BEST PRACTICES IN SEED COLLECTION AND SOURCING

By: Librada Cruz

University of Santa Cruz

June 2015

Introduction

Younger Lagoon Reserve (YLR) is located on the northern coast of Monterey Bay on the west side of Santa Cruz, California. YLR is part of the University of California Natural Reserves system and is part of a 20 year restoration project managed by UC Santa Cruz to mitigate for construction of buildings and other portions of the site (Stern, 2013). The goal of the restoration project is to restore the previously degraded land by agricultural use back to its natural habitat of coastal prairies, coastal scrub, and freshwater wetlands (Stern, 2013). As part of the restoration, YLR staff and students collect, clean, and store seeds. The seeds are then used for direct seeding and growing plants at the UCSC greenhouse to outplant to the restoration site. Using seeds for restoration purposes can be less expensive than purchasing seeds that might not be genetically appropriate for the restoration site (Budelsky, 1999). Collection also allows for the acquisition of seeds that are not commercially available (Packard and Mutel, 1997). The goal of this paper is to review the best practices for seed collection and sourcing for coastal habitat restoration at YLR. The purpose is to provide concise background information on seed collecting and sourcing for future interns that will be involved with restoration at YLR.

Collection Considerations

Before going out into the field to harvest seeds, certain considerations should be taken into account when choosing a reference site in order to obtain the seeds that are best adapted to the restoration area. Collecting from within the restoration site is acceptable but in the case of Younger Lagoon there are few native species on site. Moreover, it is best to have multiple reference sites to increase genetic diversity, which is important for restoration because it avoids genetic inbreeding allowing for the ecosystem to be more resilient to environmental changes (Maschinski et al., 2012).

In choosing a reference site it is necessary to consider geographical distance from the restored site, but there are tradeoffs between selecting more local seed and seeds that may be better adapted to future conditions. Collecting seeds from neighboring areas is beneficial because the seeds are adapted to the local environment and can restore genetic flow between populations that have been separated by urbanization. However, finding suitable neighboring site can prove to be a difficult task in highly urbanized areas like YLR, where there are no source populations. Environmental criteria to delineate seed zones, areas in which natural genetic exchange occurs, are by geographical distance, climate, latitude, and altitude (Vander Mijnsbrugge, 2010). A negative effect that can happen when gathering seed from local sources is the lack of genetic diversity available that can adapt to environmental changes or a disease outbreak (Vander Mijnsbrugge, 2010). Another option to increase genetic variation is to collect seeds from other geographical areas that differ from the restoration site in climate, latitude, longitude, and altitude. Some studies have shown that non-local species have higher fitness in their new environment (Vander Mijnsbrugge, 2010). However, there are some negative effects that can happen like maladaptation can be introduced resulting in outbreeding depression (Vander Mijnsbrugge, 2010).

When YLR staff started their restoration efforts they could not gather seeds from the restoration site because all of the native plants were removed from the land's previous usage as agriculture (Brown, personal communication). Seven reference sites were chosen from Monterey to Franklin Point which is north of Younger Lagoon within Año Nuevo State Park (Brown, personal communication). These sites were chosen for their relative abundance of native plants and that had minimum disturbance from Younger Lagoon.

A second aspect to consider is to verify the genetic diversity of the seeds being gathered at the different sites by using DNA markers to distinguish variation among populations. Using DNA markers provides data relatively quickly, is less expensive than full genetic sequencing (Maschinski et al., 2012). The Genetic Identification System in California can be helpful in using DNA markers. A more simple way to distinguish variation is to collect seeds from phenotypically different plants, for example, different stalk lengths or seed size. This method is quick and has minimal monetary cost.

Once the reference sites are chosen certain guidelines should be followed on collecting seeds from within the reference site to increase genetic diversity. It is recommended to collect from 50 maternal plants because it captures the available genetic diversity present in the population (Vitt et al., 2010). The number of maternal plants collected from can also depends on

Table 1
Seed collection protocols.

