Standard Operating Procedures (3.6): Seed Collection and Germination

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Standard Operating Procedures:
Seed Collection and Germination

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Protocol Suitability Evaluation

A habitat suitability table containing appropriate estuarine wetland habitat types (of those evaluated) to implement seed collection and germination protocols is displayed in Table 1. Specifically, the protocols focus on common wetland species and adjacent transitional habitat species. A comparative assessment of cost, effort, and data quality are shown in Table 2. A matrix of additional detailed categorical evaluations of seed collection and germination protocols can be found in Appendix 3.6A.

Table 1. Appropriate habitat types to implement seed collection and germination protocols.

	Habitat Types					
Survey Protocol	**Tidal Channel**	**Mud/sand flat**	**Emergent salt marsh**	**Non-tidal salt marsh**	**Salt pan**	**'Degraded' / fill**
Seed Collection			X	X	X	X

Table 2. Categorical assessment of cost/effort and data quality for seed collection and germination protocols.

	Evaluation Metric	Seed Collection	Notes
Time / Effort	Office Preparation Time	10-30 minutes	Site selection and any GPS locations; print data sheets
	Equipment Construction Time (one time)	10-30 minutes	To gather supplies
	Field Time	> 60 minutes	Dependent on quantity of seeds to be collected and number of locations
	Laboratory Time (per transect)	> 60 minutes	Seed cleaning, processing, and watering in greenhouse
	Post-Survey Processing / QAQC Time	10-30 minutes	----
	Minimum Repetition (site-dependent)	Many Repetitions	Germination success data are highly variable
	Relative Cost (equipment and supplies)	< $15	----
Survey / Data Quality	Accuracy (at a survey area level) *	Not Applicable	----
	Precision (at a survey area level) *	Not Applicable	----
	Qualitative-Quantitative Score *	Not Applicable	----
	Subjectivity-Objectivity Score *	Not Applicable	----

**Seed collection and germination protocols are not a traditional survey method and do not yield specific data*

Resulting Data Types

Seed bank collection and germination protocols do not qualify as a survey type and do not yield any specific data, but rather, present a methodological approach to the direct collection and propagation of native plant species. The application of these protocols will help increase the probability of success when collecting and germinating native plant species. Many of these protocols were written concurrently, and are similar to, those found in Barton et al. 2016.

Objective

The majority of wetland restoration projects incorporate a vegetation plan, or protocol, that dictates how the specified marsh plain, as well as surrounding transitional and upland areas, will be re-vegetated during the restoration process. To facilitate re-vegetation efforts during wetland restoration projects, native seeds are often collected, stored, and propagated. Collection and use of local seeds and cuttings in restoration projects is preferred to use of nursery stock as locally collected individuals are best adapted to local environmental conditions (Vander Mijnsbrugge, Bischoff, and Smith 2010), will maintain local genetic information, may improve the long-term sustainability of the site, and may enrich the diversity of the wetland plant community (Zedler 2001). As wetland complexes naturally support a variety of brackish, freshwater, dune, and salt marsh plant species, restoration plant palettes attempt to mimic natural diversity and incorporate plants from a variety of habitat types (Johnston et al. 2012).

This document outlines the basic seed collection and germination strategies to be employed within southern California estuarine and adjacent upland habitats. For more detailed information on specific plant species, see Barton et al. 2016, published in the Bulletin of Southern California Academy of Sciences, which lists available information for 84 native plant species common to southern California restoration efforts.

Equipment

Equipment and supplies needed for seed collection, cleaning, and germination varies depending on the specific species of interest. The following equipment is recommended:

Field Equipment:

- Collecting bins or paper bags
- Sealable plastic bags
- Pens/pencils/markers
- Paper clips/binder clips
- Field Data Collection Sheet (Appendix 3.6B)
- Clipboard
- Background documentation on species locations (e.g., reports, vegetation maps) (recommended)
- Mesh screens/sieves (optional)
- Tarp(s) (optional)
- Gloves (optional)
- Gardening shears (optional)
- Jepson manual (optional)

Lab/Greenhouse Equipment:

- Sieves of varying sizes (ranging from 2 mm- 500 um)
- Paper envelopes
- Freezer and oven
- Refrigerator

- Growing medium (species specific)
- Sterile petri dishes (species specific)
- Ethylene source (ethephon or sliced apple) (species specific)
- Hydrogen peroxide (H_2O_2) (species specific)
- Nail clippers (species specific)
- Mothballs (species specific)

Field Preparation

Prior to seed collection, a list of target plant species should be developed. The field equipment necessary (from the list above) to collect seeds from the plants on the list should be gathered before the field shift. Species-specific scientific documents, reports, and maps should be studied prior to collection to identify areas where target species are most likely to occur.

Field Methods

Pre-Collection:

Figure 1. *Isocoma menziesii* plant with seeds of varying degrees of maturity. Yellow and amber flowers (center) have immature seeds. White, fluffy flower heads (bottom, left and top, center) have ripe, tan seeds.

