garden sullivantia
Symphyotrichum molle	soft aster
Synthyris ranunculina	Charleston Mountain kittentails
Telesonix jamesii	James' telesonix
Thalictrum heliophilum	sun loving meadowrue
Thamnosma texana	Dutchman's breeches
Thelesperma caespitosum	low greenthread
Thelesperma pubescens	hairy greenthread
Thelesperma subnudum var. alpinum	hairy greenthread
Thellungiella salsuginea	salt lick mustard
Thelypodiopsis juniperorum	juniper tumble mustard
Thelypodium paniculatum	northwestern thelypody
Thelypodium repandum	wavyleaf thelypody
Thelypodium sagittatum	slender thelypody
Tonestus alpinus	alpine serpentweed
Tonestus kingii var. barnebyana	Barneby's serpentweed
Tonestus lyallii	Lyall halopappus
Townsendia aprica	Last Chance Townsend daisy
Townsendia condensata var. anomala	cushion Townsend daisy
Townsendia fendleri	Fendler's townsend daisy
Townsendia glabella	smooth Easter daisy
Townsendia jonesii var. lutea	Last Chance Townsend daisy

Townsendia jonesii var. tumulosa	Jones' Townsend daisy
Townsendia rothrockii	Rothrock townsend daisy
Townsendia strigosa	strigose Easter daisy
Trautvetteria caroliniensis	tasslerue
Triantha occidentalis ssp. Brevistyla	sticky tofieldia
Trichophorum pumilum	little bulrush
Trifolium andinum	mountain clover
Trifolium andinum var. podocephalum	Intermountain clover
Trifolium kingii	King's clover
Trifolium leibergii	Leiberg's clover
Trifolium macilentum var. rollinsii	Rollins' clover
Trillium ovatum	western wakerobin
Triodanis leptocarpa	slimpod Venus' looking glass
Triteleia grandiflora	largeflower triteleia
Truellum sagittatum	arrowleaved tearthumb
Unamia alba	prairie goldenrod
Urtica gracilis ssp. holosericea	stinging nettle
Utricularia intermedia	flatleaf bladderwort
Utricularia minor	lesser bladderwort
Utricularia ochroleuca	northern bladderwort
Vernonia baldwinii ssp. interior	Baldwin ironweed
Vernonia fasciculata ssp. corymbosa	fascicled ironweed
Vernonia marginata	plains ironweed
Viburnum opulus var. americanum	American cranberrybush
Viola charlestonensis	Charleston Mountain violet
Viola franksmithii	
Viola lithion	rock violet
Viola pedatifida	prairie violet
Viola selkirkii	Selkirk violet
Virgulus novae - angliae	New England aster
Woodsia neomexicana	New Mexico cliff fern
Woodsia plummerae	Plummer's cliff fern
Zosterella dubia	grassleaf mudplantain

From:

USDA, NRCS. 2016. The PLANTS Database (http://plants.usda.gov)
National Plant Data Center, Baton Rouge, LA 70874-4490 USA.
Accessed Oct. 2016

Colorado Natural Heritage Program, 1997+, CO Rare Plant Guide,
www.cnhp.colostate.edu. Latest update: November, 2015.