- Collect from a minimum of 50 maternal plants to capture 95% of the genetic diversity
- Collect no more than 10–20% available seed on any given day, to ensure that collection efforts do not impact vital rates of the target populations
- Collect across any obvious environmental gradients
- Collect both from within the center of population density AND from the periphery to ensure the greatest genetic diversity and to ensure collection from individuals that may perform better in marginal portions of the habitat
- Search out and collect even the smallest plants, because they may contain quantitative trait variation that would pre-adapt them to an alternate site
- In general, collections are bulked within a population, but maternal lines may be stored separately in some target species
  – to facilitate research efforts
  – in species with naturally low fecundity
  – to ensure equalization of founders
  – when collecting from small or marginal populations
  – when collecting species known to be self-incompatible
- Collect a minimum of 3000 seeds, with an optimal target of 30,000. It may be necessary to collect across years in the same populations. If so
  – collect no more than 10% of seeds
  – consider maternal-line collections versus bulked
  – separate years should be accessioned individually
- Collect at peak seed maturity, recognizing that some phenotypes (and sires) will be excluded, or collect on multiple days
- Collect from within the entire inflorescence, recognizing that proximal patterns of maternal plant development as well as patterns of embryo development might be influenced by genetic makeup of the embryos, and therefore skew genetic contributions
- Collect voucher specimens
  – herbarium vouchers allow expert confirmation of species identification
  – a leaf tissue sample can ultimately become a DNA voucher
- Collection information is critical to establish provenance of each accession. Standard collection protocols that include collectors name, locality information (particularly GPS coordinates), property ownership, terms of the collecting permit if it limits the use of the seeds, etc., are essential. Information on the habitat that might be critical for habitat matching includes basic soil type, description of the terrain and hydrologic qualities of the site, as well as community dominants and other associated plant species. Additional information on the status of the target population should include an estimate of population size, percentage of reproductive plants, and the number of plants from which the seeds were collected, which is particularly important when the seeds are not separated by maternal line

Table retrieved from Vitt, P., Havens, K., Kramer, A.T., Sollenberger, D., & Yates, E. (2010).

plant reproduction; it is suggested to gather seed from 30 outbreeding sexual plants or 59 self-fertilizing species (US Department of Interior, 2012). It is also advised to only collect 10% of available seed to minimize impact and diversity loss to reference site. Randomizing collection and collecting from environmental gradients increases the amount of genetic variation gathered. Another consideration is to collect seeds on multiple day to capture the seeds that were excluded (Vitt et al. 2010). Table 1 provides an easy to read guide for seed collection protocol.

Timing is another aspect to consider before seed collecting, as it is very important to harvest seeds at the right time in order to not waste time and resources. Knowledge of the life cycle of the target plant would allow for the perfect timing to head out to the field for seed collection when the plants are actually in seed. Seeds have two stages, soft seed and hard seed. Soft seed refers to seeds that have high moisture content that can be easily squashed with the thumb and forefinger (Young, 1986). At the soft seed stage seed are not fully matured and have low viability. When collecting seeds at their soft stage it is usually recommended to let the seeds dry (preferably on the stalk) so they can reach maturity. Hard seed describe seeds that have already reached maturity and have less moisture content. Collecting mature seeds that have fallen on to the soil surface is not advisable because those seeds are of low-quality and can be contaminated with fungus and pathogen (Young, 1986). Seed set can vary at each reference site due to microclimates (Young, 1986). Most of the grasses and forbs at YLR start dropping seeds in late summer and early fall (Brown, personal communication) but the timing of seed collection varies depending on temperature and rainfall, so it is important to be prepared.

Finally before seed collection takes place permission for harvesting seeds needs to be obtained. For example, collecting seed from public land managed by the Bureau of Land Management is excluded by the National Environmental Policy Act (NEPA) unless proper permission has been granted (US Department of Interior, 2012). Most of the sites that YLR collects seeds from are from California State Parks. The stipulations that the CA State Parks places on YLR is that they must collect seed 10 ft (~3 m) away from a trail to create a buffer, and secondly they can only collect 10% of the seeds in a population (Brown, personal communication). Another piece of legislation that must be considered is the Endangered Species Act. The Endangered Species Act makes it illegal to buy, sale, carry, or transport plants that are listed as threatened or endangered (Young, 1986). If a seed is being collected that is endangered or if an endangered animal relies on those seeds to survive an ESA permit must be acquired from the Fish and Wildlife Services.

