USE OF MICROPROPAGATION TECHNIQUES TO IMPROVE GERMINATION SUCCESS IN SIX SPECIES OF CACTI

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DEDICATION

I dedicate this thesis to my family. My dad, Stewart Simon, for being supportive and an influence in my efforts towards my life goals and my person. My sister, Lesha, for being my best friend while I was in my worst times. My mother, Cindy Simon, who gave part of herself for me, literally, so I could survive and for fighting for me so I could fight for myself. And my husband, Jeremy Warren, who, when I needed a dose of reality or a shoulder, I can always rely on for anything.

ABSTRACT

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Cacti have been a major contributor to the ecosystem of desert fauna as a constant food source during dry seasons. However, this resource has reached a point of dwindling due to many human interferences. *In situ* conservative efforts have not been stable enough to maintain the efforts of preserving population genetics. Therefore, in *vitro* techniques will be required to counter the effects. Following previous studies, micropropagation techniques were analyzed to optimize germination number, time, and rate around three variables; difference in nutrient media, gibberellic acids, and species. Two trials were run at intervals of eight weeks; the second trial a few weeks after the first had ended. Results showed a strong significance in emergence and germination affected by species type for both trials. There were other significant factors including interactions between variables. Overall, this experiment showed overwhelming evidence towards the need to treat species to separate protocols in micropropagation techniques.

KEY WORDS: Cactus, Micropropagation, Botany, Conservation, Seed, Germination techniques, Sam Houston State University, Texas

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CHAPTER I

Introduction

“Take the rose—most people think it very beautiful: I don’t care for It at all. I prefer the cactus, for the simple reason that it has a more interesting personality. It has wonderfully adapted itself to its surroundings! It is the best illustration of the theory of evolution in plant life.”

Charles Proteus Steinmetz, 1913

Ecological Functions

Cacti, a family of angiosperm plants, are some of the more unique plants within desert landscapes. They have many adaptations for survival in xeric, or water-limiting, environments. These plants are able to retain water in the cortical cells of their stems making them “stem succulent”. Cortical tissue in the succulent stem holds not only water, but malate used from the CAM pathway of photosynthesis, and compound sugars (Nobel 2002). Most cacti stems are able to contract and expand with changing water availability through the morphological adaptations of ribs or tubercles. Ribs are large furrows of stem tissue that run longitudinally from the tip of the plant through to the base. Tubercles are extrusions of tissue along the stem (Mauseth 1995). These ribs and tubercles can allow the cactus stem to expand without rupturing when water is plentiful, and contract without collapsing when water is scarce. Along with the adaptation of water retention there are anatomical features that give these plants an advantage. Cacti have tightly packed fibers and sclereids for structural integrity (Figure 1). A thick cuticle covers the epidermis of cacti to avoid desiccation through evaporation as well as to reflect damaging sunlight. Many also have wool that sprouts from the areoles, or nodes, that can capture water from the air and siphon it to the roots via rib canals. All these allow cacti to survive in dry, hot deserts.

Figure 1. *Oreocereus celsianus* (Cactaceae) cut latitudinally. Notice the ring of fibers associated with the vascular tissue and the large ribs running through the length of the stem. Outside this layer is the cortex layer that can hold volumes of water, acids, and sugar.

Cacti are keystone species of deserts (Casas *et al.* 2015). They are nesting grounds for small birds, such as owls and wrens, and mammals, such as hares and rodents. Their flowers are important sources of nectar and pollen for bats, butterflies, honeybees, and birds (Steenberg and Lowe 1977; Fleming et al. 1996; Fleming *et al.* 2001). Reproductive biology in Cactaceae is very complex. There can be night bloomers or day bloomers. There are spring bloomers, summer bloomers, and winter bloomers (Mauseth 1995). To say that all species of cacti are uniform in their reproductive mechanisms is under-representative. Though, within the region of most cactus diversity, around the Chihuahuan Desert (Hernández *et al.* 2001), cacti typically flower during the early summer months from May to June right after the rainy season (Nerd & Mizrahi 1997). Cacti can bloom every year at least once in drier or hotter years during times of extreme environmental stress while maintaining a large clutch of blooms many times in

rainy years (Nobel 1988). Some species of cacti, such as *Ferocactus wislizeni*, bloom through the dry-weather months, times that other plants do not flower thus cornering the market on pollinators (McIntosh 2002). This strategy provides resources for animals during the critical dry season (Fleming & Valiente-Banuet 2002). They are also essential sources of fruit for bats, mammals, and birds (Steenberg & Lowe 1977; Casas *et al.* 1999). As much as 85-91 percent of the diet of two bat species consisted of pollen and seeds while many other bat species had diets of exclusively cactus (Petit, 2006).

Despite the sometimes-large spines that deter animal herbivores, cacti provide essential high-nutrition food sources. Many ant species use these nectaries as sources of carbohydrates, amino acids, lipids, and other small essential compounds to maintain themselves during the summer temperatures (Assunção *et al.* 2014). Because sources of nutrition may be scarce during dry seasons, numerous animals including bats, insects, birds, and lizards eat the nectar from the flowers as sources of sugar and water (Fleming & Valiente-Banuet 2002). For example, *Ferocactus gracilus* contains extrafloral nectaries, glands in auxiliary meristems that give off nectar that feed 50% of the ant communities in the area (Blom & Clark 1980).

Conservation

Cacti have many uses to humans including food sources, ceremonial components, and fences. Due to their significant water storage, unique morphological features, and attractive and unique flowers, they have become popular ornamental plants in landscape designs (Powell & Weedin 2004). Unfortunately, many species of Cactaceae are near extinction. Factors contributing to this decline include narrow niche constraints, habitat destruction after human encroachment, human poaching of mature individuals for

horticultural interests, and extreme temperature changes and low rainfall associated with climate change (Nobel 1988; Hernández & Godínez 1994; Nobel 2002; Téllez-Valdés & D¡Villa-Aranda 2003; Lema-Ruminska & Kulus 2014; Orum *et al.* 2016). Cactus populations also have a propensity towards low genetic diversity due to decline of seed vectors such as bats (Rivera- Marchand & Ackerman 2006), restricted gene flow (Clark-Tapia *et al.* 2005), clonal abundance, and inbreeding of clonal populations (Nassar et al 2001; Palliero *et al.* 2006). Many cacti in the wild have lower abundance of cross-fertilized progeny due to lack of pollinators and indirect effects of humans on seedlings such as introduction of invasive predators and pathogens (Rojas-Aréchiga *et al* 2001; Mandujano *et al* 2013; Arroyo-Cosultchi *et al* 2016).

According to the International Union for Conservation of Nature and Natural Resources, 85% of Cactaceae species are on the IUCN Red List, 10% of which are endangered and 6% of which are critically endangered (Poole & Riskind 1987; IUCN 2017). The U.S. Fish and Wildlife Service (USFWS) includes 109 species within the Environmental Conservation Online System (ECOS). As of 1973, the Endangered Species Act prohibits unauthorized taking, possession, sale, or trafficking of endangered plant species on federally owned land. Conversely, according to the Institute of Renewable Natural Resources at Texas A&M, 95% of Texas lands are privately owned (Joiner 2017). This represents one of main conflicts of conserving species of cacti in the U.S. as native populations can be subject to destruction without consequence. Natural cacti populations have narrow niche constraints that include temperature and sunlight requirements. Due to these factors, a third of all cacti species may be on the brink of extinction (Goettsch *et al.* 2015).

Though cacti demonstrate adaptations against xeric conditions, these are most functional at maturity. In the natural habitat, cacti species tend to experience high mortality in their seeds due to predation (Turner *et al.* 1969; García-Chávez *et al.* 2010) and other factors including climate change (Aragón-Gastélum *et al.* 2018), yet the population dynamic requires a variation of age and genetics to survive variable environmental changes (Parker 1987). There are many explanations for low germination rate of cactus seeds. One of these is the ability to go through dormancy. Dormancy is defined as a seed's inability to germinate without a specific cue. Germination is the first step towards successful population health. During germination, imbibition of water is essential for breaking open the seed coat and allowing the embryo to grow (Mayer & Shain 1974), even for cactus species with a thick seed coat offering a level of dormancy (Álvarez-Aguirre & Montaña 1997). Factors favorable for seedling survival include water availability, protection from disease and predation (Godínez-Alvarez & Valiente-Banuet 2004) and infection, high speed of growth, and reduced competition for sources of light.

Cacti seeds will also not germinate unless they are outside the range of any fruit structure, also known as innate dormancy. They require some type of zoochory, or seed ingestion, to break the seed from the fruit structures (Rojas-Aréchiga & Vásquez-Yanes 2000). Other factors that decrease the amount of seed progeny in the wild include incompatibility. Some cacti will avoid inbreeding depression through self-incompatibility mechanisms (Clark-Tapia *et al.* 2005); once clonal, such cactus populations would incapable of sexual reproduction (Boyle 1996; Casas *et al* 1999; Boyle & Idnurm 2001; Boyle 2003).

Population dynamics in cacti species have been studied extensively. Once cacti reach their reproductive age, fecundity increases with increasing size (Godinez Alvarez et al. 2003); however, few germinated seedlings live to reproductive age (Nobel 1988, 2002). For example, a cactus can produce between 50 to 350 seeds per plant per year (Godínez–Álvarez & Valiente-Banuet 2004; Arroyo-Cosultchi *et al.* 2016) but can take anywhere from 3-5 years in cultivation or 10-50 good rainy-season years in the wild to flower (Clayton *et al.* 1990). Some species like saguaros (*Carnegiea gigantea* (Engelm.) Britton & Rose) can take 33 years to produce its first flowers or up to 50-100 years if environmental conditions are not favorable (Steenbergh & Lowe 1977; Pérez-Molphe-Balch *et al.* 2015). This presents a conservation problem; drought and high temperature years can be expected to occur more frequently due to climate change and seedlings of cacti tend to die early due to these factors (Orum *et al.* 2016). Most successful cacti will grow underneath a "nurse plant" that hides it from most of the heat and allows for sustained moisture (Leirana-Alcocer & Parra-Tabla 1999; Flores & Juardo 2003). If species abundance decreases in deserts, seed vectors would not be available to ingest the fruit and spread the seeds and nurse plants would not be available to shade the seeds. Conservation of genetically healthy cactus populations requires that these factors are well understood.