First, using flowers, seed, stems, leaves, and/or root structures, verify that the parent plant is the desired species. If unable to identify a species in the field, take a voucher specimen, with flowers, seeds, and stems if possible, to key out in the office following the identification techniques and strategies described in The Jepson Manual 2nd ed. (Baldwin et al. 2012). Once the plant has been confirmed as the target species, carefully examine seeds to assess seed maturity (Figure 1). Avoid collection of immature seed, as premature collection may result in low seed viability (John et al. 2010). Generally, seeds are considered ripe if one or more of the following conditions is met: seed capsules are dry and dark tan/brown in color, seed capsules detach easily from the parent plant, and/or fruit is soft and detaches easily from the parent plant. For many common wetland species, more detailed descriptions of mature seeds are listed in Barton et al. 2016. See Appendix 3.6C for a list of species-specific collection times. While most seeds should be collected when ripe, seeds or inflorescences from certain dehiscent species, particularly those that explosively release ripe seeds, should be collected early (Teel 2011). The proper procedure for doing so is outlined in the 'Dehiscent seeds/inflorescences' section below.

To maximize the range of genetic diversity represented in the collection, seed should be collected from a large number of parent plants, ideally 50-100, if possible (John et al. 2010). When collecting, it is advantageous to sample populations, or individuals, that grow in distinct environmental conditions as these individuals likely exhibit genetic variability. Effort should be made to sample as randomly and evenly from the plant population as possible (Rancho Santa Ana Botanic Garden 2014). Additionally, if a

species is known to be dioecious (e.g., *Croton californicus, Baccharis* spp*., Salix* spp. *Populus fremontii, Distichlis spicata, Monanthochloe littoralis, Atriplex lentiformis)*, care should be taken to ensure that sufficient collections from both male and female plants are made (Clarke et al. 2007). It should be noted that when collecting seeds, less intense, more frequent seed harvests are preferable to infrequent, intense harvests (Rancho Santa Ana Botanic Garden 2014). To practice safe harvesting, take no more than 5% of seed from a given species/geographic area (Zedler 2001).

The lack of published information regarding collection and propagation for many native species often forces restoration managers to rely on information from the genus or other closely related species or prompts exploratory studies (Dreesen and Harrington 1997). While Barton et al. 2016 lists collection and germination information for many common species included in southern California estuarine wetland restoration plant palettes, knowledge gaps exist for many listed species. In these instances, consult literature for the genus or family when possible. Additionally, frequent visits to collection sites are suggested to assess seed stage (i.e., ripe, unripe). More specifically, if multiple scouting trips are made, it is advisable to note the percentage of seed that is early/unripe, ripe, and exhausted per species per date. Detailed field notes are essential for the successful collection of seeds. Noting and analyzing this information will help managers focus on the ideal collection window for each plant species.

Seed Collection

Once the seeds of a target species are deemed ripe, the collection process can begin. Collection / isolation of seed varies based on plant anatomy. Observe the plant and note if the species has berries or dry fruits, dehiscent or indehiscent seeds, and note if seeds are in seed heads or seed clusters. Once this information has been determined, and the plant has been classified, find the appropriate guild below and use the subsequent information to aid collection.

Moist/Wet Fruits/Berries

Hand pluck fruits (Rancho Santa Ana Botanic Garden 2014). Place fruit into a sealed plastic bag labeled with species name, date of collection, and location of collection.

Dehiscent seeds/inflorescences

If seeds are wind-dispersed, cut entire stalk/inflorescence from plant with gardening shears in the field prior to seed maturation. Store developing seed heads or stalks inside a covered box or paper bag so that when released, ripe seeds will remain in the vessel for easy collection (Teel 2011). Alternatively, cloth bags can be secured around ripening stalks in the field. Dispersed seed will be captured by the bag. Bag will need to be checked for seed periodically and recollected at a later date (Rancho Santa Ana Botanic Garden 2014).

Seed heads

Cut entire stalk off plant. Place stalks in paper bag and shake to release seed (light crushing of the seed heads may be required) (Teel 2011). Alternatively, shake ripe seed directly onto a tarp or collection bag underneath the target plant (Rancho Santa Ana Botanic Garden 2014).

Tight Seed Clusters

For tight seed clusters, such as *Baccharis salicifolia* (Figure 2), remove entire seed cluster from plant. Remove as much flower material/chaff as possible. Use of sieves can be helpful.

Figure 2. *Baccharis salicifolia* plant and seed. Photo courtesy: RSABG.org.

Data Collection/Field Notes

Information about collections should be recorded on appropriate data sheet (Appendix 3.6B). Record all relevant information. Additionally, properly label individual paper collection bags or envelopes. Indicating the species name, date of collection, and location of collection. It is advisable to bring paperclips and/or binder clips into the field to ensure that collection bags are properly sealed and to prevent unnecessary seed loss/mixing.

Laboratory Methods

Cleaning Seeds

Once back in the lab, seed cleaning can begin. Seed cleaning removes floral parts, seed coats, pods, fleshy fruit material, or other debris from seeds (Jorgensen and Stevens 2004). Before beginning the cleaning process, identify which of the following guilds the species of interest falls into:

Moist/Wet Fruits/Berries

Place collected fruit in a sealed plastic bag. Ensure bag is well sealed and then mash the berries. Let fruit decay until the pulp is fairly watery (this process will usually take a few days) (Teel 2011). During this time, store fruit in a cool, shady place as overheating can damage seed (John et al. 2010). Rinse the pulp from the seeds in a large bowl of fresh water. Pulp should float, and the seed will sink. Repeat the process until the seeds are clean. To disinfect clean seed, use a diluted hydrogen peroxide solution (1 H_20_2: 5 H_20) (Teel 2011). Dry seed at room temperature, unless otherwise noted (e.g., Barton et al. 2016). Once seed is thoroughly dry, it is ready for storage.