Propagation Protocol *Mimulus gemmiparus* W.A. Weber

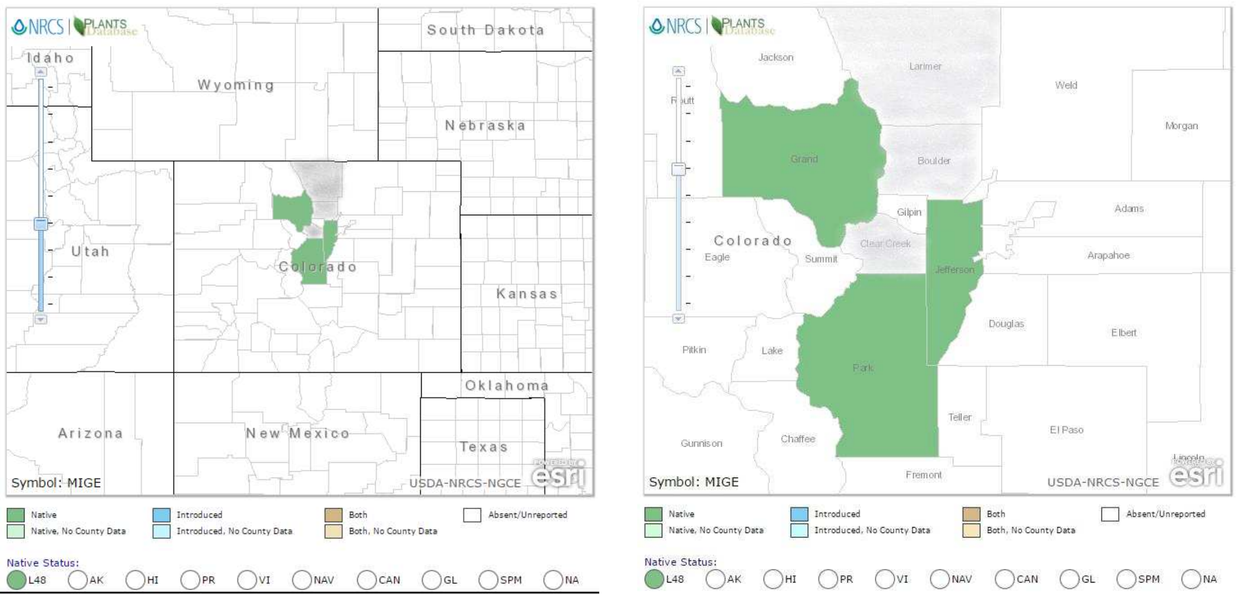

Figure A.1. Distribution of *Mimulus gemmiparus* modified by D. Harden from USDA Plants Database distribution map[1]. Modifications are marked by light charcoal coloring to indicate updated native distribution.

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Family Scientific Name: Scrophulariaceae (Phrymacea)

Family Common Name: Figwort Family (Lopseed Family)

Genus and Specific Epithet: *Mimulus gemmiparus* W.A. Weber

Synonyms: Erythranthe gemmipara W.R. Barker[2]

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[1]USDA, NRCS. 2016. The PLANTS Database (http://plants.usda.gov). National Plant Data Center, Baton Rouge, LA 70874-4490 USA. Accessed October 2016.
[2]Barker, W.R., G.L. Nesom, P.M. Beardsley, and N.S. Fraga. 2012. A taxonomic conspectus of Phrymaceae: A narrowed circumscriptions for Mimulus, new and resurrected genera, and new names and combinations. Phytoneuron 2012- 39, p. 1–60.

Common names: Budding monkeyflower, Weber monkeyflower, Rocky Mountain monkeyflower

Species Code: MIGE

Geographical Range: Narrow endemic found in Front Range region of Colorado in Arapaho and Pike National Forests, Rocky Mountain National Park, and Staunton State Park in Boulder, Clear Creek, Douglas, Grand, Jefferson, Larimer, and Park Counties on both eastern and western slopes of Continental Divide in Rocky Mountains. See distribution map.

Ecological Distribution: Found in upper montane and subalpine ecosystems in seeps on granite rock outcroppings, or under protection of rock ledges, or less frequently observed in sandy alluvial sites of spruce-fir and aspen forests.

Climate and Elevational Range: In situ populations collections propagated for protocols native site elevations; HG 2,560m (8,399')[3] / EC 3,048m (10,000')[4]/ SV 3,085-3,127m (10,121')[3] GP 3,390 m (11,122')[4]. Other native populations not propagated ex situ as of 2016 include North Inlet, East Inlet, Black Mountain, Horseshoe Park (last observation 2013), Fall River Road (last observation 1980) range in elevations from 2,609 m to 3,133 m[3].

Local Habitat and Abundance: Strict microhabitat requirements: persistent moisture, partial shade to full sun. Abundance varies with month of observation and recruitment [3]. Rated G1/S1 by Colorado Natural Heritage Program, indicating risk of extinction (CNHP, 1997+, CO Rare Plant Guide, www.cnhp.colostate.edu. Latest update: November, 2015).