Solutions

To combat damage from human encroachment, institutions propagate mature cacti in controlled environments and transplant them back into the wild. One of the conservation teams, the Cactus Rescue Project, was initiated by John Oberhausen and Joe Newman (2017) to encourage the public to save Santa Fe cholla, also known as

Cylindropuntia viridiflora, and educate about the importance of cacti in the wild. They have worked with the Ladybird Johnson Wildflower Center, the Lower Colorado River Authority, and various other botanical gardens of the southwest to host volunteer efforts to relocate and propagate cacti from development sites to local greenhouses. Although this method of conservation is good, it does not consider either the transplantation back to wild systems or preserve the genetic diversity that a population needs to adapt to environmental change.

As such ex situ conservation may not be a practical option for preservation of cacti species. This solution may be improved by ornamental horticulture techniques. Tissue culture is effective means of proliferating plants and it has been considered as a method to recover endangered plants that undergo crassulacean acid metabolism (Malda *et al.* 1999). This process relies on the use of meristematic tissue placed on an artificial medium, usually agar, mixed with nutrients and sometimes hormones to cultivate and propagate new cells and living tissue (Vasil & Vasil 1972). Previous studies have used tissue culturing to multiply individual cacti through use of axillary bud or explant proliferation to increase populations (Johnson & Emino 1979a; Johnson & Emino 1979b; Mauseth 1979; Escobar *et al.* 1986; Clayton *et al.* 1990; Hubstenberger *et al.* 1992; Pérez-Molphe-Balch *et al.* 2002; Giusti *et al.* 2002; Chavez *et al.* 2006; Estrada-Luna *et al.* 2008; Pérez-Molphe-Balch *et al.* 2015). However, culturing of mature individuals produces clones of the parent which does not remedy the problems of genetic diversity in natural populations when transplanted.

Seed micropropagation is a method of using agar as a substrate for growing seeds in culture plates. Seed micropropagation improves germination in cactus seeds (Clayton

et al. 1990; Chávez *et al.* 2006) compared to conventional propagation in soil. Because it is more than 95% water, agar allows for seeds to imbibe water and nutrients necessary to break dormancy. Previous studies have also used Murashige and Skoog powder and agar to optimize germination rate and provide ideal conditions for the longevity of the seedlings (Lema-Rumińska & Kulus 2014; Civatti *et al.* 2017). Murashige and Skoog powder was first introduced in 1962 as a nutrient supplement in agar micropropagation and is industry standard for plant tissue culturing.

Hormones dissolved in agar could also influence the dormancy of many species of cacti. There are three types of plant growth regulators; cytokinins, auxins, and gibberellins. Cytokinins were discovered by Skoog and Miller (1957) and were shown to regulate the expanse of cellular division in plant tissue culture. Auxins are plant hormones that promote growth responses such as phototropism and development of organs. Auxins are typically used in tissue culturing as a hormone to induce root formation.

Gibberellic acids are plant growth regulators of stem elongation, flowering time, and senescence of leaves and fruits. Gibberellic acids also induce germination in seeds. One of the first studies to use the hormone on cactus seeds (Alcorn and Kurtz 1959) showed that addition of gibberellic acids might help seeds grown in less-than-ideal light conditions, specifically lower wavelength light and darkness. Their study did find that gibberellic acids may affect the way that seeds germinate, but their results and data did not show a significant correlation between the light quality. In normal light conditions with no gibberellic acids, the seeds displayed the most germination of all treatments. Ortega-Baes and Rojas-Aréchiga 2007 found the greatest rate of germination for

Trichocereus terscheckii was recorded at 25°C with 0 ppm gibberellins and white light, the same as shown previously. A number of studies (Arias & Lemus 1984; Rojas-Aréchiga *et al.* 2001, 2011; Olvera-Carrillo *et al.* 2003) show the same pattern and contribute to the idea that gibberellins may not have anything to do with the germination process at all. However, these studies did not determine multispecies interactions and media effects on how the gibberellin acids affect germination. Different media may affect the way the hormone is absorbed and other taxonomically distinct species may react to gibberellic acids variably.

Propagation by seed is ecologically superior to vegetative propagation of tissues by traditional means or tissue culture, because resultant embryos may differ genetically from either parent, thus maintaining genetic diversity in the population. Seed propagation offers another advantage. Cuttings for tissue culture are difficult to disinfect due to the abstract shape of the explant (Chávez *et al.* 2006). Seeds, with smaller, simpler surfaces, can be decontaminated more easily. The seed is protected by a layer of cellulose and other components of the testum unlike the explant which may be harmed during sterilization. On the other hand, *in situ* propagation is not ideal. Seeds have low probability of survival in natural settings due to desiccation, (Fraiser 1989; Dubrovsky 1996; Dubrovsky 1998), light quantity changes due to lack of nurse plants (plants that provide shade for seedlings) (Flores & Juardo 2003), and both seeds and seedlings suffer predation from animals (Godínex-Alvarez and Valiente-Banuet 2004). There is evidence to support that growing cacti as well as many other types of plants *in vitro* improves seed germination and overall health of the seedling (Rubluo *et al.* 1993; Chávez *et al.* 2006). Micropropagation also requires less space than soil propagation. In short, the propagation

of cacti in a laboratory setting should show a more rapid onset of biomass as well as a greater chance of survival when transplanted back into the wild.

Study Questions

In this study we are exploring the variables of media and concentrations of gibberellic acids on the emergence and germination of six species of cacti. The concept of the experiment reveals the actuality of reproducing cacti on a large scale through micropropagation techniques. Three different media will be used: Murashige and Skoog (1962) agar, nutrient-less agar, and absorbent filter paper. These types of media were chosen based on the availability of nutrients and moisture availability. The MS agar gives nutrients and constant moisture while the agar is missing the nutrients and the filter paper is missing constant moisture and nutrients. The second variable will be concentration of gibberellic acid within the media being used. Four concentrations will determine effectiveness; 0 parts per million, 500 ppm, 1000 ppm, and 1500 ppm. These concentrations are based on a study done by Rojas-Aréchiga *et al.* 2011.

This study will clarify two questions regarding *in vitro* seed micropropagation; 1. What media should be used in the process of micropropagation for the optimum emergence and germination of six species of cacti; 2. What concentration of gibberellic acid has the most positive effect on the emergence and germination of six species of cacti? Interactions between species, hormone, and media effects will be measured as well. This will be tested through two crucial developmental stages in cacti; emergence and germination (Arroyo-Cosultchi *et al.* 2016).

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CHAPTER II

Methods

The protocol for *in vitro* germination experiment was devised through my own pilot experiments and incorporating protocol elements from published studies (Johnson & Emino 1979; Clayton *et al.* 1990; Giusti *et al.* 2002; Rojas-Aréchiga *et al.* 2011). The species being used in this experiment are *Carnegiea gigantea* (Engelm.) Britton & Rose (Saguaro), *Echinocactus grusonii* Hildm. (Golden-Barrell cactus), *Echinocereus reichenbachii* (Terscheck ex Walp.) Haage f. (Hedgehog cactus), *Mammillaria parkinsonii* Ehrenb. (Pincushion cactus), *Hylocereus undatus* (Haworth) Britton & Rose (Dragonfruit cactus), and *Opuntia engelmannii* Salm-Dyck ex Engelmann (Texas prickly pear cactus). These species were chosen for several reasons. The first is to best represent multiple levels of IUCN index of endangerment. *C. gigantea* has been known to be in a state of decline due to human influence, yet the IUCN still reports the species as "Least Concern" (Burquez Montijo *et al.* 2017*).* This is most likely attributed to the high clonal population numbers within their range. However, the range is fragmented because of lack of conservation control in certain areas and there has been evidence to support a massive decline in populations of saguaro (Orum *et al.* 2016). *Echinocactus grusonii* is considered "Endangered" and protected on the national species list in Mexíco due to high population fragmentation (Guadalupe Martínez *et al.* 2013) and illegal collections (IUCN 2013). *Echinocereus reichenbachii* is listed as "Least Concern" in the IUCN, however the species is considered endangered in the Texas Parks and Wildlife listings. *Mammillaria parkinsonii* is characterized as endangered by IUCN with only a few wild individuals known to exist in one part of one state in Mexíco.

The other two species, *Hylocereus undatus* and *Opuntia engelmannii* are not endangered but will be used as mechanisms for common-type cactus germination.

Opuntia engelmannii is not expected to emerge as the species has a very thick testa that prevents it. *Hylocereus undatus* is a common crop plant that is used frequently in both seed and tissue propagation.

The second reason for selecting these six species is that they cover a broad taxonomic sampling, each representing a different tribe within Cactaceae. *Carnegiea gigantea* is in Pachycereeae, *Echinocactus grusonii* is in Cacteae, *Echinocereus reichenbachii* is in Pachycereeae, *Mammillaria parkinsonii* is in Cacteae, *Hylocereus undatus* is in Hylocereae, and *Opuntia engelmannii* is in Opuntieae. Using an array of different life history types gives a generalized method of how species within Cactaceae can germinate and how they grow in response to variations from *in vitro.*

The first experimental treatment exposes seeds to four concentrations of gibberellic acid: 0 parts per million, 500 ppm, 1000 ppm, and 1500 ppm. These concentrations have been shown to produce different germination percent for species even within the same genus (Rojas-Aréchiga *et al.* 2011).

The second treatment is variation in media. Three levels of substrate will include filter paper moistened with water, 1% agar possessing little to no nutrients or salts, and 1% Murashige and Skoog agar (MS) with a uniform quantity of fertilizer (Johnson and Emino 1979; Mauseth 1979; Escobar *et al.* 1986; Chavez *et al.* 1990; Clayton *et al.* 1990; Hubstenberger *et al.* 1992; Giusti *et al.* 2002; Estrada-Luna *et al.* 2008; Pérez-Molphe-Balch *et al.* 2015).

The full experiment consists of two trials. The first trial started on April 11, 2018 and ended eight weeks later on June 6th. The entire experiment was repeated with a

second trial with the same seeds and started on September 7th and ended in eight weeks on November 1st.

Substrate Preparation

The agar plates contained 1% (w/v) bacteriological grade agar in deionized water. The Murashige and Skoog agar was made with 4.3g L^{-1} MS following the supplier's recommendation (Carolina, Burlington) and 1% bacteriological agar with deionized water. Agar-containing mixtures as well as the water used for the filter paper were autoclaved before use. After four weeks the agar-containing plates require replacement to maintain adequate water storage. Entire transplantation of seeds and seedlings happened at this time. Gibberellic acid in powder form was added to the appropriate concentration of either none, 500 ppm, 1000 ppm, or 1500 ppm. Due to the thermosensitive nature of gibberellic acids, the concentrated powders were not added until after the agar solution had come out of the autoclave otherwise the acids could degrade (Hodson and Hamner 1971). The solutions were added to 9 cm diameter petri dishes with about 25 ml in each. The filter paper treatment consisted of a circle of filter paper fitted to the size of the petri dishes and sprayed with sterile water mixed with the required amount of gibberellic acid. Each treatment consisted of twenty seeds for one of six species placed in one of three media containing one of four GA concentrations, for a total of 72 treatments. The entire experiment was duplicated, requiring 2,880 seeds planted in 144 dishes.