Dehiscent seeds/inflorescences

If dehiscent inflorescences are collected early or bagged in the field as suggested above, ripe seeds or seed capsules will be released directly into the storage bag. If seed is contained in a capsule, gently crush the capsule by hand or with a rolling pin to remove the seed. Rub seeds over a sieve to remove excess chaff (Figure 3). Use stacked sieves of varying sizes to expedite the process (Figure 4). To use this technique, stack a sieve with larger openings (e.g., 1-2 mm) over a sieve with smaller pores (e.g., 500-750 µm). Rub plant material over the tower to remove both large and fine chaff from seeds. Ideally, seeds will be isolated in the middle of the tower. This methodology can be adapted based on exact seed size or sieve availability. Once seeds are isolated, only keep seeds that look ripe (i.e., dark brown/tan in color, healthy looking). Discard sickly or deformed seeds.

Figure 3. *Encelia californica* seeds and chaff over a single sieve.

Figure 4. Stacked sieves of decreasing screen size.

Seed heads

If seed is contained in a capsule, crush capsules to isolate the seed. Removal of woody capsules, as seen in *Abronia* spp*.,* may be aided with the use of generic nail clippers (P.M. Drennan, personal communication) (Figure 5). To separate seeds from chaff, pour bag over an appropriately sized sieve for your specific seed (Teel 2011). Rub seeds over a sieve to remove remaining chaff. Stacking sieves into a tower, as described above, may expedite the process. Only retain seeds that look healthy and ripe.

Figure 5. *Abronia maritima* capsules and seed photo. Photo courtesy: RSABG.org.

Tight Seed Clusters

Gently crush capsules by hand or with a rolling pin over an appropriately sized sieve. Sift chaff/seed mixture with a sieve to remove chaff and isolate seeds (Teel 2011). Use a sieve tower if desired. Again, only retain sees that appear ripe and healthy. For more detailed procedures on seed cleaning for specific species, see Barton et al. 2016.

Storing Seeds

For the greatest germination yield, storage time should be minimized and use of newer seeds should be prioritized. While seed longevity varies by genus and/or species, a number of seeds in are known to be short-lived. For example, seeds of *Lycium californicum, Limonium californicum, and Heteromeles arbutifolia* are viable for a year at most. While seeds of other species (e.g., *Atriplex* spp*., Astragalus* spp*.,* and *Lupinus chamissonis)* will remain viable for much longer (i.e., 4-10 years), the germination rate of seeds in long-term storage will likely decline over time. See Appendix 3.6D for more information regarding seed longevity. The longevity of certain seeds can be increased if specific storage rules are followed for the species and/or general seed storage rules are applied. After cleaning seeds and organizing them into appropriately labeled paper envelopes/bags, Vierhelig suggests storing seed packets in a large, sealed, collective container with a number of mothballs for 1-2 days to kill remaining insects and their eggs (Vierheilig 2014). To further increase longevity, keep seed dry and store in a

stable environment with low temperature and humidity (Jorgensen and Stevens 2004). Certain species will store better if kept at lower temperatures in a refrigerator or freezer. See 'Seed Storage' in Barton et al. 2016 to see suggested storage temperatures and other species-specific storage information. In addition to reducing germination rate, long-term storage will often induce seed coat or embryo dormancy, and seeds may need to be treated prior to planting to break dormancy.

Greenhouse Methods

Seedlings of a variety of marsh angiosperm species have been successfully grown in greenhouses. Transplanting greenhouse-grown seedlings is an effective re-vegetation strategy and often offers restoration ecologists a greater degree of success than simple seeding. Seedlings of appropriate size can be transplanted to the restoration site (Broome, Seneca, and Woodhouse 1988).

Germination Considerations

Successful propagation of native marsh and dune vegetation species requires a deep understanding of seed germination ecology. Naturally, seed germination is dependent upon a number of evolutionary and ecological factors, factors which generally must be observed, and often replicated, in the lab or greenhouse to successfully grow propagules. These factors include but are not limited to the following: germination timing/seasonality, environmental conditions, such as temperature, moisture, soil salinity, and light availability, seed age, and dormancy state, both at the time of maturation and dispersal (Baskin and Baskin 2014).

For many species, germination is only possible during a particular season or for a small fraction of the year. For instance, it is ideal to plant *Atriplex lentiformis* in winter. For other species, germination is possible almost year-round (Baskin and Baskin 2014). Understanding germination timing is important to determine the best environmental conditions to promote germination in the greenhouse or lab.

Understanding germination timing will in turn often indicate what temperature, or range of temperatures, best promote germination. Further, the germination rate of certain species is enhanced with simulated temperature fluctuations, rather than constant temperatures. While response to fluctuating temperatures is species-specific, a few generalities exist. Both small seeded species and forbs tend to respond well to fluctuating temperatures while larger seeded species and graminoid species do not show as marked a preference for temperature fluctuations (Liu et al. 2013). If information regarding the necessary conditions or procedures to promote germination is not readily available, it is advisable to run simple tests/experiments using a variety of the possible treatments.