Associated Species: Documented nearby species include *Abies sp., Achillea lanulosa, Amelanchier utahensis, Amerosedum lanceolatum, Antennaria sp., Aquilegia sp., Aquilegia saximontana,*

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[3]Beatty, Brenda L., Jennings, William F., and Rawlinson, Rebecca C., 2003, Mimulus gemmiparus W.A. Weber (Rocky Mountain monkeyflower): A technical conservation assessment, USDA Forest Service, Rocky Mountain Region, Species Conservation Project

Ceratsium sp., Artemesia ludoviciana, Brickellia microphylla, Chamerion danielsii, Campanula rotundifolia, Cystopteris fragilis, Dodecatheon sp., Erigeron subtrinervis, Heuchera bracteata, Jamesia Americana, Micranthes rhomboidea, Mimulus floribundus, Mimulus floribundus, Mimulus guttatus, Mimulus rubellus, Montia chamissoi, Oreochrysum, Packera plattensis, Polygonom minimum, Pentaphylloides floribunda, Picea sp., Populus tremuloides, Pseudotsuga menziesii, Rhodiola integrifolia, Rosa woodsii, mosses and graminoids, mosses, liverworts, algae, ferns[3,4].

Plant Stategy Type/Successional Stage: MIGE is a clonal vegetative annual that has never been observed in wild to reproduce sexually from seed production, and has only one documented observation of viable seed production ex situ. There is some evidence that it may be a poor competitor, as it is found in monocultures with observations of reduced occurrence with an increase in interspersion of other species[3].

Plant Characteristics: MIGE is the only known plant with petiole enclosed bulbils. Annual forb that has also been described as an herbaceous perennial due to persistence in soil of bulbils which produce plant clones. Patches of plants ephemeral in nature with patch extinction/emergence variable. Diminutive, inconspicuous, and rarely flowering in wild where it can be found growing in dense mats, typically resulting in bulbil dispersion densities > 100 bulbils per dm^2 or less frequently observed more widely spread or singly[5]. Larger and profusely flowering ex situ. Leaves are opposite, entire, ovate, and glabrous with swollen petioles. If present, yellow 5 lobed bilabiate corrolas on short pedicels, calyces campanulate, toothed, incurved, with prominent red spots and pubescence in throat. Style length positions stigma beyond tip of stamens, so over and in front of anthers.

Stamens glabrous, anthers dehiscent.

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[3]Beatty et al, 2003.

[4]Smith, Pam, Colorado Natural Heritage Program, 2012, Survey East Inlet.

[5] Beardsley, Mark, 2014, Establishing new patches of *Mimulus gemmiparus* in Staunton State Park:assisted establishment as a tool for reducing the risk of extinction, research report prepared for the Colorado Natural Areas Program.

Propagation Goal: multiplication of propagules (bulbils) for out planting, ex situ propagation, research, or storage

Propagation Method: vegetative

Product Type: propagules (bulbils)

Time to Grow: 8 to 12 weeks from sowing to harvest

Target Specifications: ≥1,000 bulbils per mature propagated plant

Bulbils/kg: Approximately 16,700 bulbils per kg pure based on average 100 bulbil pure weights of .06 g

Germination: First emergence 5-7 days post sowing with continued emergence 45 or more days post sowing. Observations of greenhouse emergence of plants from sown field and greenhouse collected bulbils from highest to lowest emergence: Saint Vrain 60%, Elk Creek 54%, Guanella Pass 42%, Hankins Gulch 38%. Viability of greenhouse pot grown propagated bulbils determined by germination test were EC 92%, GP 92.5%, HG 92% 5-13 days post harvest , and SV 94.5% 57 days post harvest.

Propagule Collection: Obtain permission for collection of regulated species of concern propagules. Field collections take place in late summer to fall, generally in August, contingent upon development of plants. Larger bulbils may have higher rate of viability[6]. Collect from fully mature plants with signs of senescence such as browning foliage, inflated red to brown petiolar encased bulbils, and some shattering of propagules (separation from plant) when touched. Pull entire petiole encased bulbil from plant by hand. Do not remove entire plant or disturb soil. Allow bulbils to remain attached to full leaf or any leaf fragments that separate along with bulbil. Do not collect more than 30% of propagules from any plant, patch, or population from non-sensitive species[7]. For collection of sensitive species, follow

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[6] Beardsley, M., 1997, Colorado's rare endemic plant, Mimulus gemmiparus, and its unique mode of vegetative reproduction, thesis, Colorado State University.
[7] Rocky Mountain National Park, 2011, Propagation Protocols Manual, Division of Resource Stewardship, Estes Park, Colorado

guidelines of resource manager.