Sterilization

Methods for sterilizing seeds vary in the literature. Castro *et al.* (2011) washed *Nopalea cochenilifera* (Cactaceae) seeds in low concentrations of sodium hypochlorite, 0.5%, 1%, and 1.5%, for ten minutes and washed three times to produce a sterile

treatment set. Balch *et al.* (1998) washed seeds five times with 0.1% Extran, a liquid detergent, then disinfected for 1 minute in 70% ethanol, 25 minutes in 2% sodium hypochlorite, and rinsed four times with sterile distilled water.

For this experiment, seeds were placed 20-25 at a time into 1.5 ml microcentrifuge tubes and washed for one minute in 80% ethanol and then drained of the ethanol. Then the seeds were washed in 5% sodium hypochlorite with 0.1% Tween 20 for 10 minutes and then drained of the solution. The seeds were then washed three times with sterile deionized water to remove any extraneous detergent.

Once the seeds were completely sterile and the plates were cool, they were plated to maximize distance between adjacent seeds. The plates were taped along the sides to prevent opening and contamination. Seeds were grown under Taopu 40W E27 full-spectrum light bulbs. The chamber was lined with reflective material to avoid position bias in the chamber. Daylight conditions were set at a daily schedule of 14 hours (Dubrovsky 1996, 1998; Rojas-Aréchiga *et al.* 2001).

Measurements and Analysis

Observations were recorded every day for 8 weeks for emergence and total germination. Emergence was recorded as soon as the radicle or any part of the embryo broke from the testum of the seed. Germination was recorded when the seedling was completely separated from the testum and free-living. These measurements are defined by the difference between only imbibition of water and nutrients versus total health of the plant. Variables were as follows in table 1 with species with six types, hormones with four concentrations, and media with three types.

Table 1

Experimental Variables in Type

Main effect variable	Variable Name
Species	1. *Carnegiea gigantea*
	2. *Echinocactus grusonii*
	3. *Echinocereus reichenbachii*
	4. *Hylocereus undatus*
	5. *Mammillaria parkinsonii*
	6. *Opuntia engelmannii*
Hormone	1. 0 ppm
	2. 500 ppm
	3. 1000 ppm
	4. 1500 ppm
Medium	1. Filter paper
	2. Agar
	3. MS + agar

To obtain mean emergence and germination, the sum of the counted data was calculated as the total number divided by the duration of the experiment (Ranal *et al.* 2006). This data was conglomerated in four databases; Trial 1 Emergence, Trial 1 Germination, Trial 2 Emergence, and Trial 2 Germination. I first combined the data between the two emergence trials and termed the "Trial" effect as a block comparison to verify significance between trials. I also did this between the two germination trials to verify significance. If there was significance, I could treat them as separate. If not, they must be treated as similar trials and analyzed together. After that, the data was analyzed using an analysis of variance under a general linear model with $p<0.05$ in SAS (Ranal *et*

al. 2009). Under the general linear model, each main effect was compared separately and then compared between each other variable such as species versus media, species versus hormone, and hormone versus media to understand interactions in the variables. I also compared the three variables together to determine significant interactions. Once interaction significance was found between variables, contrast analysis was used to show where in the data the interactions took place. The contrast statements were written using the main effects' significance as a reference to judge where the interaction would lie. For example, if the MS media was significantly higher than the filter paper, I would compare species versus media by comparing MS higher than filter paper. This was paralleled by a least significant difference (LSD) test with "slice" option in SAS at $p<0.05$ to examine effect differences within LSD mean interactions. I analyzed the data of the main effects using Tukey's pairwise comparison to compare how the means of each effect grouped together. This helped in establishing how different each mean was from another. The Tukey's test results were edited with Microsoft PowerPoint within the graph of average emergence or germination.

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CHAPTER III

Results

Data from trials were analyzed separately due to the significant difference in trial effects in the trial block test ($p < 0.0001$) (Table 2; Table 3). The trial effect had interactions on other variables such as species and media as well as an interaction with all three variables. Species had a very significant effect ($p < 0.0001$) in both emergence and germination for both trials. Media and hormone had varying effects on each development step and trial that will be explained further in this section.

Table 2

ANOVA results of emergence with Trial block

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Trial	**1**	**1001.281250**	**1001.281250**	**260.97**	**<0.0001**
Trial*Species	**5**	**1879.739583**	**375.947917**	**97.98**	**<0.0001**
Trial*Hormone	3	24.427083	8.142361	2.12	0.1000
Trial*Media	**2**	**26.645833**	**13.322917**	**3.47**	**0.0337**
Trial*Species*Hormone*Media	**61**	**411.656250**	**6.748463**	**1.76**	**0.0032**

Note. Significant results are in bold.

Table 3

ANOVA results of germination with Trial block

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Trial	**1**	**75.0312500**	**75.0312500**	**49.91**	**<0.0001**
Trial*Species	**5**	**148.3645833**	**29.6729167**	**19.74**	**<0.0001**
Trial*Hormone	3	1.7048611	0.5682870	0.38	0.7690
Trial*Media	2	2.4375000	1.2187500	0.81	0.4466
Trial*Species*Hormone*Media	30	37.2569444	1.2418981	0.83	0.7240

Note. Significant results are in bold.

Trial 1

Emergence.

Species had a significant effect on the number of emergents ($p<0.0001$) as did hormone ($p = 0.0006$) (Table 4). Every species exhibited significantly different number of emergents than every other species, with the exception of *Echinocereus reichenbachii* and *Opuntia engelmannii*, both of which had near zero emergence number (Figure 2).

Table 4

ANOVA results of Trial 1 emergence

Source	DF	Type I SS	Mean Square	F Value	Pr > F
species	**5**	**4771.638889**	**954.327778**	**225.28**	**<0.0001**
hormone	**3**	**82.000000**	**27.333333**	**6.45**	**0.0006**
media	2	18.097222	9.048611	2.14	0.1255
species*hormone	15	81.250000	5.416667	1.28	0.2382
species*media	**10**	**139.986111**	**13.998611**	**3.30**	**0.0014**
hormone*media	**6**	**131.791667**	**21.965278**	**5.19**	**0.0002**
species*hormone* media	**30**	**278.458333**	**9.281944**	**2.19**	**0.0035**

Note. Significant values in bold.

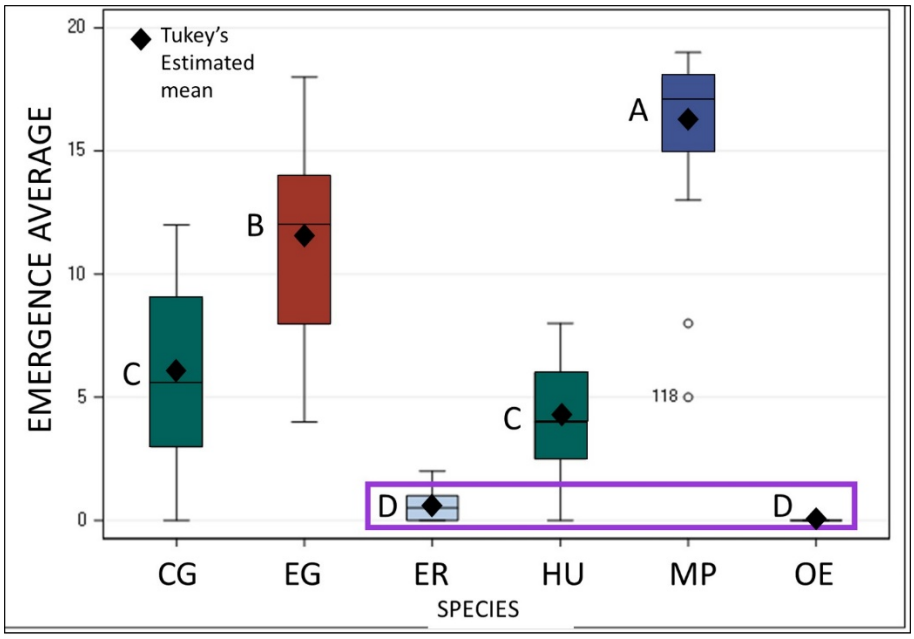

Figure 2. Distribution of Trial 1 emergence by species. The letters illustrate the grouping for means of species from the Tukey's test. *Carnegiea gigantea (CG), Echinocactus grusonii (EG), Echinocereus reichenbachii (ER), Hylocereus undatus (HU), Mammillaria parkinsonii (MP),* and *Opuntia engelmannii (OE).* In the box and whisker plots, the line in the center of the boxes represent the median, the total box represents 50% of the range of scores, and the whiskers represent scores outside the 50% range.

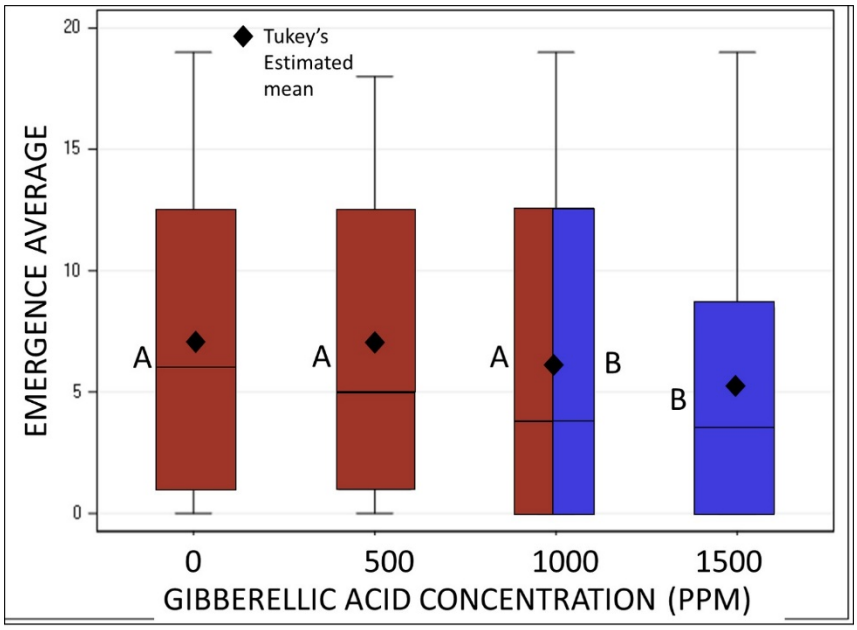

Figure 3. Distribution of Trial 1 emergence by hormone concentration. In the box and whisker plots, the line in the center of the boxes represent the median, the total box represents 50% of the range of scores, and the whiskers represent scores outside the 50% range.