Seed Collecting

Hand collecting of seed, the primary method of seed collection at YLR, allows for accurate selection of the preferred seed, if the personnel are well trained. The benefit of this method is that the healthy mature seeds will be picked and no seeds from unwanted plants will be mixed in. This method is best for plants that are widely dispersed in areas that are inaccessible to machines (Packard and Mutel, 2007). The hand harvesting method is a slow process and requires plenty of labor, but several approaches can help speed up the process. Clippers can be used to cut the seed head. Another option is to shake the seed stalk and collect the seeds in a container as they drop (Young 1986). This method is faster than hand harvesting each seed, however, it still requires plenty of labor.

One of the ways to speed up the manual method this to use a hand held machine, such as the seed stripper. The seed stripper has a high speed rotating wheel that combs seeds off the plants then drops them into a bag (Packard and Mutel, 2007). The downside is that the stripper catches the seeds of unwanted plants. Another method is to use a hand held machine that would cut the stalk at seed level. This method is faster, however, it makes it difficult to store and transport the material to the restoration site. Heavy machinery is not recommended because of the damage it will inflict on the reference site (Vander Mijnsbrugge, 2010).

Figure 1: Seed Stripper. Photo taken from Internet.

Another method for collecting seed is to build a seed farm. A seed farm is when plants are grown for the purpose of harvesting their seeds. This could be done at YLR in small plots on the reserve to regenerate seeds. Regeneration is when seeds reaching their storage capacity are planted to renew their seeds (Querol, 1993). Seed farms would allow for a more efficient manner in harvesting seed because the seeds are densely packed together. In this type of set up, harvesting can be done quicker with a small hay harvester because it can be driven over the plot to collect and clean seeds at the same time. The downside is that this machinery is expensive. Another method is to use a mechanical trimming device to cut the crop at seed level. This method while slower than using a hay harvester it is less expensive. Another benefit is since these cuttings are at the restoration site it is much easier to gather and store the stalks underneath some sort of house cover for short storage before cleaning. Stalks are gathered then dried to let the soft seed mature because the range of seed maturity differs on the same stalk (Young, 1986).

Figure 2: Seed farm plot at Watsonville Wetlands Watch. Photo taken on site

Cleaning

Seed cleaning is an important process in order to remove floral chaff, undesirable seeds that were unintentionally collected, and unhealthy seeds. Seeds are separated by methods that take advantage of seed texture, shape, weight, and size (Wall and MacDonald, 2007). The range of cleanliness of the seeds depends on the seeding method used. Younger Lagoon thoroughly cleans their seeds for planting individual plugs at the greenhouse (Brown, personal communication). Other benefits of thoroughly cleaned seeds are that they increases storage longevity and reduces mold growth (Wall and MacDonald, 2007). There are two general methods used to clean seeds, threshing and winnowing.

The threshing method involves agitating the floral chaff to releases seeds. One threshing method, commonly used at YLR, is hand cleaning with sieves. Separation of the seeds from the

Figure 3: Seed cleaning with sieves. Photo taken from Mary Paul, Watsonville Wetland Watch.

Figure 4: Different sized screens. Photo taken at YLR

chaff is done by rubbing the material over a screen. The seeds drop through the screen while the chaff stays on top. Depending on the size and shape of each seed the screens can be changed. Other threshing methods involve using modified blenders with nylon line or taped blades to macerate fleshy fruits to separate the seeds (Wall and MacDonald, 1997). Maceration can be done with nightshade, barberry, and gooseberry (Emery, 1988). In fire adapted systems, heat is used to opens seed cones, so they can release the seeds inside.


Figure 5: Seed Blower. Taken from the Internet

The winnowing methods used for seed cleaning utilize air flow to separate seeds from chaff. The air cleaning process can be done with a seed blower that has a motor driven fan to supply air pressure on a vertical plastic cylinder that blows away the light chaff to leaves behind the seed (Young, 1986). Seed blowers can be expensive but be relatively cheaper if bought used (Packard and Mutel, 1997). Natural wind, a small fan, or a hair dryer, can be part of the winnowing process as they serve the same purpose, however, the hair dryer should be used with the heat turned off because high heat can damage seeds (Young, 1986).

Another method used is flotation where chaff is separated from the seed by water (Wall and MacDonald, 2007). For smaller seeds textured plate can be used to separate the seed from the chaff with the help of a small brush (Wall and MacDonald, 2007). This method is best used for smaller seeds and can be a tedious process. After seeds are cleaned they should be stored.