Greenhouse Harvest: Greenhouse propagated bulbil collections can occur year round contingent upon dates of propagation and plant development. After 8 to 12 weeks of development, harvestable plants should be senescing, with red to brown coloring of bulbils, and some shattering of bulbils if touched. Entire potted plants can be placed in open, upright paper bags to capture any shattering bulbils as plants senesce. Bulbils allowed to dry on the mother plant for a greater length of time may have higher degree of desiccation resistance. 100% collection allowable for ex situ propagated plants. Remove fully mature, senesced plant from soil and separate bulbils from plant by hand or by gently shaking plant inside paper bag.

Avoid rough handling, crushing, piercing, or compression of bulbils. Place collected propagules in paper bags or envelopes. Place paper bags in cooler for transport. Maintain constant temperature and humidity close to ambient conditions, 20^{o}C and 40%RH, until utilization or cold storage instigation. Do not repeatedly cool and reheat propagules before utilization. Propagules should be allowed to after ripen in paper packs for 5-10 days at ambient temperature and RH, then should be planted or cold stored as soon as practicable.

Propagule Characteristics and Processing: Reproduces asexually with vegetative propagules, the only known examples of unique petiole encased bulbils. Bulbil is analogous to a seed and embryo. Enclosing petiole serves as seed coat, fully formed plant clone resembles true seed embryo with thickened cotyledon-like leaf primordia, root axes, and apical meristem. Native population bulbils shatter in July to October and survive up to nine months before spring germination with winter cold exposure temperatures as low as -30^{o}C[3,8].

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[3]Beatty et al., 2003.
[8] Personal observations East Inlet Population, 2016

Brood bulbils other taxa: *Poa alpina*, *Allium spp*., *Agave spp*., *Lillium spp*., *Dentaria bulbifera*, *Remusatia vivapara*, *Ranunculus facaria*, *Saxifraga granulate* [9].

Pre-Planting Treatments: None required. Ideally, use bulbils 5-10 days post harvest from senesced plants, or when bulbils are red to brown, or brown in color.

Growing Area Preparation: Preferred method found in pilot study for maximum bulbil production, largest, most fully expressed plants displaying marked morphological and growth habit differences was 4" pots, 1-2 bulbils spaced 2.5 cm apart sown per pot for density of 1-2 mature plants per 10 sq cm maintaining separation between populations. Pots arranged on trays in checkerboard pattern. Trays nested in 34 Liter watering tubs lined with a 2.5 cm layer of clean pea gravel. Alternative potting methods tried include nested round black plastic pans, and nested clear triangular tubs, both with drainage/uptake holes in bottom.

Growing Media: Fafard 4P potting mix, 45-55% Canadian Sphagnum peat moss, vermiculite, bark, dolomite lime, wetting agent. Fafard 4P, Fafard Super Fine Germinating Mix, Proxmix HP high porosity growing medium with biofungicide and mycorrhizae. There was no appreciable change in tendancy for damping of plants between the Promix with fungicide compared to Fafard 4P when recommended watering and drainage procedures followed . Preferred method Fafard 4P with fine layer of Fafard Germinating mix, potatoes and hygiene for pest control.

Establishment Phase: Bottom water soaking 2-3 times per week with replacement of water. Drain time for multi-day soakings at least 12 hours, up to 2 days depending on temperature and PAR conditions of

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[9]Moody, Amber, Pamela K. Diggle and David A. Steingraeber, 1999, Developmental analysis of the evolutionary origin of vegetative propagules in *Mimulus gemmiparus (Scrophulariaceae)*, *American Journal of Botany*, Vol. 86, No. 11 (Nov.), p. 1512-1522.

greenhouse. Overnight drainage less likely to result in accidental over drying of plants. Thinned or salvaged plants transplanted into 4" pots with same conditions. Transplanting successful when performed on newly emerged plants. Minimal success with transplants of plants that have already developed multiple nodes, and long branching root system that may be interspaced with adjacent plant roots. Germination rates EC; 83% pot grown, 92% tub grown, GP; 96% pot grown, HG; 92% pot grown, and SV 94% pot grown.

Active Growth Phase: Grow in greenhouse cool temperatures on south facing bench closest to pad and fan cooling system. Grown successfully at Colorado State University (CSU) University Greenhouse at 17-22^{o}C (62^{o}-72^{o}F) day, 16^{o}-22^{o}C (61^{o}-68^{o}F) night, with 16 hours supplemental light from high intensity sodium discharge lamps. Morning light 290 – 320 watts per meter squared, 600-700 late afternoon. Bottom watered in mixing tubs, thoroughly drained and clean water replaced 2-3 times per week.

