Germination, survivorship and growth of an invasive desert grass, Schismus arabicus, under varied shade, moisture and soil-nitrogen regimes

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Germination, survivorship and growth of an invasive desert grass, *Schismus arabicus*, under varied shade, moisture and soil-nitrogen regimes

by

Sarah E. Emeterio

A thesis submitted to the graduate faculty

in partial fulfillment of the requirements for the degree of

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Program of Study Committee:
Kirk A. Moloney, Major Professor
W. Stanley Harpole
Lee Burras

The student author, whose presentation of the scholarship herein was approved by the program of study committee, is solely responsible for the content of this thesis. The Graduate College will ensure this thesis is globally accessible and will not permit alterations after a degree is conferred.

Iowa State University

Ames, Iowa

2018

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Finally, thanks for my family for all of their support, encouragement, and lifesaving care packages full of chocolate and caffeine.
The overarching goal from each of the thesis chapters in this document is to provide information on survival, growth and germination responses of *Schismus arabicus* when exposed to multiple fertility island microhabitat conditions simultaneously. Data from these studies may help identify key interactions which help explain contradictory findings in the literature, facilitate or direct future research, or potentially be incorporated into conservation plans or fire models.

In the first study, three questions were addressed: in the absence of competition and possible allelopathy: (1) how does *Schismus arabicus* survival and growth respond to varied shade, moisture and soil-nitrogen treatments combinations? (2) Which microhabitat features (shade, moisture, soil-nitrogen) exert the greatest influence on *S. arabicus* survival and growth when experienced in combination? And (3) do the results support the assertion that competitive or allelopathic influences outweigh the potential benefits of fertility islands and drive *Schismus* distribution away from the shrub canopy at the Desert Flame project Mojave site, as proposed in Schafer *et al.* 2012? Results of generalized linear mixed effects models and visual interpretation of graphs indicated that many multilevel interactions influenced the survival and growth of *Schismus arabicus*. However, even in the absence of competition or allelopathy, survival and growth variables demonstrated a strong preference for dry open conditions similar to the shrub interspace.

The goal of the second study was to create a set of inexpensive, time efficient and effective methods for germinating *Schismus* from seedbank soil. Generalized linear mixed model analysis indicated that moisture was the primary driver of germination. As long as an
optimal moisture threshold was met, shade and watering periodicity were only influential insomuch as they helped reach and maintain soil moisture levels within the optimal range. The simplest, cheapest and most time effective method for germinating *Schismus arabicus* from seed bank soil was to apply 9, 12 or 15 ml of water per 40 g of soil every other day in open shade conditions.
CHAPTER 1. GENERAL INTRODUCTION

Introduction

It is well established that the invasion of habitats by non-native species, also referred to as alien or exotic species, poses a threat to numerous systems at the global scale (Davis, Grime and Thompson 2000; Goodwin, McAllister and Fahrig 1999; Gordon 1998; Leung et al. 2002; Pimentel et al. 1999; Pimentel et al. 2001; Pimentel, Zuniga and Morrison 2005; van der Velde et al. 2006, Vitousek et al. 1996). Invasive species, defined as non-native species which have successfully spread and reproduced prodigiously outside of their native range (Daehler 2003; Richardson et al. 2000; Williamson and Fitter 1996), are problematic because of the damage they cause local economic and ecological systems. Damages include the high costs of their management as weeds, the loss of local biodiversity and habitats, and the disruption of ecosystem functions they can provide (Davis, Grime and Thompson 2000; Goodwin, McAllister and Fahrig 1999; Gordon 1998; Pimentel et al. 1999; Pimentel, Zuniga and Morrison 2005; van der Velde et al. 2006). One particular ecological impact of invasive species on the invaded habitat is their potential to alter fire regimes.

In desert systems, the presence and distribution of native and non-native annual plant species can have profound impacts on the severity and frequency of fires (Brooks et al. 2004). Once non-natives are established, they accelerate fire cycles in a self-reinforcing manner by fueling fires and then colonizing the newly disturbed landscape (Archer and Predick 2008, Brooks et al. 2004). According to Archer and Predick (2008), non-native plant species encourage fire in desert ecosystems where fire is historically rare. As the predicted effects of climate change and increased land use in the Southwestern United States include an
increased abundance of non-native, invasive species (Abatzoglou and Kolden 2011; Archer and Predick 2008), understanding the dynamics between alien invasive species and the native desert communities is essential to conserving present desert plant communities.

One plant community native to the Mojave and Sonoran Deserts is the creosote bush scrub plant community. These shrubs create small islands of fertility by concentrating nutrient and moisture resources under their canopies. However, while native annuals take advantage of the island of fertility and grow under the canopies of creosote bushes (*Larrea tridentata*), some non-native invasive grasses and shrubs grow between the creosote bushes (Brooks 1999; Brooks *et al.* 2004; Brooks and Berry 2006; Schafer *et al.* 2012).

Research exploring the reasons behind the different microhabitat preferences demonstrated by the native and invasive fertility island annuals is relatively limited, and the results are likely not universally applicable (Ehrenfeld 2003). For instance, the foundational study for this thesis – hereafter referred to as the Desert Flame study - found that a dominant exotic invasive annual, *Schismus arabicus* (Nees) grew in different fertility island microhabitats in a Mojave Desert site than in a Sonoran Desert site (Schafer *et al.* 2012, unpublished data). In addition to the preference for interspace growth observed for invasive annuals at the Mojave Desert Flame site, the native annuals also demonstrated a preference for growth away from the under canopy habitat. Schafer *et al.* (2012) suggest the possibility that competitive or allelopathic interactions with the focal *L. tridentata* shrub might be stronger than potential positive benefits of the fertility islands.

Such results highlight the importance of continued study of invasive-native-microhabitat interactions, especially given the fact that these interactions influence the growth, distribution, microhabitat conditions and fire regimes in fertility island habitats. They
also expose the need to identify how specific environmental or competitive factors influence the survival and growth of individual invasive species. For instances, understanding how, and under what circumstances, shade levels influence the survival and growth of *S. arabicus* plants in the Mojave Desert Flame site may allow for more accurate estimates of fire effects or *S. arabicus* distribution modeling for the site. However, such data is extremely limited overall, and should not be applied beyond the scope of the experiment. For those reasons, the relationship between microhabitat conditions and specific invasive species should be studied at the site of interest. Detailed in this thesis are two projects designed to help address the knowledge gap by examining how varied quantities and combinations of three creosote bush fertility island microhabitat features (shade, moisture and soil-nitrogen) influence the germination, survival and growth of *S. arabicus* in the absence of competitive and allelopathic interactions.

**Objectives and Research Questions**

Two experiments were completed for this thesis. The overarching goal of both projects is to provide information on the survival, growth and germination responses of *Schismus arabicus* when exposed to multiple fertility island microhabitat conditions simultaneously (i.e. shade, moisture, soil nitrogen). Each experiment had a different objective. The objective in the first study was to explore potential microhabitat features that might explain *S. arabicus*’ growth preference for open microhabitats in the Mojave Desert Flame site. The first objective was to be achieved through experimentally studying the effects of varied shade, moisture and soil-nitrogen level combinations on the survival and growth of *S. arabicus* individuals in the absence of competition and potentially allelopathic influences.
The hypothesis was that there would be an influence of shade, moisture and soil-nitrogen on the survival and growth of *S. arabicus* seedlings. Specifically, three research questions were addressed: (1) how did the fixed effects (shade, moisture and soil nitrogen treatment) influence the response variables, (2) which treatments fostered or hindered the growth or survival of the plants the most for each response variable, and (3) did the results provide support for the idea that microhabitat feature interactions may have driven *S. arabicus* growth away from the under canopy in the Mojave Desert Flame site?

As further studies of *S. arabicus* are needed to address these and other questions, the second experiment in this thesis had the objective of filling a gap in the literature through generating a set of cost effective, time efficient, and simple methods for germinating *S. arabicus* from seed bank soil. Based largely on the results of several informal pilot studies, the hypothesis was that the greatest germination would occur in high shade, high moisture treatments, when watering on alternate days. The second objective was achieved by pursuing three research questions: (1) what watering and shade regime combinations result in the greatest amounts of *S. arabicus* seedling emergence from seed bank soil under greenhouse conditions? (2) are there differences in the timing of seedling emergence due to differences in watering and shade regime combinations? and (3) does the periodicity of watering (every day or every other day) influence the abundance or timing of *S. arabicus* seedling emergence from seed bank soil?

**Thesis Organization**

This research-based thesis will begin with a review of relevant literature, including general microhabitat conditions and ecology of fertility island systems, fire system ecology
in the Mojave and Sonoran Deserts of the American southwest, the Desert Flame study, and the ecology of the study species *Schismus arabicus*. The second chapter is a manuscript for the primary experiment, which examined *S. arabicus* survival and growth under varied shade, moisture and soil-N treatment combinations. The third chapter is a manuscript detailing the *S. arabicus* germination methods experiment. Manuscript authors for both chapters two and three are part of the Desert Flame research team who made significant contributions to the design, implementation and analysis of the experiments. Dr. Erika L. Mudrak is an author for aid in project design and statistical expertise. Dr. Kirk A. Moloney is an author for design and consultation contributions. Following the two data chapters will be a chapter of general conclusions. The thesis will end with a terms and nomenclature section, followed by two appendices containing annotated R code used in the analyses for chapters two and three, respectively.