Storage

After initial collections, seeds should be stored in a in a dry environment to allow seeds to dry and mature. Seed collected that are small enough can be placed in a paper bag to dry. Plastic bag should not be used because they prevent moisture from escaping which can spoil the seed and promote mold growth (Young, 1986). When drying seeds that are still attached to their stalk they should be piled on a tarp outside to dry. It is important to ensure to cover the pile at night and to protect them from birds and rodents (Emery, 1988).

After seeds are dried and cleaned, seed can be stored in an opaque glass jar in a dry room. Opaque glass is used to prevent direct light from reaching seeds to prevent premature germination and to reduce the temperature inside the jar. Two important factors that affect seed longevity are seed moisture content and temperature. It has been shown that storage potential doubles with every one percent reduction in seed moisture and every 5.6 °C drop in temperature (Querol, 1993, Young 1986). It is important to control these factors because moisture and warm temperature stimulate germination and fungal growth (Young, 1986). A good way to ensure moisture does not seep into the storage container is to place a desiccant packet within the storage container.

Figure 6: Opaque jar for storing seeds. Taken at YLR

There are different temperature at which to store seed, which depends on each individual species' life history and dormancy patterns. For example, grassland seeds are stored in dry-warm climate while wetland seeds are better stored in a dry-cold climate (Budelsky, 1999). Another storage method is to have a temperature controlled room where seed can be stored in a frozen state, also known as cryopreservation. Seeds in cryopreservation are stored at -40°C and have a seed moisture content below 14%, so no small ice crystals can form inside the seed (Querol, 1993). This method is already being used by the Millennium Seed Bank (MSB), which is a facility that collects and stores a huge amount of the world's plant species for preservation. But, cryopreservation is not as important at YLR, where seeds are stored mostly for one or a few years before being used for restoration. An important part of storage to remember is labeling;


Figure 7: Cryopreservation storage facility. taken from MSB website.

labels should include the date of collection, location, and species name in Latin nomenclature (Figure 6).

Viability Testing

There are a number of ways to test seed viability. One way to assess viability is based on shape. A healthy seed is determined by its plump shape that is filled from edge to edge with firm moist tissue and an undamaged seed coat (Wall and MacDonald, 2007). Seed viability testing

Figure 9: Seed excision test. Photo taken from the Internet

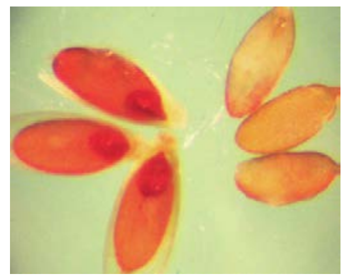

Figure 10: Tetrazolium test. Photo taken from the Internet

can also be done with embryo excision which involves cutting the seed in half to examine the tissue inside (Young, 1986). The problem with the embryo excision method is that the seeds are rendered unviable when they are cut (Young, 1986). When using the embryo excision a small sample of the seeds collected should be examined. Tetrazolium staining is another method used to test seed viability. This test determines if the seed are alive by staining the embryo pink or red. When the seed do not take up the red stain it means that the seed are dead (Querol, 1993). However, a red stained seed does not guarantee that the seeds will germinate even if dormancy factors are removed (Young, 1986). Both these methods are a way of estimating the percent of damaged seeds which can be a good confirmation factor that the majority of the seeds are mature and fully formed (US Department of Interior, 2012).

Quantifying germination rates is important to know the amount of seeds that will germinate when planting an area and determine the seeding rate in order to achieve desirable amounts. Seeds tend to lose their viability the longer they are stored, and the length of viability varies greatly among species, so seed viability has to be tested again after storage by measuring germination. A germination rate is achieved by sowing 100 seeds on a tray to see the percentage of seeds that germinate (Brown, personal communication). Germination rates should be done periodically to test the viability of seeds after storage to determine how long seeds are viable. Young (1986) recommends doing four replicates of 100 seeds to to calculate a statistically dependable germination rates. A Germination rate around the range of 80% are desirable (Packard and Mutel, 1997).