Hormone concentration was also shown to affect emergence significantly. As gibberellic acid concentration increased, emergence decreased (Figure 3). There is a significant drop between the highest concentrations, 1500 ppm, and the three lower concentrations, 0 ppm and 500 ppm, as seen in the Tukey's Studentized Range Test.

There was a significant interaction between species and media treatments ($p=0.0014$) (Table 4). All species demonstrate little difference between emergence on agar or Murashige-Skoog media (Figure 4). Variance in emergence on filter paper produces significant interactions in *Carnegiea gigantea, Echinocactus grusonii*, and *Mammillaria parkinsonii* (Figure 4).

Table 5

Emergence contrast between Species with respect to Media

Contrast	DF	Contrast SS	Mean Square	F Value	Pr > F
CG	**1**	**27.5625**	**27.5625**	**6.51**	**0.0129**
EG	**1**	**18.0625**	**18.0625**	**4.26**	**0.0425**
ER	1	0.25	0.25	0.06	0.8087
HU	1	0.0625	0.0625	0.01	0.9037
MP	**1**	**72.25**	**72.25**	**17.06**	**<0.0001**
OE	1	0	0	0	1

Note. Significant values in bold.

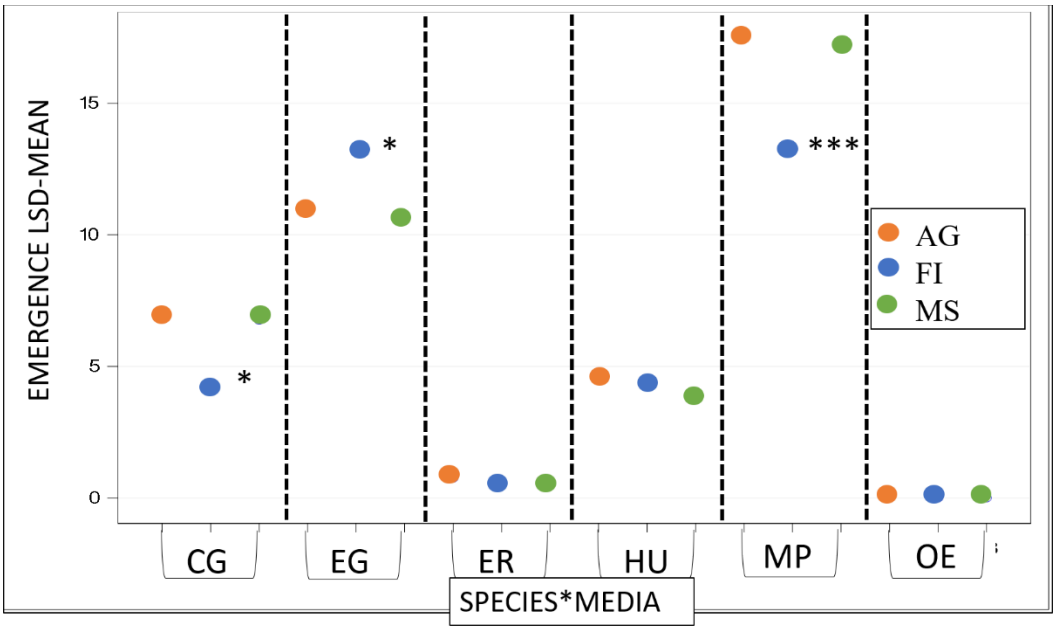

Figure 4. Species versus media least significant difference of Trial 1 emergence. *Carnegiea gigantea (CG), Echinocactus grusonii (EG), Echinocereus reichenbachii (ER), Hylocereus undatus (HU), Mammillaria parkinsonii (MP),* and *Opuntia engelmannii (OE).* (FI) filter paper, (AG) agar, and (MS) Murashige and Skoog with agar.

Similarly, significant interactions were also detected between hormone and media (p=0.0002). The most significant interaction is with GA at 1500 ppm, which results in significantly lower emergence on filter paper than other hormone treatment levels (p<0.0001; Table 6; Figure 5).

Table 6

Emergence contrast between Hormones with respect to Media

Contrast	DF	Contrast SS	Mean Square	F Value	Pr > F
0 ppm	1	4.166667	4.166667	0.98	0.3246
500 ppm	1	0.375	0.375	0.09	0.7669
1000 ppm	1	0.375	0.375	0.09	0.7669
1500 ppm	**1**	**108.375**	**108.375**	**25.58**	**<0.0001**

Note. Significant results in bold

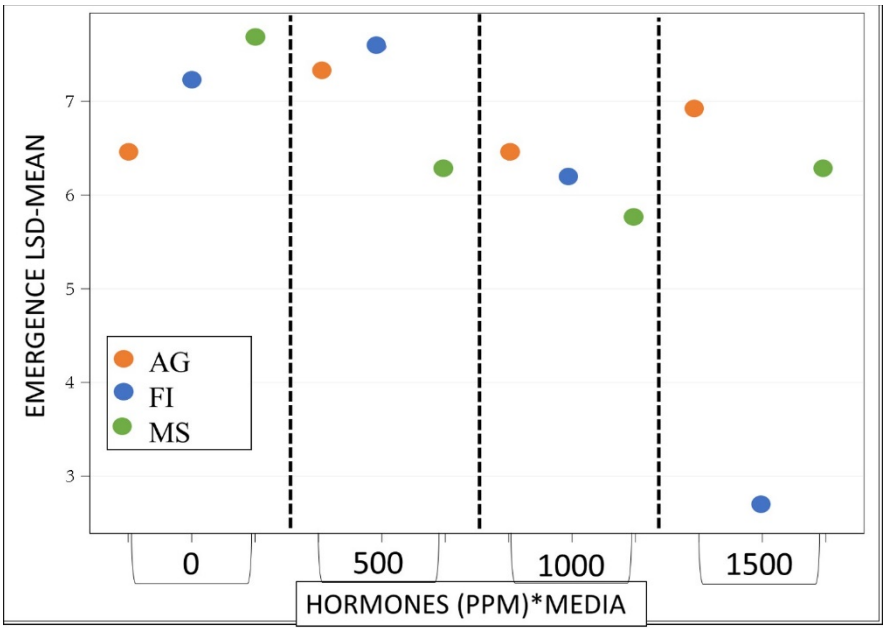

Figure 5. Hormone versus media least significant difference of Trial 1 emergence. (FI) filter paper, (AG) agar, and (MS) Murashige and Skoog with agar.

Finally, there was a three-way interaction between species, hormone, and media ($p=0.0035$) (Table 4). In *C. gigantea* the interaction the most effect in 0 ppm GA over the three media types and had the lowest significant decrease in emergence at filter paper and 1500 ppm GA ($p=0.0009$) (Figure 6). Filter paper and 1500 ppm GA had a similar effect in *E. grusonii* ($p<0.0001$) and *M. parkinsonii* ($p<0.0001$).

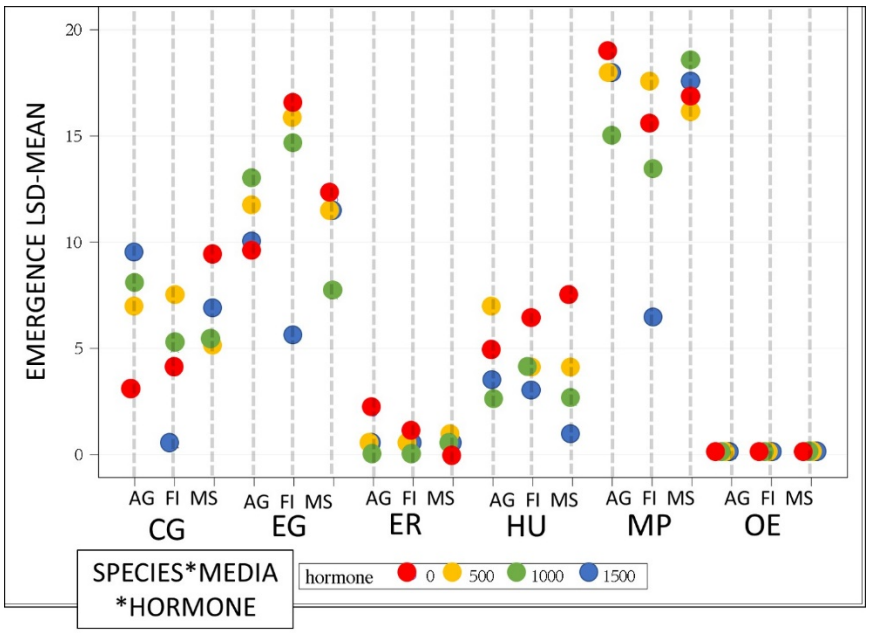

Figure 6. Species, media, and hormone least significant difference of Trial 1 emergence. *Carnegiea gigantea (CG), Echinocactus grusonii (EG), Echinocereus reichenbachii (ER), Hylocereus undatus (HU), Mammillaria parkinsonii (MP),* and *Opuntia engelmannii (OE).* (FI) filter paper, (AG) agar, and (MS) Murashige and Skoog with agar.

Much of this three-way interaction is the result of the varying effects of filter paper treatment and high GA (1500 ppm) treatment. For example, there were significant differences in number of emergents when seeds were grown with 1500 ppm GA on agar vs. filter paper for both *C. gigantea* and *M. parkinsonii*, but none of the other species. Similarly, the number of emergents in *E. grusonii* was lowest on filter paper with 1500 ppm GA, but highest on filter paper with 0 ppm GA. *Mammillaria parkinsonii* also showed the lowest emergence on filter paper with 1500 ppm GA.

Germination.

Species had a significant effect on germination ($p<0.0001$) as did media ($p<0.0001$) (Table 7). A post-hoc Tukey's test indicates significantly different groups; *M. parkinsonii* had the highest germination followed by *C. gigantea* and *E. grusonii*. The other three species had negligible germination and grouped together.

Filter paper resulted in significantly lower germination than either agar or Murashige and Skoog media (Figure 8).