**Literature Review of Fertility Island Ecology**

While the complex interactions between *Larrea* individuals, soil biogeochemistry, and native and invasive annual plants are poorly understood, the effects those interactions may have on the spatial distribution of plants - and in turn their impact on fire regimes in the Southwestern United States - is worthy of investigation (Ehrenfeld 2003; Whitford, Anderson and Rice 1997). In this literature review, four general topics will be discussed. Firstly, the ecology of fertility island ecosystems will be described. Secondly, I will discuss fire system ecology in the Mojave and Sonoran Deserts of the Southwestern United States. Thirdly, the foundational project for this thesis (Desert Flame) will be described. Lastly, the known
ecology of the study species, *Schismus arabicus*, will be detailed. This literature review will end by outlining some brief conclusions based on the review.

**Fertility Island Ecology**

**Physical microhabitat environments**

In many arid and semi-arid ecosystems, nutrients, organic matter and soil moisture are concentrated in small loci which are directly associated with the scattered occurrence of desert shrub vegetation (Schade and Hobbie 2005). Loci with higher soil nutrients, moisture and organic matter are termed fertility islands (Gutierrez *et al.* 1993). The changes in nutrient levels are often abrupt and occur over very small distances between the resource-rich under-canopy of shrubs, and the areas between shrub canopies, termed inter-shrub spaces or interspaces (Figure 1; Bolling and Walker 2002). The formation of fertility islands is primarily a biological process, through which mineral nutrients from a large area are concentrated in the island along with biomass from the resident plants and local animals. In addition, island formation is affected by the central shrubs’ and local plants’ processes such as growth, loss and decomposition of roots and leaves as well as by decomposition of animal biomass, and the physical movement of organic matter by animals (Bolling and Walker 2002; Ehrenfeld 2003; Fisher *et al.* 1987; Lajtha and Schlesinger 1986). Specifically, plants absorb nutrients through their root system and redeposit them in the soil as organic residues (Gutierrez 1992). At the same time, the activity of decomposers is enhanced by the more moderate temperature, increased infiltration and retention of soil moisture that prevails under the shade of desert shrubs (Bolling and Walker 2002; Gutierrez *et al.* 1993).
Many of the most notable characteristics of fertility islands relate to soil nutrient characteristics. It is well documented that phosphorous, soil organic matter, soil moisture, and in particular soil nitrogen are more available under shrubs of fertility islands than in inter-shrub spaces (Bolling and Walker 2002; Esque et al. 2010; Gutierrez 1992; Gutierrez et al. 1993; Lajtha and Schlesinger 1986; Mudrak et al. 2014). Total nitrogen, available phosphorus and organic matter were more spatially variable than other soil parameters measured in several studies (Bolling and Walker 2002; Mudrak et al. 2014). It should also be noted that in addition to horizontal distribution of soil nutrients, there is also a strong link between vertical distribution of soil nutrients and the distribution, composition and biomass of vegetation.

According to Gutierrez et al. (1993), nutrients may become the limiting factors to production of biomass when moisture is not limiting to plant growth. Because of this, the difference between soil nutrients over very small distances is important since nitrogen and phosphorous are often considered the most limiting elements to plant growth in arid regions (Bolling and Walker 2002). From a plant distribution stand point, this is also influential since annual plants – plants which compete their lifecycle within a single year - that live relatively close to each other may be exposed to very different rates of nutrient supply (Gutierrez et al. 1993).

Influence of microhabitat resources on annual plant distribution

It has been suggested that the heterogeneity in soil resources, such as soil moisture or nutrients, may affect the distribution of annual plants beneath and around the fertility islands (Brooks 1999; Gutierrez 1992; Schafer et al. 2012). In the Mojave and Sonoran deserts,
winter annuals appear to be “So dependent on nutrient availability that the relatively nitrogen-rich fertility islands beneath shrubs support much larger plants with higher production efficiency” than those supported in the nutrient-poor interspaces (Gutierrez et al. 1993). However, the distribution and growth of annuals along the under canopy – interspace gradient varied substantially by the species being studied. Despite the greater overall plant density under the canopy, some individual plant species had greater density and/or abundance outside of the canopy, while others demonstrated the opposite trend (Gutierrez et al. 1993). For instance, one study found approximately four times as many plants (Porlieria chilensis) outside of a desert shrub canopy than underneath it (Gutierrez et al. 1993). In addition, despite the higher nutrient availability under the shrub, species richness was lower under shrub canopies. This paradox of enrichment, which is the inverse relationship between production and species richness, may be the result of the dominant species being able to better take advantage of the increased nutrient concentrations and increase their biomass at the expense of the other annual species (Gutierrez et al. 1993).

While some plants may be better able to utilize nutrients than others, nutrients are not necessarily the main factor limiting neighboring plant growth. For example, light availability may be limiting the growth of a shade intolerant plant growing under the shrub canopy regardless of the presence of a nutrient-competitive neighbor (Gutierrez et al. 1993). These findings suggest that interactions between alien and native species may be different between different species of annuals, and between different plant communities. Other studies have found that some annual invasive species, including S. arabicus, respond to lower thresholds of soil moisture than natives, resulting in increased biomass following even small increases in precipitation or irrigation (Gutierrez 1992). Due to the species and location specific nature
of species interactions in fertility island landscapes, studies that document the effects of competition should not try to generalize their findings beyond the spatial and temporal context of the study, since the multiple mechanisms at play may vary across space and time (Brooks 2000; Ehrenfeld 2003).

Specific to the Mojave and Sonoran Deserts of the southwestern United States, the growth, densities and biomass of winter annuals appear to be either dependent on or associated with the nutrient availability beneath *Larrea* shrub canopies in some studies (Gutierrez *et al.* 1993; Parker *et al.* 1982 in Gutierrez *et al.* 1993). However, others have found that for some winter annuals, such as *Schismus* spp. and *Erodium cicutarium*, the locations at which they dominated varied among the shrubland landscapes sampled during years with contrasting rainfall (Brooks 1999). This may be due to the fact that both facilitative and competitive effects of the fertility island focal shrubs occur simultaneously (Facelli and Temby 2002; Schafer *et al.* 2012). This suggests that the importance of one shrub-related aspect of fertility island microhabitat over another may depend on the intensity of the current abiotic stress (Bertness and Callaway 1994 in: Schafer *et al.* 2012). Results such as these provide further support for the suggestion that broad landscape patterns may not be applicable to local environments (Schafer *et al.* 2012).

**Non-native annual plant influence on local plant communities**

In addition to the aforementioned influence of the microhabitat resources and focal shrubs on annual plant distributions along the under canopy-interspace gradient, fertility island plant communities are also impacted by the presence and distribution of non-native annuals. Non-native annual vegetation can affect native plant communities through resource
competition, alteration of fire regimes, alteration of biogeochemical cycles, and by changing the rates of leaf litter accumulation (Brooks 2000). In a literature review of the mechanisms underlying the impacts of biological invasions, 17 of the 20 articles that examined impacts of invasive plants on the native community suggested competition was the process responsible for the invasive species’ impacts (Levine et al. 2003).

Despite the fact that all 20 of the studies explored in Levine et al. (2003) documented “Strong competitive effects of the invasive species on the growth, reproduction and resource allocation of native residents,” the resources for which the invaders were competing have remained largely unclear due to lack of explicit testing. However, some studies have begun to identify the specific fertility island resources being exploited by various desert annual species. For instance, the invasive non-native annual grass *Bromus tectorum* has been found to reduce the soil water available to native plants (Levine et al. 2003; Melgoza, Nowak and Tausch 1990). However, the effects of invasive species on local nutrient cycling vary between species. For instance, while *B. Tectorum* reduced nitrogen mineralization rates through having larger carbon-nitrogen and lignin-nitrogen ratios than native species, similar litter-quality effects did not explain the reduced nitrogen mineralization under the invasive grass *Hieracion* (Levine et al. 2003). Such highly variable differences suggest that different mechanisms operate for different species (Levine et al. 2003).

Another key way in which aliens affect the native fertility island community is by altering the structure of the native plant communities (Brooks 2000). As demonstrated by Brooks (2000), alien annual grasses can have significant effects on the density and biomass of native annual seedlings. As the competition from alien annual grasses continues over several years, there may be large changes in the native annual seed bank, causing
fundamental changes in the annual plant community structure and trophic dynamics (Brooks 2000).

Such changes were found in the Mojave Desert, where the biomass of alien annual species has been negatively correlated with the biomass and species richness of native annuals (Brooks 2000). Brooks (2000) proposes that such a result suggests that the Mojave alien annuals may be affecting the community structure of local natives possibly through competition between the species. Two of the most widespread and abundant alien annuals in the Mojave are the grasses *Bromus rubens* and *Schismus* spp., and both have been found to affect the native annual communities by promoting wildfire and possibly by competing for water and nitrogen (Brooks 2000). Specifically, alien annual grasses compete with native seedlings for water and nutrients as they become less abundant towards the end of their growing season. For instance, *Schismus*, *Bromus rubens* and *Erodium* can all assimilate nitrogen faster than native annuals in the Mojave, while *Bromus tectorum* can acquire water more quickly than natives in the Great Basin Desert (Brooks 2000). As nitrogen and water are commonly identified as the primary factors limiting plant growth in the Mojave, the overall competitive superiority of those aliens may be linked to the ability of those aliens to outcompete natives for nutrients (Brooks 2000).