Dormancy Considerations

Much in the same way that environmental germination requirements should be mimicked in the greenhouse, if a species is known to have dormant seeds, understanding which environmental conditions are necessary to naturally break seed dormancy, and thus must be manipulated in the greenhouse, is vital. If a species undergoes seed coat or embryo dormancy at any point in its life cycle, its seeds will need to be treated prior to sowing to break dormancy (Vierheilig 2014). A variety of methods can be used to break dormancy and prepare seeds for planting. These methods include:

scarification, submersion in hot water, treatment with dry heat, exposure to fire, acid, mulch treatment, cold stratification, warm stratification, and exposure to light. Unfortunately, there is not a uniform method to break seed dormancy. Instead, methods vary based on the life history of the species. Species that typically germinate in early spring after a cold and/or rainy winter, such as *Platanus racemosa,* will often need cold, moist stratification to break dormancy to mimic natural wintering. Other species, such as *Acmpispon glaber* require heat treatment to break dormancy.

Please see Barton et al. 2016 for detailed seed treatment information. Please note that this information is incomplete due to gaps in published literature and some experimentation may be necessary. However, treating seeds to break dormancy, is not enough to guarantee germination. Germination requirements must also be considered.

Germination Techniques and Methods

To promote or ensure germination, seed dormancy must be broken (if applicable) and seeds must be sown in an appropriate set of environmental conditions (Baskin and Baskin 2014). To grow seedlings, clean, viable seeds should be planted in mixtures of sand, top soil, and peat moss or vermiculite (Broome, Seneca, and Woodhouse 1988). To achieve the greatest germination rate, the exact composition of the mixture should be tailored to the individual plant species of interest. Life history and preferred habitat of the species should be considered when determining optimal soil conditions. For instance, *Abronia maritima,* which naturally occurs on sandy dunes, should be sown in soil consisting largely of sand, or other coarse grains. Similarly, seeds of halophytic species should be sown in mediums that contain an appropriate level of salt or allowed to sprout directly in a saline solution, while salt intolerant species should not be sown in such conditions.

If germination studies need to be performed, it is preferable that they are conducted shortly after collection, within 7-10 days, to ensure that seeds have not entered dormancy. While a germination data sheet has not been generated as part of this SOP, when designed it should include the following items:

- Species name
- Dormancy treatments performed
- Date seed planted
- Planting medium
- Percentage germination at varying time points (e.g., 1, 2, and 3 weeks post-planting)

Data Entry and QAQC Procedures

Data should be entered in the laboratory using the appropriate data sheet (Appendix 3.6B). All required fields should be completed in full, and the data recorder should assign their name at the top of the document(s). Data should be transferred to the appropriate electronic database within three days, and the hard copies filed in labeled binders. Electronic copies of all data should be housed on an in-house dedicated server and backed up to a cloud-based or off-site server nightly. Hard copies should be saved for five years. Electronic copies should be saved indefinitely.

Data Analyses

Results of germination experiments should be carefully analyzed. Suggestions for analyses include: assessing the percentage of seed germination as a function of collection location, species, or growing medium, analyzing the percent germination as a function of time in storage, and analyzing the percent germination as a function of dormancy treatment(s).

Results Summary:

Table 3 is an example of information collected and summary results of several species of seed collections made at the Ballona Wetlands Ecological Reserve in spring and summer 2014.

Table 3. Example of summary results for several species collected at the Ballona Wetlands Ecological Reserve.

Seed Information	Seed Maturity	Field Information				
Species Name	**# Plants Sampled**	**Date(s)**	**Early**	**Ripe**	**Late**	**BWER Area**
Baccharis pilularis (female)	1	1-Jun	X	X	X	FW marsh
Baccharis salicifolia (female)	3	1-Jun		X		FW marsh
Camissonia cheiranthifolia	3	11-Jul	X	X		BWER Dunes
Encelia californica	10	19-May		X		FBW Dunes
Eriogonum fasciculatum	2	19-May	X		X	FBW Dunes
Frankenia salina	3	11-Jul	X	X		BWER Dunes
Heliotropium curassavicum	15-20	1-Jun	X	X	X	FBW dunes
Juncus acutus	1	11-Jul		X	X	FW marsh
Lupinus chamissonis	20	19-May	X			FBW Dunes

Health and Safety Precautions

While the plants on the southern California plant palette are safe for human handling, individuals should exercise caution in the field as certain native and non-native marsh species are known to be toxic. For more information on a specific species, reference Calflora.org, which lists toxicity ratings for all plant species (Calpoison.org 2014; “Calflora: Information on California Plants for Education, Research, and Conservation” 2014).

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APPENDIX 3.6A

	Evaluation Metric	Seed Collection and Germination	Notes
	Correlation to L2 CRAM	Not Applicable	----
Personnel Requirements	Specialty Equipment or Clothing Required	Many Specialty Items	Most specialty items are related to greenhouse processing and cleaning methods
	Ease of Transport (amount or weight of supplies)	Some Items / Moderate	----
	Ease of Implementation	Moderate	----
	Expertise / Skill Level	Some Technical Knowledge	Familiarity with species identifications is required
	Number of Personnel	2	----
	Training Requirements	None	----
	Seasonality of Survey Time	Year round	Species dependent
	Suggested Frequency	As needed	----
Survey / Data Quality	Type of Output	Not Applicable	Seed collection and germination protocols are not a traditional survey method and do not yield specific data
	Active or Passive Monitoring Style	Active	----
	Specialty Computer Software Required	Not Applicable	----
	Availability of Online / External Resources	Many resources	----
Potential Limitations	Wetland Type Applicability	All	----
	Images or Multi-Media Required	Images Required	----
	Degree of Impact / Disturbance	Moderate Disturbance	----
	Vegetation Height Limitation	Overhead (~2m)	Must be able to reach seeds
	Appropriate for Tidal / Wet Habitats	Yes	----
	Tide Height	Low Tide Only	----
	Regional or Broad Implementation *	Frequently Used	Especially for restoration project
	Potential for Hazards / Risk	Low to No Risk	----
	Restrictions	Special Status Species	----