Table 7

ANOVA results of Trial 1 germination

Source	DF	SS	Mean Square	F Value	Pr > F
species	**5**	**3546.06250**	**709.212500**	**301.26**	**<0.0001**
hormone	3	6.854167	2.284722	0.97	0.4115
media	**2**	**65.791667**	**32.895833**	**13.97**	**<0.0001**
species*hormone	**15**	**90.187500**	**6.012500**	**2.55**	**0.0042**
species*media	**10**	**198.208333**	**19.820833**	**8.42**	**<0.0001**
hormone*media	**6**	**54.375000**	**9.062500**	**3.85**	**0.0022**
species*hormone *media	**30**	**138.958333**	**4.631944**	**1.97**	**0.0102**

Note. Significant values in bold

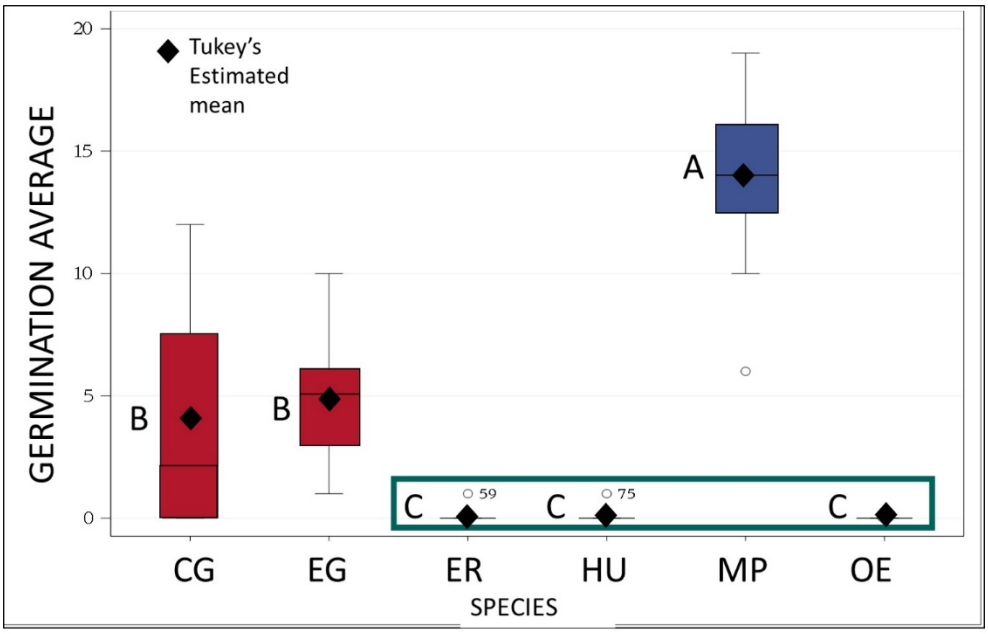

Figure 7. Distribution of Trial 1 germination by species. The letters illustrate the grouping for means of species from the Tukey's test. *Carnegiea gigantea (CG), Echinocactus grusonii (EG), Echinocereus reichenbachii (ER), Hylocereus undatus (HU), Mammillaria parkinsonii (MP),* and *Opuntia engelmannii (OE).* In the box and whisker plots, the line in the center of the boxes represent the median, the total box represents 50% of the range of scores, and the whiskers represent scores outside the 50% range.

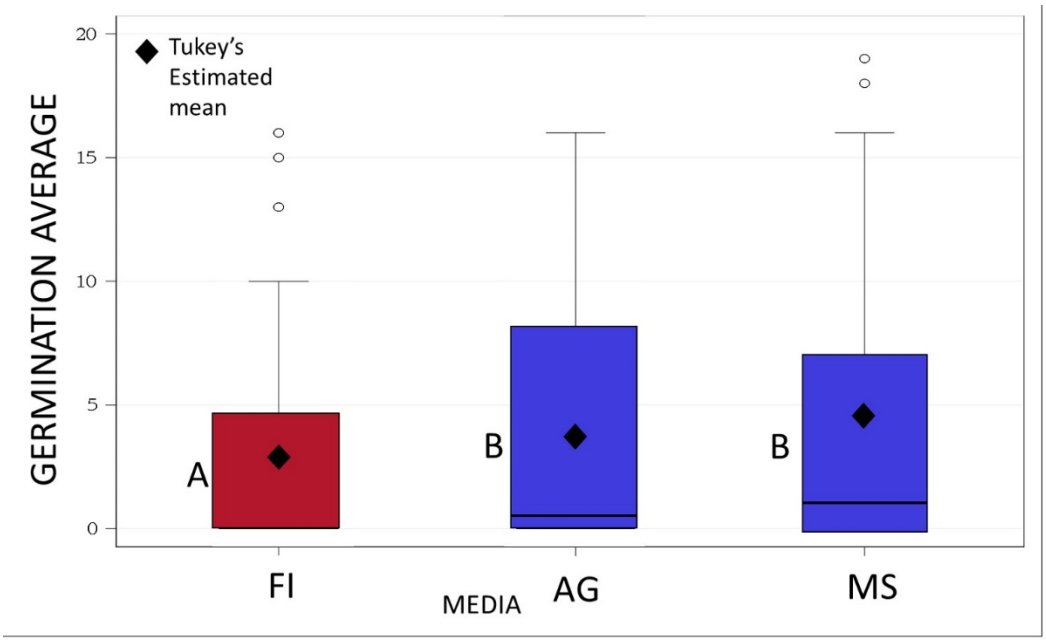

Figure 8. Distribution of Trial 1 germination by media. The letters illustrate the grouping for means of species from the Tukey's test. (FI) filter paper, (AG) agar, and (MS) Murashige and Skoog with agar. In the box and whisker plots, the line in the center of the boxes represent the median, the total box represents 50% of the range of scores, and the whiskers represent scores outside the 50% range.

There was a significant interaction between the species type and hormone (p=0.0042) due to the different effect of GA within one species, *Mammillaria parkinsonii* (p=0.0004) in which the lowest germination was produced by 1000 ppm of gibberellic acids (Figure 9).

Table 8

Germination contrast between Species with respect to Hormone

Species	DF	Contrast SS	Mean Square	F Value	Pr > F
CG	1	0.166667	0.166667	0.07	0.7909
EG	1	3.375	3.375	1.43	0.2351
ER	1	0.041667	0.041667	0.02	0.8945
HU	1	0.041667	0.041667	0.02	0.8945
MP	**1**	**32.66667**	**32.66667**	**13.88**	**0.0004**
OE	1	0	0	0	1

Note. Significant results in bold

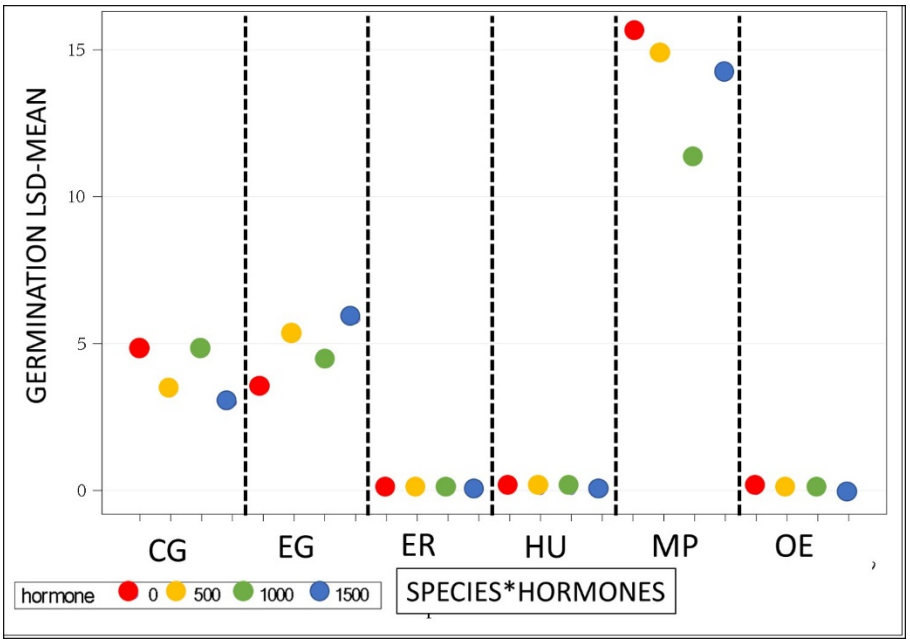

Figure 9. Species versus hormone least significant difference of Trial 1 germination. *Carnegiea gigantea (CG), Echinocactus grusonii (EG), Echinocereus reichenbachii (ER), Hylocereus undatus (HU), Mammillaria parkinsonii (MP),* and *Opuntia engelmannii (OE).*

A significant interaction also existed between species and media This was due to variation in *Carnegiea gigantea* ($p<0.0001$) with the lowest germination on filter paper, and *Mammillaria parkinsonii* ($p<0.0001$) with highest germination on MS agar (Table 9).

Table 9

Germination contrast between Species with respect to Media

Species	DF	Contrast SS	Mean Square	F Value	Pr > F
CG	**1**	**144**	**144**	**61.17**	**<0.0001**
EG	1	0.25	0.25	0.11	0.7455
ER	1	0.0625	0.0625	0.03	0.871
HU	1	0.0625	0.0625	0.03	0.871
MP	**1**	**52.5625**	**52.5625**	**22.33**	**<0.0001**
OE	1	0	0	0	1

Note. Significant results in bold.

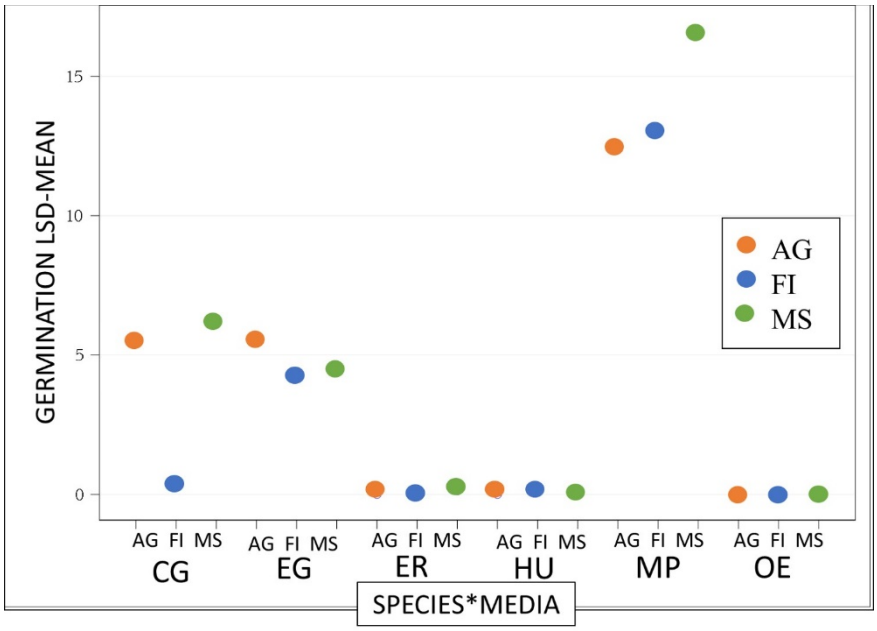

Figure 10. Species versus media least significant difference of Trial 1 germination. *Carnegiea gigantea (CG), Echinocactus grusonii (EG), Echinocereus reichenbachii (ER), Hylocereus undatus (HU), Mammillaria parkinsonii (MP),* and *Opuntia engelmannii (OE)*

There was also interaction between hormone and media (p=0.0002) due to effects at 0 ppm GA (p<0.0002), 1000 ppm GA (p =0.137), and 1500 ppm GA (p=0.0192) (Table 10). So many significant contrasts make the joint effects of hormone and media on germination unpredictable (Figure 11).