Differential mortality during fire events is another cause of community change, as it immediately affects community composition as well as subsequent composition by its influence on resprouting and reproductive efforts. The differential mortality of plants during fire events is influenced by species specific characteristics such as meristem location, plant age and phenology (Melgoza, Nowak and Tausch 1990). In addition, fire events and fire regimes create changes in the overall physical microhabitat, nutrient availability, and the
post-fire vegetation dynamics where the plants are growing (Melgoza, Nowak and Tausch 1990). Those rapid changes in community composition and growing conditions associated with fire events alter the competitive interactions among species. For instance, competition faced by invasive grasses is reduced when native, fire intolerant shrubs, such as *L. tridentata*, die during fire events, or through the subsequent reduction of nutrient competition due to the increased nutrient availability.

In addition to impacting the native plant community through nutrient resource competition or differential mortality during fire events, which they may have promoted, non-native species can also impact the community by changing the availability of soil moisture resources. Hydrology alone can be altered through changing rates or timing of evaporation or runoff in a region, often due to differences between native and invasive plant transpiration rates, phenology, biomass or photosynthetic tissue or root depth (Levine *et al.* 2003). While many studies found rates of evaporation were changed through the loss of soil moisture due to invasives, it should be noted that some invasive species have been found to decrease community water use. In one such study, *B. tectorum* communities were found to lose 70mm less soil moisture per year than adjacent communities dominated by native shrubs (Levine *et al.* 2003). Root system have been another way in which hydrology was affected by non-native species, as stands dominated by exotic species were found to have shallower root systems than the native communities they are replacing (Levine *et al.* 2003). However, other studies have found exotic annual grasses in California alter hydrology through displacing deeper rooting native perennials (Levine *et al.* 2003).

Phenological differences between native and non-native species can also impact local plant communities by altering resource availability. This includes the fact that non-native
plants have been found to be photosynthetically active for a shorter portion of the year (Levine et al. 2003). In addition, exotic winter annual grasses have been found to transpire for a shorter period in later winter and spring, while the native perennials transpire into summer months, which changes the timing and magnitude of water demand by plants in the community (Levine et al. 2003). As many alien grasses germinate in the fall and dry out by early spring, which is the opposite of the native summer annual flora, they also extend the fire season (Brooks et al. 2004).

**Fire ecology and regimes in the American Southwest**

Fire regimes are affected by the spatial and temporal variations in topography, climate and fuel. As regional climate can change within decades, and fuel conditions can change as rapidly as a day following major disturbance, the features affecting fire regime can be fairly dynamic (Brooks et al. 2004). Invasive alien species can change the fuel properties of a natural ecosystem. By affecting the fuel properties, they can affect the behavior of the fire, which changes the fire regime characteristics, e.g., frequency, intensity, extent and seasonality (Brooks et al. 2003; Esque et al. 2010; Levine et al. 2003).

As grasses invade more woody communities, they create a more continuous horizontal fuel bed (Figure 1; Brooks et al. 2004; Levine et al. 2003). The *Larrea* shrub plant community is an example of this phenomenon. In the western United States, both the Mojave and Sonoran deserts have had dramatic increases in fire frequency in the last one hundred years due to invasion of Mediterranean grasses such as *Schismus arabicus*, *Bromus rubens* and *Bromus tectorum* (Esque et al. 2010; Levine et al. 2003). One result of the invasions and subsequent changes in community structure, fuel load, and fire disturbance has been the
widespread conversion of shrub lands to grasslands (Levine et al. 2003). Another example is *B. tectorum*, which has increased the horizontal continuity of fuel in western shrub lands of North America, which has increased the frequency and extent of fires, since it can grow in the previously unoccupied interspaces between fertility islands (Brooks and Matchett 2003). In addition to the increased frequency of fires, the invaders have also increased fire intensity due to their greater productivity then the native species they are replacing, which increases the fuel load (Levine et al. 2003).

One serious potential outcome of the changed fire regime is the potential for the new regime to promote the dominance of invaders over native plants. If that occurs, an invasive plant-fire regime cycle may be established in an ecosystem (Brooks et al. 2004). As an increasing number of the ecosystem’s components and interactions are altered, restoring the ecosystem to its pre-invasion condition becomes increasingly difficult and intensive (Brooks et al. 2004). In addition, the invasion of communities by fine textured plants such as grasses can increase the length of the fire season and promote fire ignitions during the heat of the fire season. Such a phenomenon is caused through the production of standing dead fuel, which dries quickly as soil moisture and atmospheric humidity decreases, which in turn promotes ignition earlier in the spring and later in the fall (Brooks et al. 2004).

In some cases, the addition of an invasive species, such as alien annual grasses *Bromus tectorum* or *Bromus rubens* increases the fuel load, which increases the fire frequency past the point from which native shrub-steppe species can recover (Brooks et al. 2004). As a result of this, animals that require that type of plant community are negatively affected as well. This ripple effect of changed fire regimes due to invasive aliens permeates the ecosystem and makes restoration efforts more difficult (Brooks et al. 2004). In addition,
efforts to restore affected native plant communities to pre-invasion conditions could be hampered by additional changes the alien species cause to spatial or temporal distributions of soil nutrients or the high density of the seed bank of the invader (Brooks et al. 2004). For instance, the disturbance caused by fire affects properties to the ecosystems such as soil erosion or formation, as well and the pathways and timing of nutrient cycling patterns and energy flow (Brooks et al. 2004). Furthermore, disturbance regimes may act as a selective force affecting life history traits of individual species and the composition, structure and emergent properties of whole groups of organisms (Brooks et al. 2004). Several general traits commonly exhibited by invasive desert annuals favor rapid reproduction, effective dispersal and increased reproductive efforts due to fire; this sets up a system where invasive species first cause changes to fire regimes by altering fuel conditions and then flourish under the new conditions they create (Brooks et al. 2004). In conclusion, the combination of new fire regimes and loss of native plants creates opportunities for other plants to colonize new areas or increase cover in sites where they were less common as many invasive annuals are well suited for rapid dispersal into altered landscapes and persistence under altered disturbance regimes (D’Antonio, Dudley and Mack 1999 in Brooks et al. 2004).

The Mojave and Sonoran Deserts have both been influenced by fire effects caused by non-native annual plants. Most of the Mojave and Sonoran deserts are hot desert shrub lands dominated by creosote bush fertility islands at low elevations. Precipitation is relatively low and occurs mostly during the winter, and native vegetation types generally exhibit low productivity and fuel loads (Brooks and Chambers 2011). Those conditions fostered an infrequent fire regime pre-settlement, as the fine fuels were largely from the sparsely growing winter annuals.
However, following settlement and subsequent invasion of both the Mojave and Sonoran deserts by invasive exotic annual Mediterranean grasses (specifically *B. rubens* and *Schismus barbatus*), fire fuel loads have significantly increased in the creosote bush scrub systems (Brooks and Chambers 2011). The increased fuel loads have created conditions conducive to fire spread (Brooks 1999). Partly as a result of this, there was an increase in fire frequency in the Sonoran desert between 1955 and 1983, as well as an increase in fire frequency in the Mojave between the 1980s and early 1990s (Brooks and Chambers 2011). Given the extreme influence of non-natives on desert fertility island communities and fire regimes, it is essential to understanding the processes underlying the documented effects of exotic plant invasions. This includes increasing our understanding of how specific suites of microhabitat resources influence non-native plant germination, survival and growth, so that we can better predict their distribution and fire effects under various environmental conditions. Doing so may allow managers to prevent or restore damages their presence may cause (Levine *et al.* 2003, Williamson and Fitter 1996).

**Foundational Study**

It has been suggested that a better understanding of the mechanisms underlying invasive species distribution around fertility islands and their impacts on the native community through competition and fire regime modification is essential in order to prevent further damages and create more effective restoration practices for affected landscapes (Brooks and Berry 2006). The basis for my thesis projects was developed from the preliminary results of a 2010-2013 study which worked from those premises.
The study examined the impacts of exotic annuals on fire frequency in *Larrea tridentata* fertility islands landscapes on two military facilities in the Southwestern United States. Both facilities are sites where military training activities, such as field exercises involving the deployment of live ordinance, may act as sources of ignition in local Mojave and Sonoran Deserts landscapes. The study was carried out by the labs of Dr. Kirk A. Moloney of Iowa State University in Ames, Iowa, and the lab of Dr. Claus Holzapfel of Rutgers University in Newark, New Jersey. While the ultimate goal is to produce a model that would allow for the bases to identify how plant distribution around fertility islands (e.g. fuel source, abundance and physical characteristics) at specific locations under various environmental conditions would respond to fire so that preventative measures can be more effectively employed, preliminary data allows other information about the distribution and resource availability to be assessed.

Two sites were selected for study. The Mojave Desert site (FTI) was located on Fort Irwin National Training Center, north of Barstow California (35°9’21” N, 116°53’6” W). The site was on an east facing bajada with “Coarse-loamy, mixed, superactive, hyperthermic, Typic Haplicalcids” soils. The soils have low to very low runoff, which are “Somewhat excessively drained” or “well drained” (USDA 2005). The mean annual rainfall between 1973 and 2006 is 147mm, mostly falling during the winter and spring, and the 30-year mean annual temperature is 17.7°C (Data for Goldstone Echo 2, 22km N of the Mojave study site, Western Regional Climate Center, www.wrcc.dri.edu, *in:* Schafer et al. 2012).

The Sonoran site (BMG) was located within the Barry M. Goldwater Range, south of Gila Bend, Arizona (32°41’49”N, 112°50’22”W). Soils are “Coarse-loamy, mixed, superactive, hyperthermic, Typic Haplicalcids” with low runoff, which are “Somewhat
excessively drained” (USGS 2005). The mean annual precipitation between 1992 and 2010 was 153mm, falling predominantly during a summer and a winter wet season. Mean annual temperatures for the same time period is 22.7º C (Data for Gila Bend, AZ, -29km NNW of the site, Western Regional Climate Center, www.wrcc.dri.edu in: Schafer et al. 2012). In addition to Ambrosia dumosa (A. Gray) Payne at the Mojave site, creosote bush is a dominant shrub species in both the Mojave and Sonoran sites, and has been found to have “Obvious spatial effects on soil properties (fertile islands)” (Bolling and Walker 2002).