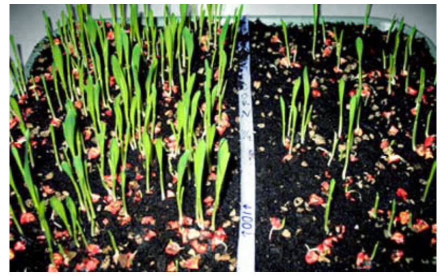

Figure 11: Variations in germination rate. Photo taken from Internet

Seeds that are alive and fail to germinate are in dormancy. Dormancy allows the seed to stay viable for a long period of time without germinating in optimal conditions (Querol, 1993). Dormancy of seeds should be broken before germination, which can be challenging as the triggers to break dormancy are highly variable across species. There are two types of seed dormancy, Seed coat dormancy (hard seed) and internal dormancy (Emery, 1988). Internal dormancy occurs when chemicals inside the seed prevent germination (Querol, 1993). A common strategy used to break this type of dormancy is stratification. Stratification is used by exposing the seed to cold temperature. This strategy changes the biochemical processes inside the seed by mimic seasonal changes to break seeds dormancy (Emery, 1988). In some grass species both hot and cold temperatures are use in oscillation to break dormancy (Querol, 1986). Scarification is used on hard seeds. Scarification involves agitating the seed coat to allow water and oxygen to penetrate the seed. This is done in many ways like using sandpaper, hot water, acid, and heat. Some seeds have both types of dormancies, in these cases, scarification should be done first then stratification (Emery, 1988).

Seeding Methods

The seeding method that YLR has primarily used is that of direct transplanting. The UCSC greenhouse grows the seedlings that are transplanted at YLR (Velzy, personal communication). Growing seedlings at the greenhouse effectively uses seed since they are not lost to predation or desiccation of recently germinated seedlings in the field. The transplanting

Figure 12: Direct Transplanting. Photo taken from Mary Paul, Watsonville Wetlands Watch

method works well for YLR because there are plenty of workers and it minimizes the amount of seed that is needed.

Another method for seeding is broadcasting. Broadcasting spreads the seed directly on to the desired area. This can be done by hand or with a belly grinder. Broadcasting with the belly grinder allows for faster setting of seed and requires less labor than transplanting. The downside of broadcasting is that it does not provide the required seed to soil contact for germination and exposes many seeds to predation. Broadcasting is impractical on windy days since seeds would blow away, however, weighing down seeds with straw can prevent seeds from blowing away while providing mulch (Packard and Mutel, 2007).

Figure 13: Broadcasting with belly grinder. Photo taken from Internet

A third method is called drill seeding, which can be done with a hand held machine that digs a small hole, places the seed inside then covers it up. This method tends to result in higher germination rates since it buries the seed reducing seed exposure to predation and increases seed to soil contact. Clean seeds are best used on drill seeders to reduce jamming and since YLR already thoroughly cleans their seeds, no extra steps need be added. Drill seeding also requires less seeds than broadcasting does for the same area and can be used on windy days (Packard and Mutel, 2007). Drill seeding like broadcasting is faster and less labor intensive than transplanting. The downside to drill seeders are that they are expensive to purchase but are available for borrowing or renting. Drill seeding is the most effective seeding method of prairies (Packard and Mutel, 2007).

Figure 14: Drill Seeder. Photo taken from internet

Conclusion

Most of the methods that YLR uses for collecting and sourcing seeds are best for the type of restoration being done. The methods that are working at YLR are their usage of the seven reference sites because it increases the genetic diversity at the restoration site. Their hand collection methods are feasible because of the labor available to them while creating educational opportunities. The cleaning methods at YLR are also working because they are adaptable and make sense for the amount of seeds being cleaned. Methods that can be improved at YLR are storage and seeding. Velzy recommended that cryopreservation be used because it would help increase the germination rates at the greenhouse, but this would require cold storage facilities. For seeding methods the drill seeder seems to be the best choice. Drill seeder require less seeds than broadcasting for the same amount of area and drill seeding can be done on windy days. YLR should try small-scale drill seeding methods because the drill seeder can plant rather quickly without needing too much labor.

Annotated Bibliography and Recommended Further Readings

Baskin, C.C., & Baskin, J.M. (2014). Seeds: ecology, biogeography, and evolution of dormancy and germination. Burlington: Elsevier Science

- This source cover the types of seeds, their anatomy, dormancy patterns, and germination. It is detailed with the specifics of each seed dormancy pattern. The source also provides many tables and examples of seeds. This source is very scientific in detail and it mostly geared toward professionals in that field.

Brown, T. Younger Lagoon Reserve Steward. Interview. April 16, 2015.