Table 10

Germination contrast between Hormone with respect to Media

Contrast	DF	Contrast SS	Mean Square	F Value	Pr > F
0 ppm	**1**	**35.04167**	**35.04167**	**14.88**	**0.0002**
500 ppm	1	7.041667	7.041667	2.99	0.088
1000 ppm	**1**	**15.04167**	**15.04167**	**6.39**	**0.0137**
1500 ppm	**1**	**13.5**	**13.5**	**5.73**	**0.0192**

Note. Significant results in bold

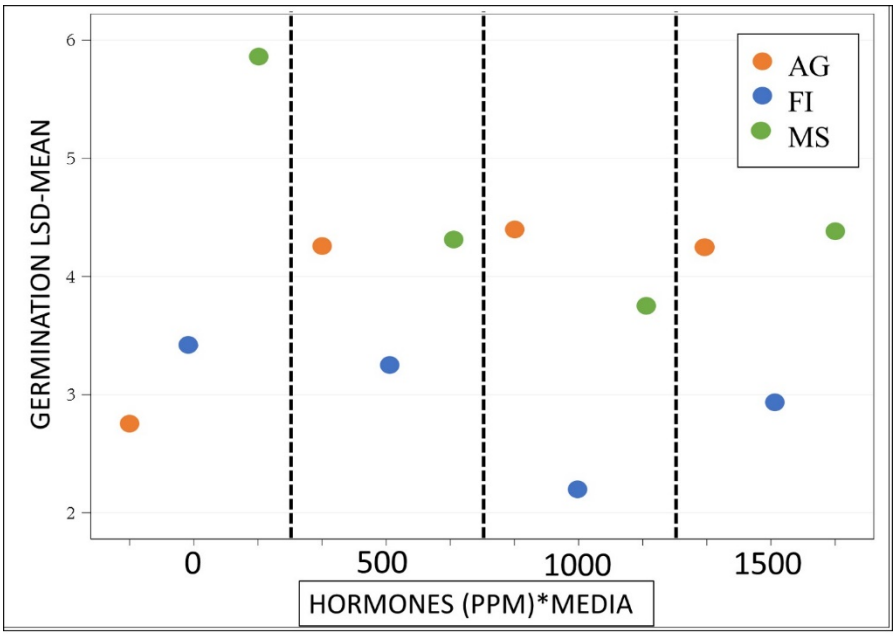

Figure 11. Hormone versus media least significant difference of Trial 1 germination. (FI) filter paper, (AG) agar, and (MS) Murashige and Skoog with agar.

The last interaction was a three-way among species, media, and hormone (p=0.0102) that can be explained through effect in three species; C. gigantea (p<0.0001), *E. grusonii* (p=0.0025), and *M. parkinsonii* (p<0.0001) (Figure 12).

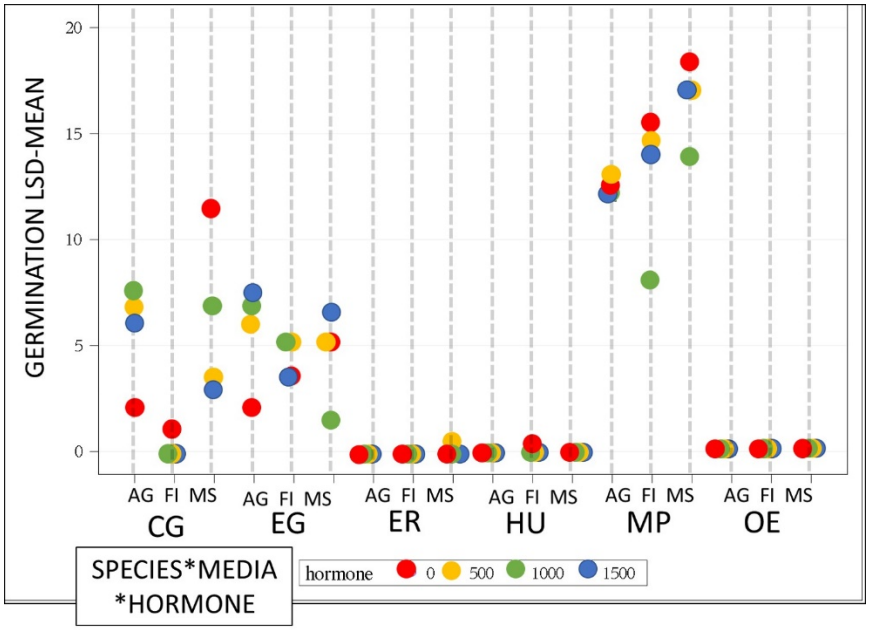

Figure 12. Species, media, and hormone least significant difference of Trial 1 germination. *Carnegiea gigantea (CG), Echinocactus grusonii (EG), Echinocereus reichenbachii (ER), Hylocereus undatus (HU),*

Mammillaria parkinsonii (MP), and *Opuntia engelmannii (OE).* (FI) filter paper, (AG) agar, and (MS) Murashige and Skoog with agar.

Trial 2

Difference between trials.

Overall the number of emergent and germinated seeds was lower in the second trial than the first. *Mammillaria parkinsonii* for instance dropped from 16 emergence to 1.92 emergence and *Carnegiea gigantea* dropped from 5.96 to 0.08 (Figure 13). *Echinocereus reichenbachii, Hylocereus undatus,* and *Echinocactus grusonii* did not change much in emergence and *Opuntia engelmannii* did not emerge in either trial as expected.

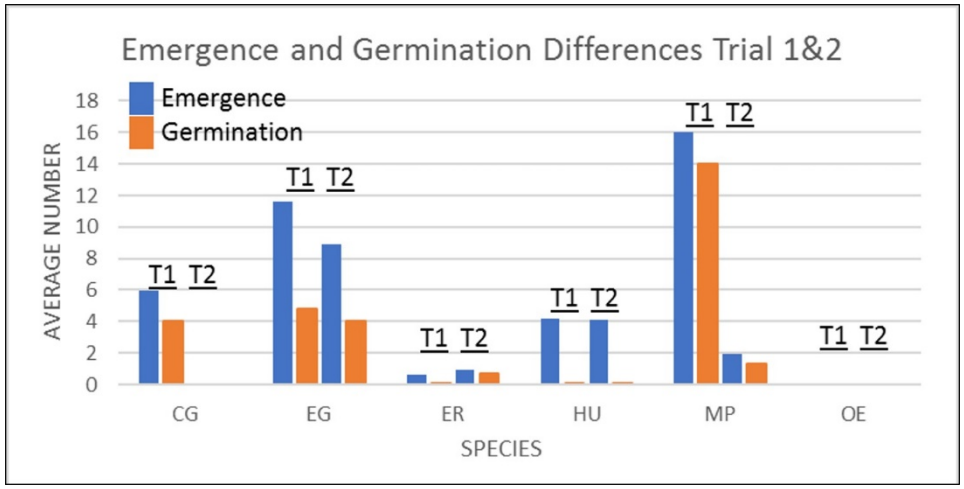

Figure 13. Emergence and Germination Differences Trials 1 and 2. This figure shows the differences between the average emergence or germination between species of the two trials. *Carnegiea gigantea (CG), Echinocactus grusonii (EG), Echinocereus reichenbachii (ER), Hylocereus undatus (HU), Mammillaria parkinsonii (MP),* and *Opuntia engelmannii (OE).*

Emergence.

Similar to the first trial, there was a significant effect of species on the emergence ($p<0.0001$) (Table 11). *Echinocactus grusonii* had the highest emergence followed by *Hylocereus undatus* (Figure 14). These species were grouped individually in the post-hoc Tukey's pairwise analysis. *M. parkinsonii* and *E. reichenbachii* grouped together as they

are near the same range of means. *C. gigantean* and *O. engelmanii* were not significantly different from *E. reichenbachii* with near-zero means.

Media also affected emergence significantly (p=0.0029) with filter paper and agar treatments resulting in significantly less emergence than Murashige and Skoog medium (Figure 15). There were no significant interactions among main effects.

Table 11

ANOVA results of Trial 2 emergence

Source	DF	Type I SS	Mean Square	F Value	Pr > F
species	**5**	**1406.368056**	**281.273611**	**81.83**	**<0.0001**
hormone	3	17.187500	5.729167	1.67	0.1818
media	**2**	**43.597222**	**21.798611**	**6.34**	**0.0029**
species*hormone	15	37.604167	2.506944	0.73	0.7472
species*media	10	65.986111	6.598611	1.92	0.0561
hormone*media	6	8.458333	1.409722	0.41	0.8700
specie*hormone* media	30	107.625000	3.587500	1.04	0.4282

Note. Significant values in bold.

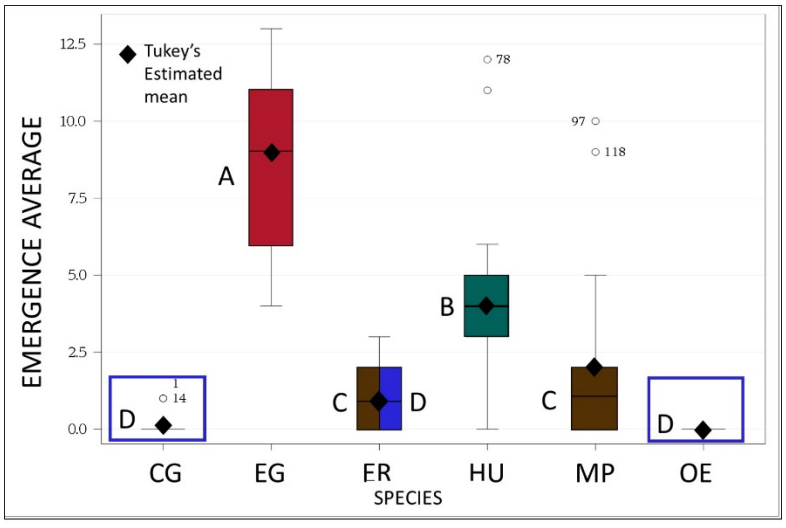

Figure 14. Distribution of Trial 2 emergence by species. The letters illustrate the grouping for means of species from the Tukey's test. *Carnegiea gigantea (CG), Echinocactus grusonii (EG), Echinocereus reichenbachii (ER), Hylocereus undatus (HU), Mammillaria parkinsonii (MP),* and *Opuntia engelmannii*

(OE). In the box and whisker plots, the line in the center of the boxes represent the median, the total box represents 50% of the range of scores, and the whiskers represent scores outside the 50% range.