In both the Mojave and Sonoran deserts, the biologically available nitrogen (measured through the use of PRS-probe samples) was highest near the shrub stems, sharply decreasing until it reached and maintained a low level as distance from the stem increased (Mudrak et al. 2014). Phosphorous, Calcium and Magnesium varied greatly within each desert. Potassium decreased with distance from the shrub stem in both deserts, most sharply in the Mojave Desert, but with a more gradual rate than the reduction in nitrogen with increased distance from the shrub stem (Mudrak et al. 2014; Unpublished data). Average nitrate at varying distances from the stem for 25 shrubs at each site revealed similar results (Figure 2).

In late March 2013, I collected shade data in order to set appropriate shade treatment levels for the first thesis experiment. Canopy openness photographs taken with a 180º hemispheric lenses approximately 20 cm from the soil surface at various under canopy, canopy drip line and interspace microhabitats of 25 unburned and 8 burned shrubs at the Mojave and Sonoran Desert Flame sites. Canopy openness was analyzed with Gap Light Analyzer version 2.0. Percent shade at the various distances from the shrub stem was very...
similar between the Mojave and Sonoran sites, despite the smaller shrubs (on average) at the Sonoran site (Figure 3).

At each site, two transects were established in a northern or southern direction from 168 focal *Larrea tridentata* shrubs into the inter-shrub space at each site. One transect was lightly turbated to simulate disturbed soil conditions, while the other transect remained undisturbed. On each transect, four 20 cm by 20 cm plots were established. The transect nearest the shrub stem was identified as the under-canopy plot (UC), the second plot was centered over the canopy’s drip-line (CD), a third was located 40 cm along the transect away from the CD plot (starting 50 cm from the canopy drip-line) and identified as the open near shrub plot (ON), and the final plot was established 60 cm along the transect from the ON plot and identified as the open-far plot (OF) (Figure 4).

A census of all living plants was taken between late March and early April in 2011, 2012 and 2013, the timing of which correspond to winter annual flowering. In the 2011 pretreatment reproductive census it was found that the distribution of invasive individuals, predominantly *S. arabicus*, and various native species differed in relation to each other and to the creosote bush canopy between sites. Mojave average invasive abundance by microhabitat demonstrated a strong preference for the open microhabitats while the native individuals demonstrated a preference for the canopy drip line and open-near microhabitats (Figure 5). However, average abundance per plot was extremely low and even between the Sonoran site invasives (nearly entirely *S. arabicus*) and microhabitats. Invasive annuals were highest in the open habitats, with averages very similar for the open near and open far microhabitats. At the Sonoran site, the average abundance of invasive individuals per microhabitat remained low and fairly even across microhabitats, but was highest in the under-canopy microhabitat.
Sonoran natives also exhibited very low and fairly even average abundances between microhabitats, with the greatest average abundance in the open-near habitat (Figure 5).

The higher densities of native annual species, which preferred non-under canopy microhabitats (i.e., demonstrated a preference for the canopy drip-line and open near microhabitats), was unexpected based on the literature, and the numerous advantages of the fertility island effect in under canopy microhabitats. The fact that there was less annual abundance in the under canopy microhabitat than in canopy drip-line or open habitats, despite the higher nutrient concentrations, soil moisture, and reduced temperature and solar radiation under the shrub, suggests that the negative effects of *Larrea* allelopathy (use of secondary metabolites to reduce competition with neighboring plants or damage due to herbivory) or *Larrea* competition with natives or some other interaction that was stronger than the positive effects of the fertility islands in our study (Schafer *et al.* 2012).

Consideration of the suggestion offered in Schafer *et al.* (2012) raises several other questions for examination. One, how strongly do *Larrea* shrubs compete directly with annuals? Another is if, or how much, allelopathy from *Larrea* affected native and invasive annuals, and under what conditions is it stronger than the potential benefits of the fertility island for annuals?

In addition to the puzzling results discussed above, the result of different distribution patterns of *S. arabicus* between the two deserts also warrants further investigation. Several literary sources discussed in previous sections identified competitive abilities allowing it to survive in harsh interspace environments. Among invasive desert annuals, *Schismus* spp. has been found to be particularly well suited for the harsh arid environments, able to thrive where few native annuals can (Brooks 1999). Its origin in arid regions may have made it better
adapted for the low nutrient and resource levels that are typical in the Mojave and Sonoran Deserts (Brooks 1999). Those predispositions may allow it to establish in the harsher environment found in the interspaces, while other invasive species, such as *Bromus rubens*, prefer more near-shrub locations (Brooks 2000). Other studies have identified environmental or resource requirements (i.e., shade intolerance, Brooks 2000), which may suggest that there is an environmental difference between sites that was not taken into account which is exerting an influence over *Schismus* spp. distribution at one of the sites.

An alternative explanation is that there may be noteworthy differences between the two *S. arabicus* populations, potentially due to being introduced from different source populations. Though less probable, it is possible that they were introduced from different parent populations or consist of different ecotypes, making them better adapted to slightly different conditions or responsive to different cues. If that was the case, the differences in their origin and history may help explain the varied patterns of distribution in the slightly different desert habitats.

*S. arabicus* seed germination differences between the two deserts may provide a third potential explanation for the microhabitat preference inconsistencies between deserts. It is possible that the seeds that germinated during the census year were from parent plants that were produced in substantially different conditions in the two deserts. Germination and seed production is extremely complex in *Schismus arabicus*, as several environmental and genetic factors exert influences. Factors such as rain, temperature, relative humidity, timing of annual precipitation, seed size, seed genotype, seed phenotype, soil moisture content during plant development and day length have been found to influence seed production, seed germinability and germination timing for *Schismus arabicus* (Gutterman 1989, 1993, 1996a,
As one of the key ways *S. arabicus* can persist in more hostile interspaces is through its ability to alter the number of seeds produced according to the moisture level of the growing year, as well as its ability to maintain an extensive seed bank in order to survive during dry years, the seeds germinated in a given year could be the propagules of plants that grew in a wide variety of environmental conditions (Brooks 1999). If the weather was sufficiently different between the two sites in the years leading up to the pretreatment census, the seeds that germinated and were measured in the census could be from individuals that were better able to grow in varied conditions, making the distribution inconsistencies appear more dissimilar than they would if conditions were uniform in previous years.

Overall, there has been relatively little study of fertility islands and annuals in the Sonoran compared to research of fertility islands in other North American deserts (Schafer et al. 2012). It is possible that there are important differences in the biotic or abiotic features of fertility islands between the Sonoran and Mojave that may have contributed to differences in *Schismus* distributions that have simply not yet been explored, such as soil texture, porosity or slope effects.

**Study Species**

*Schismus arabicus* Nees was selected as the study species due to its short life cycle (Gutterman 1989; Halvorson 2003), dominance as an invasive in the creosote bush fertility island landscape of the American Southwest (Brooks 1999; Brooks *et al.* 2004; Brooks and Berry 2006; Brooks and Matchett 2003), and the different microhabitat position preferences demonstrated between Mojave and Sonoran sites in the Desert Flame study (Schafer *et al.*
2012; Unpublished data). It should be noted that while the sites in both deserts are within the ranges of both *Schismus arabicus* and *Schismus barbatus*, only *Schismus arabicus* was found during adult censuses and in seed bank soil samples at the Mojave site. While that suggests that all *Schismus* spp. seedlings selected for the study are *Schismus arabicus* individuals, it does not preclude the possibility that some may have been *Schismus barbatus*. *S. arabicus* and *barbatus* individuals can only be distinguished by reproductive morphological structures (Brooks 2000), which made differentiation as seedlings or during the vegetative stages of my projects impossible in this experiment. Given the fact that both *Schismus* species have such similar ranges, ecology, and ecological effects on local fire and community ecology (Halvorson 2003), the potential use of *S. barbatus* instead of *S. arabicus* was not considered significant. For these reasons, the individuals used in this study will be considered solely of the species *Schismus arabicus*.

*S. arabicus* is an annual cool season grass native throughout the Mediterranean and Middle East regions (Gutterman 1989; Halvorson 2003). It reproduces solely by seed, and maintains a multiyear seed bank presence through maternal effects, which limit germination to periods of sufficient moisture during a date when the day length matches that when the mother plant germinated (Gutterman 1980; Gutterman 1994). In the Southwestern United States, *S. arabicus* germinates in early winter following sufficient rainfall of approximately one cm (Brooks 2000, Gutterman 1989). It then overwinters until approximately March, when increased rainfall and higher temperatures stimulate vegetative growth (Brooks 2000). As a facultative long-day plant, flower initiation and anthesis occur more rapidly the longer the day length is (Gutterman 1989, 1996a; Gutterman, Gendler and Rachmilevitch 2010). Depending on rainfall and day length, a seedling can develop from a seedling to a flowering
plant in as little as two weeks (Halvorson 2003). Flowering continues until water stress
triggers senescence (Brooks 2000). Soil moisture has been identified as the primary factor
affecting the growth of *S. arabicus* (Gutterman 1989), with sufficient moisture putting off
senescence regardless of increased solar irradiation and high temperatures through the
summer and early autumn (Halvorson 2003). Senesced *Schismus* plants can remain rooted
and upright for up to two years following senescence (Brooks 2000).