Budelsky, R. A, & Galatowitsch, S.M. (1999). Effects of moisture, temperature, and time on seed germination of five wetlands Carices: implications for restoration. *Restoration Ecology, 7*(1), 86-97.

- This source is a scientific article about the effects of different type of storage on wetland species. Five different sedges were stored in wet-cold, wet-warm, dry-cold, and dry-warm environment to check what effects storage had on germination. It was found that wet-cold was best for wetland species. This is a good article that contradicts the traditional method of storing seed in cold-dry environments.

Emery, D.E. (1988). Seed propagation of native California plants. Santa Barbara Botanic Garden.

- This book contains basic information about the dormancy and the propagation of native plants in California. It also has recommendations for seed treatments of native California plants. This book is meant for the average reader, so it is easy to read and well organized. This source was helpful for describing the different techniques in dormancy breaking.

Maschinski, J., Haskins, K. E., Raven, P.H., (2012). Plant reintroduction in a changing climate: promises and perils. Pages 71-403 in Neale, J. (eds) Washington: Island Press. Center of Plant Conservation (Saint Louis, Mo.), & Society for Ecological Restoration.

- This source is about genetic considerations for the reintroducing rare plants. It covers everything from biological and ecological aspects that should be kept in mind to insure genetic diversity. This source was dense with information and provided many of examples where different strategies were used.

Packard, S., & Mutel, C.F. (1997). The tallgrass restoration handbook: for prairies, savannas, and woodlands. Island Press.

- This book is a guide on the restorative practices on prairies, savannas, and oak woodlands. The books covers the range of topics from planning, collecting, seeding, and monitoring restorations sites. This source is a great reference to anyone involved in restoring grasslands.

Querol, D. (1993). Genetic resource: a practical guide to their conservation. Zed Books Ltd.

- This book is about genetic diversity, why it is important and how to maintain it. The book covers the methods that are used to keep genetic diversity through collecting, cleaning, storage, and germination. This book has more scientific in detail and would be of better use to someone with more knowledge.

Stern, Naomi. (2013). Background to younger lagoon reserve: a socio-political perspective. Senior Internship Paper: UC Santa Cruz.

U.S Department of the Interior, Bureau of Land Management. (2012). Technical protocol for the collection, study, and conservation of seed from native plant species for Seed of Success.

- The Seeds of Success program managed by the Bureau of Land Management goal is to collect seed of native plants for research, restoration, and rehabilitation efforts on public land. In order to standardize procedures of all participating organizations in the Seeds of Success program, this manual contains the rules and guideline for collecting, handling, cleaning, packaging, and shiping of seeds.

Vander Mijnsbrugge, K., Bischoff, A., & Smith, B. (2010). A question of origin: where and how to collect seeds for ecological restoration. *Basic and Applied Ecology, 11*(4), 300-311.

- This article explains the pros and cons of collecting seeds from local and non-local areas for the use in ecological restoration. It focuses on the different aspects to keep in mind when harvesting seeds from collection sites and other methods for collection. This source was helpful in providing information on things to consider when choosing collection sites.

Vitt, P., Havens, K., Kramer, A.T., Sollenberger, D., & Yates, E. (2010). Assisted migration of plants: changes in latitudes, changes in altitudes. *Biological Conservation, 143*(1), 18-27.

- This article is about the challenges of protecting certain plant species that face extinction due climate change moving their natural range poleward. It also covers the benefits and cost of translocating species across geographical ranges. This article describes why collecting and storing seeds is important for the future.

Velzy, Jim. Greenhouse manager. Interview. April 30, 2015

Wall, M., MacDonald, J. (2007). Seed Cleaning. Rancho Santa Ana Botanic Garden.

- This source explains the different methods used for seed cleaning. Seed cleaning can be done by threshing or winnowing depending on the type of seed. It also has a short section on assessing the quality of the seed. This source is organized in a easy to read way and has many pictures that demonstrate the different cleaning techniques. It is a good source as an quick introduction of seed cleaning.

Young, J., Young, C. (1986). Collecting, processing and germination: seeds of wildland plants. Timber Press.

- This book is about the various techniques for seed collection and sourcing. It covers the basics of seeds, seed collection, cleaning, storage, and germination at a much greater detail. This was a useful source that provided an overabundance of information that I could not cover in the paper. I recommend this source for those seek more knowledge on the topic.