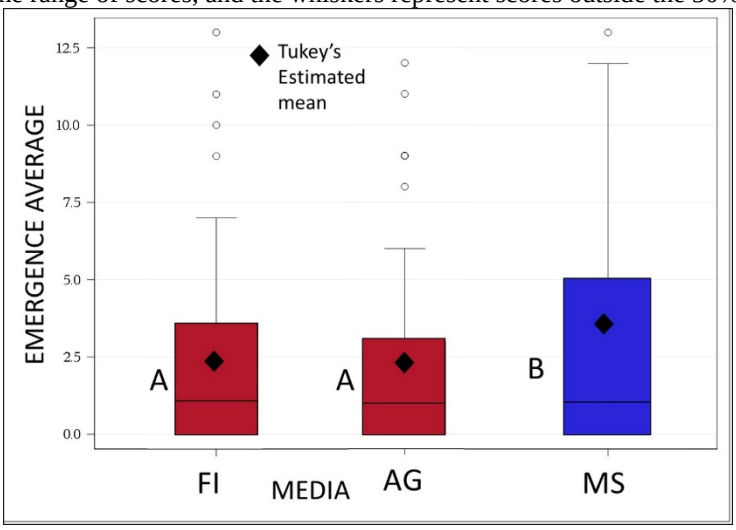

Figure 15. Distribution of Trial 2 emergence by media. The letters illustrate the grouping for means of species from the Tukey's test. (FI) filter paper, (AG) agar, and (MS) Murashige and Skoog with agar. In the box and whisker plots, the line in the center of the boxes represent the median, the total box represents 50% of the range of scores, and the whiskers represent scores outside the 50% range.

Germination.

The germination test during the second group of tests only showed a significant result through the variation of species ($p<0.0001$) (Table 12). *E. grusonii* demonstrated significantly greater germination than the rest of the species (Figure 16). There were no significant interactions among main effects.

Table 12

ANOVA results of Trial 2 germination

Source	DF	Type I SS	Mean Square	F Value	Pr > F
species	**5**	**296.7291667**	**59.3458333**	**19.74**	**<0.0001**
hormone	3	3.4097222	1.1365741	0.38	0.7691
media	2	4.8750000	2.4375000	0.81	0.4486
species*hormone	15	32.9652778	2.1976852	0.73	0.7456
species*media	10	7.7083333	0.7708333	0.26	0.9884

(Continued)

Source	DF	Type I SS	Mean Square	F Value	Pr > F
hormone*media	6	10.2361111	1.7060185	0.57	0.7549
species*hormone*media	30	74.5138889	2.4837963	0.83	0.7150

Note. Significant result in bold.

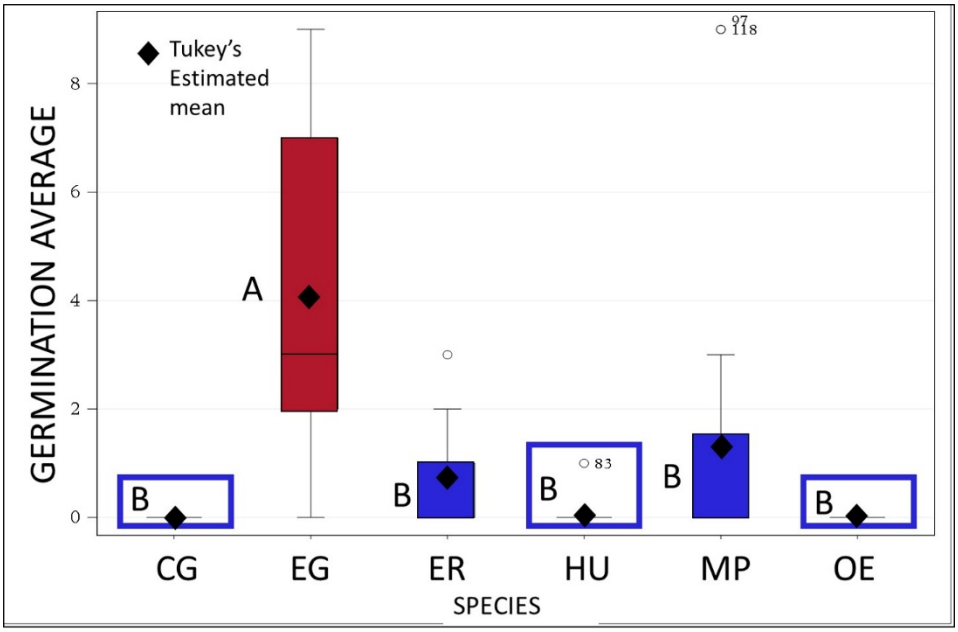

Figure 16. Distribution of Trial 2 germination by species. The letters illustrate the grouping for means of species from the Tukey's test. *Carnegiea gigantea (CG), Echinocactus grusonii (EG), Echinocereus reichenbachii (ER), Hylocereus undatus (HU), Mammillaria parkinsonii (MP),* and *Opuntia engelmannii (OE).* In the box and whisker plots, the line in the center of the boxes represent the median, the total box represents 50% of the range of scores, and the whiskers represent scores outside the 50% range.

CHAPTER IV

Discussion

Emergence

The first conclusions that can be made about this data is that species have to be treated separately with regard to treatment. *Mammillaria parkinsonii* was found to be the highest in emergence in trial 1 but failed to reach the same level in trial 2. The result in trial 1 is expected as *M. parkinsonii* has a very small seed with a thin testa, but it was comparatively different than its closest relative in this experiment *E. grusonii*. They both retain phylogenetic similarities as they are within the tribe Cacteae (Hernández-Hernández *et al*. 2011), however these phylogenetic relationships did not offer parallels to emergence as they are outside significant groupings of means. Similarly, *Echinocereus reichenbachii*, which is in the same tribe as *C. gigantea*, Pachycereeae (Hernández-Hernández *et al*. 2011), did not show parallel results. Phylogenetic relationships do not necessarily tend towards similar growth patterns or response to treatments.

Hylocereus undatus was expected to demonstrate the highest level of emergence and germination, due to its thin seed coat (Cisneros *et al.* 2011), but it did not. Similarly, *Mammillaria* seeds tend to also have a thin testum that allows for moisture absorption; they exhibited strong emergence in the first trial, but markedly less in the second. Probability of emergence clearly depends on more than the thickness of the testa.

The first trial showed that GA at high concentrations negatively affects emergence, as expected (Ortega-Baes & Rojas-Aréchiga 2007). The interaction between hormone and media might be explained by their combined effects on the ability of a seed to absorb water. Emergence was significantly lower when seeds were grown on filter paper with 1500 ppm GA. Absorption of water through the testa is likely less efficient on filter paper than either agar solution. The flow of water into a plant is governed by the

difference in osmotic pressure between the substrate and the plant itself. For water to flow into a plant, water potential in the substrate must be higher than in the plant (Nobel 1988). Filter paper holds less water than agar. To exacerbate this, adding solutes, such as GA, to this what water the filter paper does hold, decreases the strength of the osmotic gradient between substrate and plant. Osmotic pressure has only been measured in seeds of four species of cactus: *Neobuxbaumia tetetzo* at -0.66 MPa, *Pachycereus hollianus* at -0.44 MPa (De la Barrera & Nobel 2002) *Cereus validus* at -0.28 to -0.34, and *Ferocactus acanthodes* at -0.09 to -0.20 (Nobel 1988).

Although overall emergence was less in the second trial than the first, the main findings are similar; species and media main effects were significant. Echinocactus grusonii remained to a relatively moderate emergence and filter paper remained the lowest emergence in media. However, there were also differences. *Mammillaria parkinsonii* demonstrated much lower emergence in the second test than the first test. This may be because seeds were 22 weeks older when the second trial began than they were at the onset of the first trial. In the second trial, *C. gigantea* showed a decrease in emergence to near zero while *E. grusonii* only decreased slightly. *Carnegiea gigantea* was unexpected to emerge in the first trial with such an adequate amount because it does not usually in the wild (Nobel 1988), however it had an extreme decrease in emergence average to near zero. Though dormancy is a factor in the seed development of these plants, the aspect of time may alter their emergence. The time may also be altered by the sterilization process used.

Media effects from the second trial were different from the first trial grouping filter paper and agar as a decreased emergence average. As a possible explanation for this

result, older or drier seeds may require more nutrients such as the MS solution in order to emerge from their testa. This result is not very surprising as MS only adds to the foundation of the substrate what the seed needs to grow, however it is interesting from an ecological perspective. If cacti seeds require more nutrients to emerge and they live in a mostly nutrient-poor environment it begs to question how the majority of them germinate after a certain time.

Germination

Once again, the species effect had a significant influence on the germination of the seeds. In *C. gigantea*, *E. grusonii*, and *M. parkinsonii*, a high proportion of seeds that emerged also germinated. Though *H. undatus* seeds demonstrated moderate emergence, almost none of them germinated. This suggests that *H. undatus* seeds that imbibed water did not necessarily support growth of the embryo. This could be due to an evolutionary trait exendospermism (Cisneros *et al*. 2011), in which the embryo consumes the endosperm during development instead of during dormancy. Without the endosperm, the embryo without endosperm, the embryo isn't able to grow following emergence. There was also the problem that in Cisneros *et al.* (2011) sometimes the seed testa looked intact, but the embryo was missing. This could have been a problem in my experiment as well. Moisture can open the seed, but no embryo will germinate.