**Conclusions**

It is clear from the literature that desert fertility islands are locations of complex and
dynamic interactions between the physical and biological features of the landscape. Given
the large influences of non-native invasive species on the native plant community
composition, resource availability and fire regimes, it is clear that a greater understanding of
the process of individual species in the community, the spatial and temporal distribution of
individuals and resources as well as the influences of non-native invasive species on fire
regimes is necessary. As more studies similar to the ones being performed by the Moloney
lab are completed, including the work in this thesis, it may become increasingly possible to
accurately predict how specific landscapes will respond to various types and degrees of
disturbance. Greater understanding of which and how various environmental and biological
features influence each other may lead to greater predictive abilities. Both experiments
detailed in this thesis were designed to increase that understanding for the Desert Flame
Mojave site *S. arabicus* survival and growth, and for the Mojave and Sonoran site
germination. As the influences and interactions between some of the prominent microhabitat
conditions (i.e. shade, moisture, soil nitrogen, etc.) were assessed for *S. arabicus* from the
Mojave Desert Flame site survival and growth, it may be possible to more accurately model population distribution following events influencing soil nitrogen or events that alter shade availability. More accurate predictive abilities will not only allow for preemptive action to reduce the risk of further changes to natural regimes, but also allow for more targeted restoration and conservation action.
Figure 1. Invasion of interspace by invasive annual vegetation. Invasion creates a continuous horizontal fire bed between fertility islands and redistributes soil nutrients. Red = high soil nutrient concentration. Orange = moderate soil nutrient concentration. Yellow = low – moderate soil nutrient concentration. White = extremely low soil nutrient concentration. Dark green vegetation = native annual plants. Light green vegetation = invasive annual plants.
Figure 2. Average biologically available soil nitrate by probe distance from the canopy drip in the (A) Mojave and (B) Sonoran Site. Sample of three probes from each of the 25 shrubs photographed per desert. Plant root simulator probes placed 5-10cm deep at the Desert Flame Mojave site January 18 – March 22, 2011, January 28-March 15, 2011 Sonoran site. Nitrate units = µg/10 cm²/63days. Probe distance range (cm) from canopy drip-line = (distance code * 20) + 9 cm for upper range, -10 cm for lower range.

A. Mojave Site

\[ y = 0.9529x^2 - 12.229x + 45.687 \]

\[ R^2 = 0.9292 \]

B. Sonoran Site

\[ y = 46.515e^{-0.188x} \]

\[ R^2 = 0.8101 \]
Figure 3. Average percent shade by microhabitat for Mojave, Sonoran and overall at Desert Flame sites in late March 2013. Microhabitat codes: 1=under canopy (-130 to -31cm of canopy drip-line); 2=canopy drip-line (-30 to 9cm from canopy drip line); 3=open near (10 to 69cm from canopy drip-line); 4=open far (70 to 249cm from canopy drip-line).
Figure 4. (A) Shrub diagram with transects, plots, and a dotted line indicating the location of the *Larrea* canopy drip line and (B) Transect diagram with plot sizes and distances between plots within a transect. UC = Under Canopy; CD = Canopy Drip-line; ON = Open Near shrub; OF = Open Far from shrub. DM = demography plot (always toward the other transect); SL = soil plot (always away from the other transect); * indicates that this distance is variable and depends on the size of the shrub. The mid-point of the CD plot is considered to be at 0 cm, such that each transect ends 150 cm into the inter-shrub area and a possible location for the UC plot is -20 to -40 cm. Image from: Schaffer et al. 2012.
Figure 5. Mean abundance of living native, total invasive or invasive *Schismus arabicus* winter annual individuals per plot by microhabitat during a late March 2011 spring reproductive census at a (A) Mojave and (B) Sonoran Desert site. Total invasive includes *Schismus* arabicus individuals. Microhabitat abbreviations: UC = under canopy; CD = canopy drip line; ON = open near; OF = open far. Data from 2011 Desert Flame research.
CHAPTER 2.

SURVIVORSHIP AND GROWTH OF AN INVASIVE DESERT ANNUAL, *SCHISMUS ARABICUS*, UNDER VARIED SHADE, MOISTURE, AND SOIL NITROGEN REGIMES

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Abstract

*Schismus arabicus* is a dominant invasive annual grass in the Mojave and Sonoran Deserts of the Southwestern United States. Its preference for growth between creosote bush canopies creates a continuous fuel bed that connects otherwise isolated fertility islands. The fuel bed may encourage fire in a habitat where fire is historically rare, which represents a threat to local biodiversity. Limited information is available about the influence of microhabitat features (e.g. shade, soil moisture, soil nitrogen, etc.) and feature quantities on *S. arabicus* survival and growth. Additionally, some studies have proposed competitive interactions with creosote bushes or allelopathy, rather than physical microhabitat feature differences, may prompt the *S. arabicus* inter-shrub habitat preference. The purpose of this study is to help address the knowledge gap concerning *S. arabicus* ecology by examining how different levels of fertility island microhabitat conditions (shade, moisture and soil nitrogen) influence the growth and survival of *S. arabicus* in the absence of competitive or allelopathic interactions. Using a split-plot design, shade (50%, 30%, open), soil nitrogen (40ppm, 12.3ppm, 5.7ppm) and moisture treatments (96ml or 48ml total) were applied to *S. arabicus* seedlings, and the resulting growth and survival responses were analyzed using linear, and generalized linear mixed effects analysis. Survival and growth results were
significantly influenced by a number of complex, multilevel interactions. Low soil 
nitrogen/open shade/high moisture conditions favored survival (days alive) and growth (leaf 
number, above ground biomass, below ground biomass, and above ground to below ground 
biomass ratio), while high shade and medium or high soil nitrogen treatments hindered 
survival and growth. The results of this study suggest that *S. arabicus* may not be forced into 
occupying the open, nutrient poor interspace habitats, by competition with fertility island 
plants or allelopathic effects, but may instead prefer or even need the harsher interspace 
microhabitat conditions for survival and maximum growth. Further experiments are needed 
to both identify additional microhabitat and biotic influences on *S. arabicus* survival and 
growth, and to determine if the influences observed in this study are representative of 
populations in other invaded locations.

**Introduction**

**Background**

It has been well established that habitat invasion by non-native species, also referred 
to as alien or exotic species, poses a significant threat to ecological and economic systems at 
the global scale (Davis *et al.* 2000; Goodwin *et al.* 1999; Gordon 1999; Leung *et al.* 2002; 
Pimentel *et al.* 1999; Pimentel *et al.* 2001; Pimentel *et al.* 2005; van der Velde *et al.* 2006, 
Vitousek *et al.* 1996). Those threats by non-native invasive species, defined as species that 
spread and reproduce prodigiously outside of their native range (Daehler 2003; Richardson *et 
al.* 2000; Williamson and Fitter 1996), include the high costs of their management as weeds, 
the loss of local habitat and species diversity, and the disruption of ecosystem function 
(Davis *et al.* 2000; Goodwin *et al.* 1999; Gordon 1999; Mack and D’Antonio 1998, Pimentel
et al. 1999; Pimentel et al. 2005; van der Velde et al. 2006). In the desert habitats of the southwestern United States, one ecological impact of extreme interest is the potential for invasive species to alter the fire regimes of their invaded habitat (Brooks et al. 2004; Mack & D’Antonio 1998; Rogstad et al. 2009).

In desert systems, the presence and distribution of native and non-native plant species can have profound impacts on the severity and frequency of fires in habitats where fire is historically rare (Allen, Steers and Dickens 2011; Brooks et al. 2004; Brooks and Matchette 2003, 2006; Chambers and Wisdom 2009; Rogstad et al. 2009). Once established, non-native invasive species accelerate fire cycles in a self-reinforcing manner by fueling fires and then colonizing the newly disturbed landscape (Archer and Predick 2008; Brooks et al. 2004; Rogstad et al. 2009). This creates a nearly continuous fuel bed between fertility islands that are otherwise almost completely isolated (Allen, Steers and Dickens 2011; Brooks 2002; Brooks and Matchette 2006; Rogstad et al. 2009). As the predicted effects of both climate change and increased land use in the Southwestern United States include an increased abundance of non-native species, understanding the dynamics between alien invasive species and the native desert communities they occupy is essential for conserving present desert plant communities (Archer and Predick 2008, Abatzoglou and Kolden 2011).

One plant community native common to the Mojave and Sonoran deserts of the Southwestern United States is the creosote bush (Larrea tridentata, (DC.) Coville), scrub plant community. One of the most iconic features of the habitat is the widespread distribution of creosote bush fertility islands. In arid systems, perennial plants exert a strong influence on the spatial distribution of the microclimate, soil properties and local organisms (Abella and Smith 2013). Those influences combine to form fertility islands. Fertility islands
are characterized by markedly higher concentrations of soil organic matter, nutrients (N, P, K) and soil moisture, as well as reduced soil temperatures and solar radiation under the canopy of a central shrub, in contrast to the conditions in the open areas between fertility islands (Bolling and Walker 2002, Esque et al. 2010, Gutierrez et al. 1993, Schlesinger and Pilmanis 1998, Titus, Nowak and Smith 2002, Walker, Thompson and Landau 2001). Many native annual plants and native animals take advantage of the refuge from the harsh desert environment by growing under, or very near, the shrub canopies (Bolling and Walker 2002; Ridolfi, Laio and D’Odorico 2008; Walker, Thompson and Landau 2001).