** based on monitoring literature review*

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Appendic 3.6C

Seed Collection Field Sheet		
Date:	Site:	Survey Start Time:
Staff:	Weather:	Survey End Time:

Species Name	# Plants Sampled	#Seeds Collected	Fruiting Stage of plants (e.g. early, ripe, late)	Site Conditions	Description of Local Habitat	Other Notes

Appendic 3.6C

Appendix 2: Seed Collection Field Sheet		
Date:	Site:	Survey Start Time:
Staff:	Weather:	Survey End Time:

Species Name	# Plants Sampled	#Seeds Collected	Fruiting Stage of plants (e.g. early, ripe, late)	Site Conditions	Description of Local Habitat	Other Notes

Appendix 3.6D

Scientific Name	Start	End	Seed Collection Details (copy of draft palette)
Isomeris arborea	Feb	Nov	Flowers several times/year (except Dec-Jan). Ready for collection when capsules turn brown and are crisp. Strip mature fruits from plants by hand. Break apart pods by hand to remove seeds. A hammermill or coater blender can also be used for this step.
Juncus bufonius	Mar	May	Collect Mar-May. Seed capsules quickly dehisce, seeds should be collected quickly after plant death. Shake mature flowers to collect tiny seed
Stipa lepida [Nassella l.]	Mar	May	Collect seed heads in spring when flowers fade. Allow seed heads to dry on plants, remove and harvest seed. Clean prior to storage
Plantago erecta	Apr	May	The tiny capsules dehisce when mature, usually from April-May. Dehiscing inflorescences can be collected early into a paper bag and left to dry. Use sieve to clean.
Lupinus chamissonis	Apr	Jun	Collect seed April 1- June 30th. Remove seeds from receptacles, no further cleaning required.
Melica imperfecta	Apr	Jun	Collect seeds April 15th- June 1st.
Acmispon glaber [Lotus scoparius]	May	Jul	Collect seeds May-July. When ripe, strip seed pods from stems by hand. Avoid breaking seeds during thrashing. Rub pods with wooden block over #16 (medium) screen. Seeds should be removed from seed pods. Remove excess chaff with seed blower.
Astragalus tener var. titi	May	Jul	Collect May- June. Extract seeds from fruits by hand. Thresh seeds over soil sieve large enough to let seeds fall through. Extracted seeds should be run through a seed blower to remove parasitized or aborted seed.
Baccharis salicifolia	May	Jul	Dioecious. Collect seeds May-June. Collect ripe fruits by hand or shaking seeds onto canvases/tarps. Fruits can be rubbed with fingers or over a screen to remove the pappus. For cuttings, use a stem as long as your arm and as wide as your finger. Cut the bottom of the stem at an angle, strip off leaves, push the stem into soil, leaving at least 2 buds above the surface. New leaves will sprout in ~2 months.
Abronia maritima	May	Aug	Plants produce flowers and seeds throughout the year, with the majority of flower and seed production occuring in late spring/summer. Rub fruits over a medium screen, use a seed blower unit to remove chaff from sieved seeds.
Abronia umbellate	May	Aug	Plants produce flowers and seeds throughout the year, with the majority of flower and seed production occuring in late spring/summer. Rub fruits over a medium screen, use a seed blower unit to remove chaff from sieved seeds.
Eriogonum fasciculatum	May	Aug	May-Aug (best Jun-Jul). Collect inflorescences as they begin to brown and turn rusty in color. Seeds may be seperated or left in the flowers. Push seeds through a screen to remove chaff
Salvia apiana	May	Aug	Propagates more easily from seed than cuttings. Collect seeds as capsules begin to dry, but before seeds are released. Shake seeds from seed heads and/or use a sieve to isolate seeds. Dry clean seeds for a few days before transferring to refrigerator. For cuttings, gather soft wood before flowering. Cuttings should be 3-4 inches long. Remove lower leaves, dip cutting in growth medium
Suaeda taxifolia	Jun	Jul	Collect seed by stripping flowers with seed from inflorescences (Jun-Jul)
Mimulus aurantiacus	Jun	Aug	Seeds are collected Jun 1st- August 1st. Rub seed capsules over a sieve
Salvia mellifera	Jun	Aug	Seeds collected Jun- Aug, after inflorescences with calyces are dry and brown. Mature seeds can be collected by clipping, stripping, or shaking seed heads. Seed should be dried and passed through a sieve. Use of a blower is recommended
Atriplex watsonii	Jun	Sep	Seed collection: Jun-Sep
Monanthochloe littoralis (Distichlis littoralis)	Jun	Sep	Dioecious. Seeds small and difficult to collect. Seeds should be acquired over summer from Jun-Sep.
Artemisia douglasiana	Jun	Oct	Seed is ready to harvest when it can be easily removed from the heads by shaking. Clip the seed stalks and bag the material for air drying. Seeds can be threshed by rubbing the inflorescence through a screen and separating chaff with a blower.
Grindelia camporum	Jun	Oct	Harvest seed in June and again in October. Clip seed heads or shake/rub mature seeds from seed heads into a collection bag. To clean, rub seed heads over sieve. Remove chaff using additional sieves or an air separator. Air dry in oven at 203 F (room temp ok).
Hordeum brachyantherum	Jun	July	Collect seeds June 1st to July 31st. Mature inflorescences are light brown. Seed easily removed when stalks are hand stripped. No additional cleaning required
Salix exigua	Jun	July	Dioecious. Harvest when catkins change from green to yellow-brown in June-July and capsules begin to open. Seeds are then easily stripped from branches (15-36 seeds/capsule). Dried seeds will separate from cottony catkins when shaken. For cuttings, branches must be cut before seed is dispersed and placed into water buckets.
Heliotropium curassavicum	Jun		Seeds ripen from base of stalk toward tip.
Cressa truxillensis	Jul	Aug	Produces mature seeds from late summer into early autumn.
Rosa californica	Jul	Aug	Hips can be collected as soon as they are ripe, in late summer or early fall. Achenes extracted by macerating hips in water and removing floating seeds
Stipa cernua [Nassella c.]	Jul	Aug	Seed can be harvested by hand or with a flow-vac or combine. Dry seeds in paper bags kept in warm conditions.
Triglochin maritime	Jul	Sep	Collect seeds between July 17- Sept. 23rd. Mature inflorescences are brown. Seed Cleaning: Rub dry fruits between fingers to extract the seeds.
Iva axillaris	Jul	Oct	Collect late in the season. Seeds can be hand stripped or beaten into a hopper/open container. Flower material should be rubbed over a sieve/screen and run through a blower to remove chaff
Croton californicus	Jul	Nov	Dioecious. Collect July 15th- November 17th, shake chaff away from seeds by hand. Seeds encased in seed pods, pods will need to be removed. Mature seeds are round and brown with tan spots.
Vulpia microstachys	Jul	Sept*	As it is an annual, only regenerates from seed. Unknown when seeds from S. California plants mature (intermountain varieties mature in late July-- late Sept.)