Hardening Phase: Duration 3 weeks. Gradually reduce watering as plants senesce, and stop watering completely at least 1 week before harvest. Potted plants not recommended for field planting, so no acclimation of mature plants to outdoor conditions necessary. Over watering when plant growth has completed and activity is diminishing could result in damping off and loss of bulbil viability.

Harvesting, Storage, and Shipping: For harvest of entire ex situ plant, document date of harvest, pull plant from soil, separate propagules from mother plant, clean collection by sifting or by hand to remove gross inert material such as flowers and stems. Do not remove remnant leaf parts still attached to bulbil. Do not remove bulbil from encasing dried petiole. Weigh entire cleaned collection in grams. Weigh 100 bulbils + any remaining inert. Reweigh same 100 bulbils completely removed from remaining inert for 100 bulbil weight. 100 bulbil + inert weight – 100 bulbil only weight = weight of inert. Weight inert/weight bulbils + inert x 100 = % inert. Mulitply % inert by total cleaned collection weight, then subtract this number from total cleaned collection weight for an adjusted weight. Adjusted weight/100 bulbil weight x .01 or simply 1 bulbil weight = approximate number of bulbils (Miller, pers. Communication, 2015).

Example:

Elk Creek Collection

Total sample weight: 24.6731g

100b+inert weight: 0.116g

100b only weight: 0.0724g (1 bulbil weight ~ .000724g)

Inert: 0.116-0.0724 = 0.0436

(0.0436/0.116) x 100 = 37.6% inert

37.6% of Total sample weight (24.6731g) = 9.2770856

Adjusted weight: 24.6731-9.2770856 = 15.396015

Estimated # bulbils: 15.396015 / .000724 = ~21,265 bulbils

Bulbils can be held in paper bags enclosed in plastic ziplocks and transported in coolers that maintain constant ambient temperatures not to exceed 20^{o}C and humidity ranges of 38-42% RH. Bulbils that will be sown or out planted within 1 to 2 months of collection date should be maintained at constant ambient temperature and RH. If propagules will be stored for 3 months or longer, viability may be prolonged by storage at 3^{o}-5^{o}C.

Watering Regime: Soak plants only as long as needed to fully moisten soil, over the course of 1-2 days, or overnight, then let plants drain and sit in dry tubs for 12-36 hours. Clean and dry tubs between waterings to reduce pest habitat. Always use clean water to refill tubs. Plants in completely dry medium for > 5 days will senesce or die. If plants lose turgor or appear stunted in growth and development, reduce watering. Entire trays or Individual slots in pot trays can be lined with pea gravel to raise pots higher and reduce overwatering. Overwatering results in stunted plants that fail to thrive. Entire crops can be lost from damping off mortality.

Pests and Disease: Standing water in tubs and moist substrate invites pests, notably the fungus gnat. Continuously wet soil grows algae that can prevent soil aeration. Scrape away algae growth from soil surfaces. Yellow sticky pest traps can help control adult fungus gnats, and potato slices removed and replaces at least weekly on surface of media can help control larval fungus gnat infestations.

Best Method: Greenhouse grown on south facing bench backed by pad and fan cooling system with temperatures of 17º-22ºC (62º-72ºF) day, 16º-22ºC (61º-68ºF) night, and 16 hours supplemental light from high intensity sodium discharge lamps.Sown in Fafard 4P potting mix in 4" pots arranged on trays in checkerboard pattern and bottom watered in pea gravel lined watering tubs.

Protocol Author: Dyan Harden

Date Created: 11/2016

Note: Template modified by D. Harden from:

http://www.nativeplantnetwork.org/network/SampleBlankForm.asp and

University of Washington ESRM 412 Native Plant Production Protocol URL: https://courses.washington.edu/esrm412/protocols/LAJA.pdf

Other Sources: Current protocol builds upon previous practices of propagators David Steingraeber, Mark Beardsley, and Kevin Chu. Useful protocols for *Mimulus lewisii* found at:

University of Washington ESRM 412 Native Plant Production Protocol URL: https://courses.washington.edu/esrm412/protocols/LAJA.pdf and *Mimulus guttatus* at:

https://npn.rngr.net/npn/propagation/protocols/scrophulariaceae-mimulus-644/?searchterm=mimulus%20guttatus.