In contrast to the pattern observed for native species, some non-native, invasive species grow primarily from the shrub canopy drip line, defined as the furthest horizontal edge of the shrub canopy from the shrub stem, into the shrub interspace, defined as the zone between canopy drip lines (Figure 6, Brooks et al. 2004, Abella and Smith 2013, Schafer et al. 2012). Such growth is typified by the invasive annual grass species, Schismus arabicus (Nees) (Brooks et al. 2004; Rogstad et al. 2009; Schafer et al. 2012). It has been proposed that the distribution of annuals away from the under-canopy microhabitat is driven by allelopathic or competitive interactions with L. tridentata (Schafer et al. 2012).

While the complex interactions between components of the fertility island microhabitat and the associated plants are relatively poorly understood in the Mojave Desert (Rogstad et al. 2009), the effects those interactions may have on the spatial distribution of native and non-native plants, and in turn their impact on fire regimes in the southwest, warrant investigation (Brooks 2002; Whitford, Anderson and Rice 1997). For instance, it has been established that Schismus spp. (Schismus arabicus and Schismus barbatus) is a dominant exotic invasive in the southwestern United States, and that it has demonstrated a
preference for interspace growth (Brooks et al. 2004; Rogstad et al. 2009; Schafer et al. 2012). However, understanding of how various fertility island microhabitat features (i.e., soil moisture, nutrients, shade, etc.) and competition or allelopathy by the dominant shrub influence *Schismus* spp. growth and distribution remains limited. As these interactions might influence *Schismus* spp. properties as a fuel source (Brooks 2002), understanding how they influence the growth and survival of *Schismus* spp. under various physical and biotic conditions is necessary to accurately model fire effects.

Furthermore, a recent study (referred to hereafter as the Desert Flame study) found the distribution of *Schismus* spp. in relation to the creosote bush canopies differed between Mojave and Sonoran desert sites (Schafer et al. 2012, unpublished data). In the Desert Flame study, average *Schismus* spp. abundance in the Sonoran Desert was low and fairly even from under the canopy into the interspace microhabitat. In contrast, the Mojave site had a much higher abundance (up to 10 times greater than in the Sonoran), with a large increase in the average non-native invasive abundance going from under the canopy into the interspace. The greatest number of adult individuals were found in the near and far interspace study plots. These contrasting results lend further support to the claim that site-specific environmental and biological features likely influence invasive distribution, and should be incorporated into fire effect models for accurate predictions (Figure 7).

In addition to the suggested explanation of allelopathy or competition with the *L. tridentata* shrubs influencing the Mojave site, *Schismus* spp. distribution, other competitive, genetic or environmental features could have influenced native and *Schismus* spp. distribution. However, despite the prevalence and influence that *Schismus* spp. exerts in the creosote bush fertility island landscape’s fire regimes and plant community ecology, large
pieces of information regarding the influence of microhabitat features (e.g. shade, soil texture, local competition, etc.) on growth are still sparse or lacking entirely. This may be partially due to the focus on nitrogen or water competition between annuals in the literature, the difficulty proving the role of allelopathy, and the relatively limited literature available examining the impact of the numerous other environmental features on specific species growth along different gradients.

**Objectives and Research Questions**

Given the magnitude of influence invasive plant species have on desert scrub plant communities and local fire regimes, it is prudent to develop a deeper understanding of the ecology of creosote bush scrub communities and their members. In order to more accurately predict and manage how changes in specific environmental conditions and competitive interactions will impact key plant community members, a greater understanding of the influence those interactions have on key individual species is necessary. Given the multiplicative nature of an organism’s response to multiple variables, exposing an organism to several microhabitat features simultaneously is likely to yield a more comprehensive understanding of how a given organism may respond in a given environment.

Towards that end, this study utilized a multivariate design to explore the suggestion in Schafer *et al.* (2012) that competitive interactions or allelopathy, rather than less favorable microhabitat conditions, explained the greater abundance of *Schismus* spp. individuals away from the under-canopy microhabitat in the Mojave site of the Desert Flame study. Given the difficulty of proving the presence and role of allelopathic chemicals, and the methodological restrictions in conducting a multivariate competition study in a greenhouse setting, this study
was designed to examine potential non-allelopathic, non-competitive explanations for the distribution of *S. arabicus* away from the shrub canopy at the Desert Flame Mojave site. That objective was achieved through experimentally studying the effects of varied shade, moisture and soil nitrogen level combinations on the survival and growth of Mojave *Schismus arabicus* individuals in the absence of competition and allelopathic influences. Few studies have examined any one or two of those variables on *S. arabicus* survival and growth, and all have been in field conditions with limited growth observations made. To the best of my knowledge, no study has explicitly examined the influence of all three in combination, or any of the three factors in the absence of competition.

Three specific research questions were addressed. In the absence of allelopathy and competition: (1) how does *Schismus arabicus* survival and growth differ under varied shade, moisture and soil nitrogen level combinations? (2) Which microhabitat features (e.g. shade, moisture, soil nitrogen) exert the greatest influence on *S. arabicus* survival and growth when experienced in combination? And (3) do the results support the assertion that competitive or allelopathic influences outweigh the potential benefits of the under-canopy fertility island microhabitat and act to drive the *S. arabicus* distribution away from under the shrub canopy at the Desert Flame Mojave site? The central hypothesis was that there would be an influence of shade, moisture and soil nitrogen on the survival and growth of *S. arabicus* seedlings which favored survival and growth in interspace microhabitat conditions (low shade, low moisture, low soil nitrogen) despite the lack of competition or allelopathy discouraging growth in under canopy microhabitats (high shade, high moisture, high soil nitrogen).
Study Species

*Schismus arabicus* Nees is native to the Middle East and Mediterranean regions (Gutterman 1996a, 1996b, GISD 2005). It invaded the Mojave Desert region around the 1940s and was common in the Mojave Desert within a decade (O. Clarke, pers. comm. in Brooks 2000; Peebles 9080, in Burgess *et al.* 1991, in Halvorson 2003). It reproduces solely by seed (Brooks 2000), and maintains an extensive multiyear presence in the seedbank. Germinating in early winter following sufficient rainfall of approximately 1 cm (Brooks 2000, Gutterman 1989), it overwinters until approximately March, when increased rainfall and higher temperatures stimulate vegetative growth (Brooks 2000). As a facultative long-day plant (Gutterman 1980, 1989), flower initiation and anthesis occur more rapidly with increased day length, allowing for rapid growth and reproduction before the weather is too hot for the plant to survive (Gutterman 1980, 1989). Flowering continues until water stress triggers senescence (Brooks 2000). It is shade intolerant (Brooks 2000), and has demonstrated a preference for growth in disturbed and inter-shrub spaces of Fertility Island landscapes (Brooks 2000, Brooks *et al.* 2004). However, its microhabitat preference in such landscapes is not universal, as demonstrated by the Schaffer *et al.* (2012) study.

*S. arabicus* density and biomass have been found to be highly dependent on the seasonal timing of water and nutrient pulses (James *et al.* 2006). Soil moisture has been identified as the primary factor affecting the growth of *S. arabicus* (Gutterman 1989), with sufficient moisture putting off senescence regardless of increased solar irradiation and high temperatures through the summer and early autumn (Halvorson 2003). *S. arabicus* establishment was found to be greater under continuous, low water plus nitrogen resource
supply than pulsed, high water supply, a likely reason being that more of the soil resources were available at shallow depths in the field study (James et al. 2006). In a separate study, biomass of *S. arabicus* increased significantly when a low irrigation treatment was administered, however, no response to fertilizer additions were found, possibly due to the irrigation treatment levels being too low (Gutierrez 1992). In a different study, increased nitrogen was found to increase the density and biomass of *Schismus* spp. (Brooks 2003). So, while both water and nitrogen additions have been found to influence the growth of *S. arabicus*, the way in which the interaction between water and nitrogen amounts on *S. arabicus* growth occurs is complex, context dependent, and warrants site-specific study.

**Methods**

**Experimental Design**

The survival and growth responses of *S. arabicus* to contrasting levels of shade, soil nitrogen and moisture were evaluated in a split-plot greenhouse experiment conducted at Iowa State University between December 19, 2013 and March 3, 2014. Fifteen main plots were arranged on a single greenhouse bench and assigned a shade treatment (high, medium or open) by stratified random assignment for north/south and east/west sides of the bench (Figure 8.1). Subplots contained a set of all six, soil nitrogen–moisture treatments. Within each subplot, moisture treatments (high or low) were applied to individual black, 163cm³ plastic conetainers filled with nitrogen treated soil (high, medium or low) and one seedling (Figure 8.2). Each main plot was comprised of two conetainer trays surrounded by a shade enclosure. The space required for a single subplot was one half of one conetainer tray. Three
of the five main plot/shade treatments contained two subplots of seedlings, and the other two main plot/shade treatments had only one subplot of seedlings, for a total of eight replicates.

Shade enclosures were constructed with a half-inch PVC pipe frame (28in x 28in x 18in), to which the designated shade cloth was secured across the top and approximately 8in down each side. The enclosure design provided 2in clearance between the side of the container trays and the shade cloth, and 5in clearance between the soil surface in the container and the shade cloth top. The design allowed for sufficient air circulation to minimize differences in temperature and relative humidity inside and outside of the enclosures for all shade treatments and bench locations. There were no significant bench or main plot effects on mean temperature or relative humidity. Greenhouse temperatures were set to 18.3°F during the day and 15.6°F during the night. Lights above adjacent benches were set to a short-day cycle (8:00-17:00 daily), and ambient daylight increased from 9 hours and 7 minutes at the start of the experiment to 11 hours and 34 minutes/day at the end.