The interaction between species and media can be explained by *Mammillaria parkinsonii* demonstrating significantly higher germination under Murashige and Skoog medium, than agar or filter paper. It could be that germination in *M. parkinsonii* is particularly sensitive to nutrient availability while other species tend not to be. The thin testa on the seed could also explain the ready absorbance of nutrients. Four species of

Mammillaria were shown to have no physiological or morphological signs of dormancy (Benítez-Rodríguez *et al.* 2004). This could mean that the seeds are directly ready for germination once the embryo is developed. This explains the dramatic difference in germination rate of *M. parkinsonii* between trial 1 and trial 2.

Hormone versus media interaction on germination in trial 1 can be explained in the same manner as the hormone versus media interaction on emergence interaction of the same trial. Low water capacity of filter paper and low water potential of 1500 ppm GA (Ortega-Baes & Rojas-Aréchiga 2007) have an additive negative effect on germination.

Problems

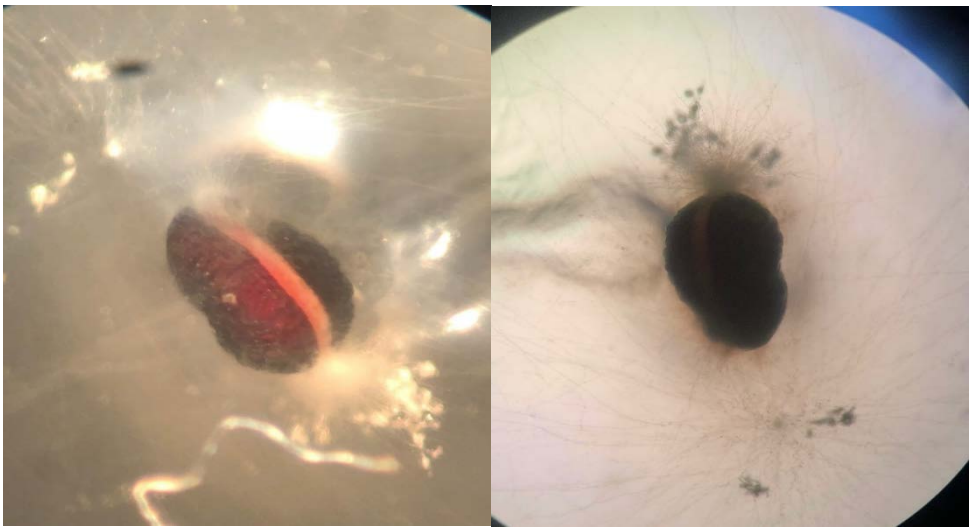

Figure 17. Hyphae on *Echinocereus reichenbachii* seeds. Left is from above; right is below. It seems to originate from within the testum of the seed

During the experiment, there were multiple instances of fungal contamination that became detrimental to the health of the seedlings. Even with sterilization, contamination occurred. The most reasonable explanation was that the spores were possibly embedded on the testum ridges or were inside the testum itself next to the embryo. There is no published support for this, but either place may have preserved spores during seed

sterilization. There is evidence of this from this experiment (Figure 17); hyphae originated from the testa of the seeds of *Echinocereus reichenbachii,* rather than elsewhere on the petri dish.

Some cacti seeds have a layer of hydrophilous subcuticular layer that can be used for absorbance. Germination of *Echinopsis thionantha* and *Gymnocalycium gibbosum* increased with the subcuticular layer intact versus the layer stripped off the seed testa (Bregman & Graven 1997). If this layer is removed during sterilization it may be decreasing the absorption rate of seeds. However, denying the sterilization method to the seeds defeats the purpose of a sterile environment in the petri plates. I do not believe current sterilization protocols are adequate to optimize the germination of these seeds.

Conclusions

The most significant conclusion to take away from this experiment is that cactus species may have very different requirements for seedling establishment. The data shows such differences even among closely related genera. If micropropagation can be used to establish seedlings, these seedlings represent genetically diverse candidates for reintroduction of endangered species into the wild. At least in regard to the species investigated here, we can make the following recommendations for achieving seedling survival in situ. *Carnegiea gigantea* is supported with MS agar without GA, *Echinocactus grusonii* is supported with filter paper without GA, and *Mammillaria parkinsonii* is supported with MS agar without GA. No particular combination of media and GA is more successful than any other for either *Hylocereus undatus* or *Echinocereus reichenbachii.* Finally, conditions other than those tested here are necessary for successful micropropagation of *Opuntia engelmannii*.

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VITA

David Ilum Warren-Hammack

EDUCATION

Degree Sought: Master of Science in Biology

- August 2016 to present
- Major Advisor: Christopher P. Randle, Ph.D.
- Committee Members: John Pascarella, Ph.D. and Chad Hargrave, Ph.D.
- Thesis: Use of Micropropagation Techniques to Improve Germination Success in Six Species of Cacti

- Degree earned:
  Bachelor of Science (May 2016) Sam Houston University,

RESEARCH INTERESTS

- Conservation Biology
- Tissue Culturing
- Plant Sciences
- Evolution
- Population Genetics

HONORS, AWARDS, AND SCHOLARSHIPS

- Delta Tau Alpha Honor Society: National Agricultural Honor Society- Member since 2015
- The Joey Harrison Biological Sciences Student Research Awards Endowment Fund, Grants-in-Aid of Research, **$1,000**.
- Received teaching award- May 2016
- Received Most Outstanding Senior Presentation in Undergraduate Research Symposium- April 2016

RESEARCH AND CAREER EXPERIENCE

Herbarium Curator; Museum Assistant, Sam Houston State University Huntsville, Texas:*May 2016- August 2017* at the Natural History Collections Museum
Competencies:

- Curation of Herbarium specimens
  - Fumigate and Freezing for sterilization
  - Mounting collected specimens
  - Organizing and databasing
- Organizing Museum Library collection
- Curation of Entomology collection
  - Alcohol preservation
  - Pin and point mounting

Research Assistant, Sam Houston State University Huntsville, Texas:

September 2012-May 2016 in the lab of Dr. Christopher Randle.
Competencies:

- Field collection
- Herbarium specimen mounting and curation
- Agarose gel Electrophoresis
- DNA extraction and purification
- PCR
- DNA sequencing
- Contig assembly and alignment using Geneious 8.0
- Phylogenetic analysis using TNT, GARLI, and MrBayes

Teaching Assistant, Sam Houston State University Huntsville, Texas: *January 2013-December 2019* under the guidance of Mrs. Lori Rose

- Taught 9 sections of General Botany Lab (BIOL 1411) Fall 2013 to Present
- Taught 4 sections of Contemporary Biology Lab (BIOL 1408) Spring 2013
- Received **Teaching Award** May 2016
- Under Department of Biological Sciences Stipend Fall 2016- Spring 2017

Independently Funded Researcher, Sam Houston State University Huntsville, Texas:

- Construction of a pressure-powered hydroponics system
- Care and research on over 250 cacti in the system
- Measurement and maintenance of 10 variables of nutrient balance in the system
- Currently being funded by the Joey Harrison Grant

Horticulturalist, Houston, Texas: *2007-present*

- Nursery manager, care of over 350 species of cacti and succulents
- Identifying, cataloging, and photographing inventory
- Seed propagation and grafting
- Testing different growing media, planting, transplanting,
- Aquaponics and hydroponics
- Received Presentation Award for research April 2016

Participant, International Research Experience for Undergraduates, Sam Houston State University, Workshop in Molecular Phylogenetics (August, 2014)

- Learned how to use Geneious 8.0, GARLI, and MrBayes
- Analyzed and combined sequence data
- Generated phylogenetic trees on TreeViewer

Participant, Rancho Santa Ana Botanic Garden, Summer Research Internship (July-August, 2013):

- Field collection and identification
- Herbarium curation
- Microscopy slide preparation
- Molecular Systematics
  - Extracted DNA out of the sample and purified it

  - Used PCR to amplify a chloroplast region
  - Sequenced the PCR product
  - Used contigs, on Geneious 8.0, from many other species within the subfamily to determine phylogeny
  - Phylogenetic analysis using TNT
  - Phylogenetic tree construction on TreeViewer
- Presentation on all research found

Attendee, Botany 2017, Omni Hotel, Fort Worth Texas (June 24-28, 2017)

Attendee, Cacti and Succulent Society of America 36th Biennial Convention, Pitzer College, Claremont, California (June 14-20, 2015)

Attendee, Cacti and Succulent Society of America 35th Biennial Convention, Austin, Texas (June 15-20, 2013)

PROFESSIONAL PRESENTATIONS

WARREN-HAMMACK, D.I., A.W. BRENEK, AND A. BYBOTH. 2016. A study in cactus conservation by means of hydroponics. "Honors College Undergraduate Research Symposium 2016", *Sam Houston State University*. Huntsville, Texas. April 23.

WARREN-HAMMACK, D. I., S.L. BALLINGER, S. BAYAT, A.W. BRENEK, J.J. MORAWETZ, AND C.P. RANDLE. 2015. Evolution and diversification of parasitism in the pantropical clade of Orobanchaceae. "Honors College Undergraduate Research Symposium 2015", *Sam Houston State University*. Huntsville, Texas. April 25.

RANDLE, C. P., J. J. MORAWETZ, S. BAYAT, **D. I. HAMMACK**, S. L. SHRUM, H. WANG, W. B. YU. 2014. Further documentation of the phylogenetic position and parasitic habit of *Brandisia* Hook. F. and Thomson. "Botany 2014" *Botanical Society of America*. Boise, Idaho. July 27-30.

RANDLE, C. P., J. J. MORAWETZ, S. BAYAT, **D. I. HAMMACK**, S. L. SHRUM, N. SINGH, E. ILUNGA, AND V. C. SOUZA. 2014. Phylogenetic investigation of diversity in the tropical clade of Orobanchaceae. "Botany 2014" *Botanical Society of America*. Boise, Idaho. July 27-30.

BAYAT, S., J. J. MORAWETZ, **D. I. HAMMACK**, S. L. SHRUM, N. SINGH, AND C. P. RANDLE. 2014. Preliminary phylogenetic investigation of the genus *Buchnera* L. (Orobanchaceae). "Botany 2014" *Botanical Society of America*. Boise, Idaho. July 27-30.

SHRUM, S. L., **D. I. HAMMACK**, J. J. MORAWETZ, AND C. P. RANDLE. 2014. Phylogenetic analysis of the tropical clade of Orobanchaceae. "Honors College Undergraduate Research Symposium", *Sam Houston State University*, Huntsville, Texas. April 24.

ORGANIZATIONS

Biological Sciences Graduate Students Organization
- Vice President August 2019- December 2019

Delta Tau Alpha- Agricultural Honors Society
- Member since August 2015

Cactus and Succulent Society of America

- Member since summer 2013
  Better Understanding of Global Sustainability at Sam Houston State University
- President (August 2015-May 2016) and Founding Member