Appendix 3.6D

Scientific Name	Start	End	Seed Collection Details (copy of draft palette)
Astragalus pycnostachyus var. lanosissimus	Jul		A. sinuatus seeds mature and should be collected in late July (http://www.centerforplantconservation.org/collection/cpc_viewprofile.asp?CPCNum=491)
Astragalus trichopodus	Jul		Other plants in genus, specifically A. sinuatus, have seeds that mature in late July
Schoenoplectus acutus	Aug	Sep	Seeds mature late August- September. Because they are easily dispersed by wind, it is important to collect seeds close to the time of maturity. Seeds must be seperated from the panicle and cleaned.
Achillea millefolium	Aug	Oct	Cut entire inflorescences, collect in paper bags. Keep seed in a well ventilated area while drying and before cleaning. Clean seeds with a hammermill, screen, and fanning mill
Oenathera elata	Aug	Oct	Collect seed from spring cultivars in October and from winter cultivars in September. Bag seed heads and allow them to dry on plant or collect early and allow to ripen in paper bags.
Jaumea carnosa	Aug	Nov	Collect seed Sept. 9- Nov. 11 while fruits are swollen and green. Seeds are linear achenes with longitudinal stripes. Rub seeds over #12 sieve to clean.
Suaeda moquinii	Aug	Nov	Seeds ready for collection when they are hard, black, and shiny. The calyces will be brown and crumbly. Seeds can be stripped from plant by hand. Seeds should be spread out to dry before being processed/stored
Baccharis pilularis	Aug	Dec	Collect Sept-Dec. Dioecious. Seeds can be collected by hand into open breathable bags or branches can be shaken with open tubs or tarps placed underneath branches. Fruit should be spread out to dry in a well ventilated room or in the sun. Dried heads and achenes can be rubbed between palms or over a screen to remove the pappus and phylliaries.
Atriplex lentiformis	Sep	Jan	Dioecious. Seeds should be collected in the fall and winter. Produces large amounts of seed
Atriplex californica	Sep	Oct	Seeds are collected from Sep- Oct. Gently rub over #18 sieve. Blow off as much chaff as possible with a seed blower.
Atriplex patula/triangularis	Sep	Oct	Seed collection: Sep-Oct. Collect seed as flowers mature. Fully mature fruit can be shaken or hand stripped from the branches and collected in bags or baskets or onto a canvas spread below the bush. Seeds will often remain on bushes until April, so later collecting is possible
Frankenia salina	Sep	Oct	Collect: September 16th- October 21st. Collect mature flowers. Rub entire flower head over #25 sieve. Use of gloves when handling the plant is advised as plant can be spiky.
Suaeda nigra	Sep	Oct	Collect seeds from mid-September- October. Seeds ready for collection when they are hard, black, and shiny. The calyces will be brown and crumbly. Seeds can be stripped from plant by hand. Seeds should be spread out to dry before being processed/stored.
Batis maritima	Sep	Nov	Collect seed Sept-Nov (best early Oct-early Nov), as fruits mature and turn from green to white. Dried fruits should fragment easily, exposing seed
Distichlis spicata	Sep	Nov	Dioecious. Collect September 11th- November 4th. Mature inflorescences are panicles, 2-8 cm long. Seed is 2 mm long and brownish-gray at maturity. Rub seeds over #18 sieve to clean.
Limonium californicum	Sep	Nov	Collect seed Sept. 9- Nov 17th (Oct best). Collect entire flower heads, flower should detach easily when ripe. Rub flower heads over #20 sieve to clean
Salicornia bigelovii	Sep	Nov	Entire nflorescences should be collected and air dried. Seeds strip easily from inflorescences after drying.
Artemisia tridentata (?)	Sep	early winter	Strip the entire inflorescence by hand. Seeds will need to be cleaned and chaff removed.
Heteromeles arbutifolia	Oct	Jan	Collect seeds from Oct- Jan. Fruits should be clipped/stripped from branches when bright red. Soak berries in water to ferment slightly (over-soaking can be damaging). Separate seeds from the pulp (flotation will help remove pulp). Allow seeds to dry before storing.
Ambrosia psilostachya	Oct	Dec	Fruits form and seeds disseminate October-December.
Isocoma menziesii var. vernonoides	Oct	Dec	Harvest seed mid-October to mid-December. Collect achenes golden in color, as seeds are usually eaten by time achenes turn brown. Shake ripe heads over open containers to collect achenes. Alternatively, remove ripe heads and keep in porous bags. For I. acradenia Wall and Macdonald recommend rubbing flowers over a large screen, using a seed blower, and sieving over a #18 screen to separate seeds from bracts
Salicornia pacifica [S. virginica]	Oct	Dec	Collect inflorescences Oct- Dec (Nov-early Dec is best). Collect when tips of plants are purple. Dry seeds on screen up to 3 mo.
Suaeda esteroa	Oct	Dec	Collect seeds Oct-Dec (best Nov/early Dec). Seeds can be harvested by collecting whole inflorescence or stripping flowers with seeds from inflorescence. Seeds remain in flower until senescence. After cleaning, seeds should be dried
Platanus racemosa	Oct	spring	Collect seedpods October- early spring by cutting them directly from the tree. Cut off the stem and break seedpod open. Let the seeds dry 2-3 days. If the seed is not ripe yet, it will be difficult to break open
Artemisia californica	Dec	Jan	Collect seeds in December and January. Strip entire inflorescence by hand. Seeds will need to be cleaned.
Salix lasiolepis	Dec	Jan	Dioecious. Seeds can be hand harvested when capsules begin opening.Hardwood cuttings collected Dec 15- Jan 31st. Cuttings should be kept moist and cool. When ready to process, dip in mild bleach soln.
Atriplex canescens			Collect utricles when mature August-September in northern territories and October- March in southern.
Baccharis glutinosa [B. douglasii]			
Baccharis sarothroides			Dioecious. Seeds can be collected by hand into open breathable bags or branches can be shaken above open tubs or tarps
Brickellia californica			Long, narrow achenes. Dark brown in color when mature.
Camissonia cheiranthifolia			