**Independent Variables**

Treatment levels were roughly based on those found in the under canopy, canopy drip line and shrub interspace microhabitats of the creosote bush fertility islands at the Mojave Desert Flame site (Figure 6). Selection of the three variables was based on their reported importance to *S. arabicus* in fertility island habitats (moisture, soil nitrogen), or their prominence in unburned fertility island microhabitats and potential use directly or as a proxy for other microhabitat conditions in future models (shade).

Shade was selected due to its tight association with distance from the shrub stem, its importance for providing solar refuge, reducing soil temperatures, and maintaining more
humid conditions under the shrub canopy. It was also selected for its potential use in models utilizing aerial photography to make *S. arabicus* population and local fire effects calculations. Shade levels used were based on canopy openness photographs taken with a 180° hemispheric lens approximately 20cm from the soil surface in the under-canopy microhabitat (47.6% shade at 60cm from the canopy drip line into the under-canopy microhabitat), canopy drip line microhabitat (21.4% shade at the canopy drip line) and interspace habitat microhabitats (12.1% shade 90cm into the interspace from the canopy drip line) of 25 unburned shrubs of various sizes at the Mojave Desert Flame sites. Canopy openness was analyzed with Gap Light Analyzer version 2.0. The shade treatment levels were high shade (50% shade cloth), medium shade (30% shade cloth) and open shade (enclosure frame only).

Moisture levels were based on the findings that 6 ml water addition on alternate days was sufficient for *S. arabicus* survival in the low shade treatments in an unpublished pilot study. The two moisture treatments were high (6 ml deionized water every other day, 96 ml total) and low (3 ml deionized water every other day, 48 ml total). A total of 16 watering events were administered over 31 days using a needleless syringe. Following the 31-day wet phase, plants entered a 19-day dry phase during which they received no water. The purpose of the dry phase was to prompt seedlings to enter their reproductive stage, which did not occur.

Soil nitrogen was selected as a treatment due to its exponential decrease in concentration (nearly all NO$^-^3$, with some NO$^-^2$ and NH$_4^+$) with increased distance away from the shrub stem (Mudrak *et al.* 2014; unpublished data), and its reported influence on *S. arabicus* growth in several studies. Treatment levels were high (40 ppm), medium (12.3
ppm) and low (5.7 ppm). The ambient nitrate in the growth medium soil was used for the low treatment. Baseline nitrate levels were obtained from a five sample mean of interspace soil, which would serve as the growth medium for the study, analyzed by flow-analyzer in December 2013. High and medium treatment levels were calculated by applying the mean ambient soil nitrate level (5.7 ppm) to the regression equation from a plot of total nitrogen by distance from the stems of *L. tridentata* shrubs. The regression equation data was obtained via Plant Root Simulator Probes (PRS probes) at a depth of 5-10cm between January 18 and March 22, 2011, at the Mojave Desert Flame site (Mudrak *et al*. 2014; unpublished data).

The soil used for the experiment was obtained from the top 10 cm of interspace soil within 50 m of the Mojave Desert Flame site border in March 2013. The soil was thoroughly homogenized, pasteurized, and allowed to air dry before the experiment. All soil components larger than 4.5 mm were removed by sieve. The required quantity of crushed fertilizer (34-0-0 by weight NH$_4$NO$_3$) was weighed and thoroughly mixed with 250 g of interspace soil for each individual container.

**Germination and Transition Phase**

*S. arabicus* seed bank soil was used to produce seedlings due to the extremely, labor intensive nature of seed collection from mature adult individuals, difficulty predicting and traveling from Iowa to California during the brief window when adult individuals would be mature but before they dropped their seeds, and the large number of viable seeds needed for the project. Seedbank soil was collected within 50 m of the sides of the Mojave Desert study sites in March 2013. Interspaces with several living and dead *S. arabicus* adults were found, and the top 1-2 cm of soil was collected. Additional soil was collected from the canopy drip
line and under canopy microhabitats by working from *S. arabicus* patch to *S. arabicus* patch in a stem-ward direction. The seedbank soil was stored in the dark at ambient room temperatures until late December 2013.

In order to evaluate the growth response of *S. arabicus* to environmental variables without the influence of competition, one *S. arabicus* seedling was transplanted into each conetainer. Prior to transplantation seeds were germinated using the following protocol: one cm of seedbank soil was spread evenly over four layers of paper towel in large seedling trays and 400-650 ml of water was sprinkled over the soil every other day for two weeks starting on December 20, 2013. As previous studies had identified temperature and light exposure as factors influencing germination (unpublished germination pilot study; per. comm. Hadas Parag 2013; Gutterman 1994, 1996a, 1996b, 200), germination was maximized by keeping soil trays under low-light conditions at cool-ambient room temperatures (65-70°F). Once seeds had germinated and attained a suitable size (see below) the seedlings were transplanted into the conetainers.

It should be noted that the Mojave Desert Flame site was within the ranges of both *Schismus arabicus* and *Schismus barbatus*, and that the two species can only be distinguished by reproductive structures (Brooks 2000). As the reproductive stage was never observed in this experiment, species identification could not be confirmed. However, only *Schismus arabicus* was found at the Mojave Desert Flame site in several reproductive censuses and seedbank composition experiments (unpublished data 2011-2013). While that suggests that all *Schismus* spp. seedlings selected from seed bank soil for this study were *S. arabicus* individuals, it does not preclude the possibility that some may have been *S. barbatus* individuals.
Based on the results of pilot studies and the available literature (Schafer et al. 2012), it was known that *S. arabicus* has high rates of seedling mortality. Transplanting pilot studies revealed that seedlings shorter than 0.5 cm rarely survived 3 days, and that seedlings, which died for other reasons, generally did so within 5 days of transplanting. To help ensure that all seedlings assigned to replicates were healthy, and that low survival or growth responses were more likely due to the experimental treatment than to transplant stress, transplanted seedlings underwent a seven day transition phase. Those that were taller than 0.5 cm were transplanted into a container of treated soil. The transplanted seedlings were kept on the greenhouse bench, but under heavy shade. Seedlings all received sufficient water to remain moist, generally 4-6 ml every other day. This allowed the healthy seedlings to acclimate to the conditions in the greenhouse and become established in the soil. It also allowed for identification and removal of unhealthy seedlings.

On the eighth day after transplanting, healthy seedlings - identified by their green color and thicker, turgid form - were randomly assigned to moisture and shade treatments. Each block was comprised only of seedlings transplanted on the same day. Any seedlings that were either unhealthy or unneeded for the blocks created were removed from the container, which was later reused in subsequent transplanting(s). Due to the high number of seedlings either in transition phase or transplanted on day 8, there was insufficient soil into which seedlings could be transplanted. For that reason, the seedbank soil was not watered on day 10. This effectively ended germination, as insufficient numbers of healthy seedlings were available to form a complete replicate block after that point. A total of 8 replicates were started. One replicate was transplanted the fourth day after germination was initiated and
placed into blocks on December 31, 2013. Three sets of replicates were initiated on January 2, 2014 using day 6 transplants, and four replicates were initiated with day 8 transplants on January 4, 2014.

**Response Variables**

For each plant, survival status and number of leaves were measured every other day, beginning on the day of the replicate’s initiation (day 0) and continuing until the plant was terminated due to death or the end of the observation period (day 50). Plants were identified as dead by a total lack of green coloration or lack of flexibility in green leaves. Three additional response variables (above ground biomass (AGBM), below ground biomass (BGBM), and the above ground biomass to below ground biomass ratio (AG:BG ratio)) were measured for plants that survived the entire observation period.

AGBM was collected at the termination of each plant. After termination, the AGBM was immediately harvested and stored at 4°C until it could be dried. AGBM was dried at 60°C for 48 hrs before being weighed. Plants that died during the experiment were largely small, single-leafed, and incredibly brittle. Many of those leaves snapped off and were lost during movement and harvesting of AGBM, and those that did not were too light to weight individually (<0.1 mg). For these reason, only the AGBM of plants that survived to the end of the project were considered.

After the plant was terminated and the AGBM was collected, the container of soil was covered and stored at 4°C until the sample could be processed for BGBM. Seedlings that died early in the project produced very little below ground biomass, most of which was extremely delicate and broke apart easily. For that reason, only the BGBM data from plants
surviving to the end of the project were used. To calculate BGBM, the soil was removed from the container and weighed. The soil was then sorted through for approximately 20 minutes, or until all large pieces of roots were collected. The soil was then thoroughly homogenized, and a subsample of approximately 15 cm$^3$ was then taken and weighed. The subsample was then processed, and all remaining, generally fine, root matter was removed. After drying the BGBM at 60º C for 48 hrs, the weight of the fine BGBM was multiplied by the proportion of the entire soil sample weight to the weight of the subsample to obtain the total mass of fine BGBM. The total fine and large BGBM totals were then combined for the total BGBM of the plant (method from Pers. Comm. Phil McGuier).

The AG:BG ratio was analyzed to provide a better understanding of how *S. arabicus* individuals that survived to the end of the project partitioned resources, potentially trading off AGBM for BGBM in more hostile environmental conditions. As both the AGBM and BGBM were only processed for those individuals which survived to the end of the project, AG:BG biomass ratios could only be calculated for those plants.