Appendix 3.6D

Scientific Name	Start	End	Seed Collection Details (copy of draft palette)
Elymus triticoides [Leymus triticoides]			
Encelia californica			Achenes are densely compressed, wedge shaped. Edges are long-ciliate and faces are flabrous or short-hairy. Seeds should be dark brown at maturity. Collection timing is critical as achenes are easily blown from plant after reaching maturity.
Eriogonum parvifolium			Other members of Eriogonum genus: flower July-August; fruit is a hard, dry, three sided achene. Achenes can be hand stripped from plants.
Euthamia occidentalis			
Hazardia squarrosa			Fruit: 5–8 mm, 5-angled, glabrous; pappus 7–12 mm, white to red-brown in color
Hordeum depressum			No information found, use H. brachyantherum information as a rough guide
Isolepis cernua [Scirpus cernuus]			
Juncus acutus ssp. Leopoldii			
Juncus balticus			
Juncus mexicanus			
Lasthenia glabrata var. coulteri			
Lycium californicum			Seeds (berries) best collected within 2 weeks of setting, otherwise birds will eat the majority. Must be picked by hand
Malacothamnus fasciculatus			
Phacelia ramosissima			Collect seed when flowers are dry and brown. Strip seed from mature inflorescences directly into collection bag.
Pluchea odorata			
Populus fremontii			Dioecious.
Potentilla anserina ssp. Pacifica			Seeds should be dried on the plant, then collected. If using, root ball divisions should be made in spring.
Pseudognaphalium californicum [Gnaphalium c.]			
Rumex salicifolia			
Salicornia subterminalis			Collect inflorescences and air dry, seeds fall out of remaining fragments
Sambucus nigra [S. mexicana]			
Schoenoplectus californicus [Scirpus c.]			Collect, dry, and store seeds in brown paper bags or burlap bags. Seeds and seed heads need to be cleaned in a seed cleaner.
Spartina foliosa			Multiple harvests may increase probability of collected good seeds prior to dispersal or herbivory loss. Seed should be refrigerated dry for 2-4 weeks and then refrigerated in the dark in salt or fresh water.

Appendic 3.6E

Species	Seed Longevity	Storage Details (copy of draft palette)
Lycium californicum	1 year	Store in cool place. Will begin to mold after 1 week at room temp or 3-4 weeks at 40 F. separated seeds can be stored up to a year
Limonium californicum	1 year (ideally)	Dry, store at cool temperatures. Best if used w/in 1 year (Zedler 2001)
Salicornia bigelovii	1 year (ideally)	Should be stored in cool termperatures. Viability is reduced after 1 year.
Heteromeles arbutifolia	1 year (or less)	Seeds have limited longevity at room temperature. Store at cool temperatures, probably orthodox in storage behavior. Shelf life of less than one year.
Baccharis sarothroides	1+ year	Cleaned seeds can be stored dry over winter (and possibly longer)
Atriplex californica	10 years	Keep dry, store at room temperature. Atriplex seeds can be stored for 10+ years in tightly closed containers in a shed/warehouse
Atriplex patula/triangularis	10 years	Store at cool temperatures. Atriplex seeds can be stored for 10+ years in tightly closed containers in a shed/warehouse
Atriplex watsonii	10 years	Atriplex seeds can be stored for 10+ years in tightly closed containers in a shed/warehouse
Salvia mellifera	1-2 years	Store in cool, dry conditions. storange in a warehouse- 41.3% germination rate. Longevity increased in cold. 1-2 years ambient temps
Isocoma menziesii var. vernonoides	1-2 years (or less)	Store in cool, dry conditions to increase longevity. Seeds are relatively short lived. After 2 years of storage, only 9.6% of seeds germinated
Frankenia salina	2 years (or less)	Keep seeds dry at room temperature. Seeds last up to 2 years.
Batis maritima	2+ years	Refrigerate (viability is 2+ years)
Salicornia pacifica [S. virginica]	2+ years	Seeds viable for 2+ years
Suaeda esteroa	2+ years	Stored at 5C. Seed germinability remains high after 2 years
Rosa californica	2-4 years	Seeds stored dry in sealed vials will retain viability for 2-4 years
Salix lasiolepis	3 years (freezer)	Can be stored for up to 10 days at room temperature or up to 1 mo in wet, refrigerated containers. Seed viability increases at cold temperatures (-10 C) and can be as long as 3 yrs- must use **double 3 mil polyethylene bags**
Salix exigua	3-4 years (freezer)	Dried to 6-10% of dry weight. Can be stored under constant humidity. Longevity: 1-5 C- 6 months, subfreezing (-10 or -20) can last up to 44 months.
Achillea millefolium	3-5 years	
Atriplex lentiformis	3-6 years	Seeds can be stored 3-6 years, have been successfully stored for 5 years (6 years is recorded max)
Malacothamnus fasciculatus	50+ (genus)	
Atriplex canescens	5-7 years	Reported good for 5-7 years in sealed containers at 21C. Dewinging may increase storability.
Abronia maritima	6 years	not indicated
Acmispon glaber [Lotus scoparius]	Long-lived	Long lived in soil seed bank and in cool, dry storage. Dried to low moisture content and stored in **vacuum vials**
Hordeum brachyantherum	Long-lived (4-5 years)	Keep dry in refrigerator
Hordeum depressum	Long-lived (4-5 years)	
Melica imperfecta	Long-lived (4-5 years)	Dry seeds should be refrigerated
Stipa cernua [Nassella c.]	Long-lived (4-5 years)	After drying and cleaning, seal in paper bags and store at 40 F and 40% RH.
Stipa lepida [Nassella l.]	Long-lived (4-5 years)	not indicated
Lupinus chamissonis	long-lived (many years)	Store dry seeds at room temperature. Longevity not noted
Astragalus pycnostachyus var. lanosissimus	long-lived (many years)	
Astragalus tener var. titi	long-lived (many years)	Maternal samples packaged in **glassine envelopes** and dried to equilibrium at 14% rel humidity. After 3 weeks, transfer seeds to heavy duty foil/plastic pouches at keep at 18 C
Astragalus trichopodus	long-lived (many years)	
Iva axillaris	Short-lived	Seeds may be relatively short lived if dry stored. Seeds should be stored submerged in water inside **lumite screen bags**
Potentilla anserina ssp. Pacifica	Short-lived	May be stored short term (longevity not noted)
Spartina foliosa	Short-lived, 4 months	Viability decreases after 4 months
Abronia umbellate		
Ambrosia psilostachya		
Artemisia californica		
Artemisia douglasiana		
Artemisia tridentata (?)		
Baccharis glutinosa [B. douglasii]		
Baccharis pilularis		Clearned dry seeds can be stored at 1.7-4.5 C in airtight containers
Baccharis salicifolia		
Brickellia californica		
Camissonia cheiranthifolia		
Cressa truxillensis		
Croton californicus		
Distichlis spicata		Seeds are kept dry and stored in refrigerator
Elymus triticoides [Leymus triticoides]		
Encelia californica		
Eriogonum fasciculatum		Store in a cool, dry place
Eriogonum parvifolium		
Euthamia occidentalis		
Grindelia camporum		
Hazardia squarrosa		
Heliotropium curassavicum		
Isolepis cernua [Scirpus cernuus]		
Isomeris arborea		
Jaumea carnosa		Store with **perlite** to remove moisture at room temperature
Juncus acutus ssp. Leopoldii		
Juncus balticus		
Juncus bufonius		
Juncus mexicanus		
Lasthenia glabrata var. coulteri		

Appendic 3.6E

Mimulus aurantiacus		Store dry in refrigerator
Monanthochloe littoralis (Distichlis littoralis)		air dried, and stored in cool temp. stolon cuttings root well in moist soil.
Oenathera elata		
Phacelia ramosissima		
Plantago erecta		Cool, dry storage
Platanus racemosa		
Pluchea odorata		
Populus fremontii		
Pseudognaphalium californicum [Gnaphalium c.]		
Rumex salicifolia		
Salicornia subterminalis		Store in cool temperatures.
Salvia apiana		
Sambucus nigra [S. mexicana]		
Schoenoplectus acutus		
Schoenoplectus californicus [Scirpus c.]		
Suaeda moquinii		
Suaeda nigra		
Suaeda taxifolia		Store in cool temperatures
Triglochin maritime		Storage Conditions: Seeds are kept dry and stored at room temperature.
Vulpia microstachys		