**Statistical Analysis**

The results were viewed primarily in the context of their implications about the influence of environmental features (shade, moisture and soil nitrogen) on the survival and growth of *S. arabicus*. All three research questions were addressed using linear (AGBM, BGBM, AG:BG ratio) or generalized linear mixed effect analysis of the relationships in question (survival time, leaf number). The analysis was performed using R (R Core Team 2014), *lme4* (Bates *et al.* 2014), *lmerTest* (Kuznetsova, Brockhoff, and Christensen 2014) and *lsmeans* (Lenth 2014). As count data was used for length of survival (days) and number
of leaves, those generalized linear mixed effects models (GLMMs) used a Poisson family distribution. The high-shade/high-moisture/high-nitrogen treatment was designated as the model intercept in all models. All results were in the form of the mean predicted response per treatment (i.e., predicted mean total number of days survived per treatment). However, the results are also referred to as just the total or the mean of the response. All comparisons were made at the 95% confidence level.

The generalized linear mixed effect analyses of the relationships between most response variables (i.e., number of days alive, above ground biomass, etc.) and fixed effects (shade, moisture and soil nitrogen treatment) resulted in significant three-way interactions. When significant three-way interaction terms are found, it is not possible to interpret any of the one- or two-way interaction terms. Plainly stated, three way interactions trump all one- and two-way interactions. Similarly, significant two-way interactions trump significant one-way interactions in the absence of significant three-way interactions. Given the difficulty such results present for interpreting the influence of a single fixed effect involved in a three-way interaction, the influence of each fixed effect was assessed using visual analysis of graphs. To do so, a lsmip graph was created for each response variable using the lattice package in R (Deepayan 2008).

For each lsmip graph, the predicted mean response values were averaged over two of the fixed effects, and then graphed by the treatment levels of the third fixed effect. For example, GLMM analysis indicated that the number of days a plant survived was significantly influenced by a three-way shade/moisture/soil-nitrogen interaction. To assess when specific interactions occurred between the fixed effects, three plots were visually analyzed. In the first, all survival day means were averaged by moisture and shade treatment,
and then graphed by their soil nitrogen treatment. This allowed for specific interactions between soil nitrogen, moisture and shade to be identified. Specifically, the more parallel the lines on the graph, the less an interaction is occurring. Conversely, the more non-parallel the lines, the greater the interaction that is occurring (Manning 2007).

Given the research questions for this project, each survival and growth response was analyzed for three things: (1) how did the fixed effects (shade, moisture and soil-nitrogen treatment) influence the response variable, (2) which treatments fostered or hindered the growth or survival of the plants the most for each response variable, and (3) did the results suggest possible microhabitat explanations to the *S. arabicus* growth away from under the canopy observed in the Mojave Desert Flame site?

**Results**

**Survival**

GLMM analysis of the relationship between the total number of days alive and the fixed effects indicated that all three-way interactions between shade, moisture and soil-nitrogen were highly significant (Table 1). Results of the maximum likelihood ratio test supported this, calculating that just over 19% of model variance could be attributed to that interaction (Table 2). Visual analysis of lsmip graphs revealed that survival time responses of the three shade treatments exhibited similar responses to nutrient treatments within moisture regime regardless of shade treatment (Figure 9). That observation was supported by results of pairwise comparison between treatments, which found that the only significant differences in survival time within either moisture treatment was in the 48-ml:40.0-ppm treatments between the 30% shade cloth (24.05 days ± 3.44) and 50% shade cloth treatments (9.58 days ±1.68). In
addition, visual analysis suggested there was a general trend of decreased survival time with increased nitrogen, particularly in the 48 ml moisture treatment at the 12.3 ppm and 40.0 ppm soil nitrogen levels (Figure 9). While the trend was also observed in the 96 ml treatment, the majority of the shortest survival times within the 96 ml moisture regime were observed at the 40.0 ppm treatment rather than the 12.3 ppm treatment.

The shortest mean survival time was 8.722 days (±1.512, CL=6.210-12.251) in the 48-ml: 12.3-ppm: no-shade-cloth treatment. However, it was not significantly lower than the four next-lowest survival time estimates, which ranged from of 9.579 days (±1.683, CL=6.789-13.516, p=1.0000) in the 48-ml: 40.0-ppm: 50%-shade-cloth treatment, to 14.454 days (±2.258, CL=10.641-19.631, p=0.0791) in the 96-ml: 40.0-ppm: no-shade-cloth treatment. Despite the lack of significant differences between the lowest mean and the four runners-up, eight of the eleven significantly different pairwise comparisons of mean survival times were significantly different when tested against the 48-ml: 12.3-ppm treatments.

Conversely, the 96-ml moisture and 5.7-ppm nutrient treatments interacted in a manner favorable for survival. All four of the longest mean survival times were predicted for 5.7 ppm nitrogen treatments. The longest survival time was predicted for the 96-ml: 30% shade-cloth treatment (46.062 days ±6.167, CL=35.430-59.886), which was only significantly longer than the shortest low-nitrogen survival time of 30.708 days (±4.267, CL=23.386-40.323, p<0.0001) in the 48-ml: 30% shade-cloth treatment.
Growth

Number of leaves at termination

GLMM analysis of the influence of moisture, soil nitrogen and shade on leaf number at termination for all specimens found no significant three-way interactions. Only two significant two-way interactions were identified, namely between the 12.3-ppm: 30% shade-cloth treatments and the 96-ml: 12.3-ppm treatments (Table 1). However, the maximum likelihood ratio test attributed less than 6% of the variation to the moisture: nutrient interaction, and just over 1% of model variance to the moisture: shade interactions. Most of the variation was attributed to the individual fixed effects of moisture, nutrient and shade (Table 2).

Interpretation of the lsmip plots revealed relatively consistent relationships between shade and nitrogen treatment levels in both moisture regimes despite the low percentage of variance explained by the interaction as calculated in the likelihood ratio test (Figure 10). There was a general trend of the largest number of leaves being exhibited at the lowest nutrient and shade regimes, with all other mean leaf numbers decreasing as nutrients increased and moisture decreased. However, the influence of shade and nutrients on leaf number was more pronounced in the 96 ml treatments; there were more total leaves at termination within the 96 ml treatment than in the 48 ml treatment, and all treatments yielded lower mean leaf numbers as shade and nutrient treatments increased. Conversely, the lowest mean leaf numbers in the 48 ml treatments were for the 12.3 ppm nutrient regime instead of the 5.7 ppm regime, and there was a slight increase in mean leaf number as shade increased. The 5.7ppm treatment also exhibited a slight increase in leaf number between the 30 and 50% shade cloth treatments.
GLMM analysis of the data from specimens which survived through the end of the growth phase found significant influence of the 96 ml moisture, 12.3 ppm nutrient, and 50% shade cloth treatments on leaf number (Table 1). However, the likelihood ratio test indicated that only shade explained more than 1.82% of the model variance, with a mean square of 50.4 (Table 2). Analysis of the lsnip plots also revealed a strong shade influence, the growth response to which differed between moisture levels (Figure 11). In both moisture regimes, there were substantially more leaves observed in the no shade cloth treatment than in either the 30 or 50% shade treatments. No specimens survived to termination in any of the 48-ml: 12.3ppm or in the 48ml: 40.0ppm: 50% shade-cloth treatments. Conversely, all treatments in the 96-ml moisture regime had survivors through the end of the experiment (Figure 11).

Given the large amount of variation attributed to shade in the likelihood ratio test, pairwise comparisons were made for survivors within shade treatments. The five highest mean leaf numbers were all in the no-shade-cloth treatment, ranging from 9.000 leaves in the 96-ml: 40.0-ppm treatment (±3.000, CL=4.682-17.299) to 16.200 leaves in the 96-ml: 5.7-ppm treatment (±1.800, CL=13.029-20.142). Of the survivors, the lowest numbers of leaves were all predicted in the 50% shade-cloth treatment. The lowest predicted means ranged from 2.400 leaves in the 96-ml: 5.7-ppm treatment (±0.693, CL=1.363-4.226) to 4.000 leaves in the 96-ml: 40.0-ppm treatment (±2.000, CL=1.501-10.659).

Analysis of the final plant leaf number for individuals that did not survive to the end of the observation period yielded only non-significant fixed effects or interactions between fixed effects (p=0.383-p=1.000). Similarly, less than 1.5% of the total variation in the model was attributed to any single or interacting fixed effect in the maximum likelihood test.
Above ground biomass (AGBM)

Linear mixed effect analysis (LMM) of above ground biomass for survivors indicated several interactions significantly influenced AGBM (Table 1). Two-way interactions between the 96 ml moisture regime were significant for the 40.0 ppm, 30% shade cloth, and 50% shade cloth treatments (Table 1). Additionally, nutrient: shade interactions were significant for the 12.3 ppm: 30% shade-cloth and 40.0 ppm: 50% shade-cloth treatments (Table 1).

Both visual examination of lsmip and scatterplot graphs and the likelihood ratio test indicated that shade was highly influential in AGBM production, with no shade promoting substantially more biomass per individual than the 30% or 50% shade-cloth treatments in all nitrogen and moisture treatments (Table 1, Figure 12). Shade appeared to interact particularly strongly with nutrients in the 5.7 ppm: no-shade-cloth treatments. Pairwise comparisons of mean AGBM within shade treatments further supported that interaction, as all five of the greatest predicted AGBM means were in no-shade-cloth treatments. However, shade was not influential in differentiating between high and medium shade treatments, evidenced by the close proximity both shade treatments maintained across all moisture: nitrogen treatments which had survivors to the end of the project.

The five largest mean AGBMs were all in no-shade-cloth treatments. The highest mean was yielded in the 96-ml: 5.7-ppm treatment (13.608mg ±1.292, df=15.00, CL=10.831-16.385), which was not significantly greater than the second and third greatest AGBM predictions of 8.446 mg in the 48-ml: 40.0-ppm treatment, and 7.498 mg in the 96-ml: 5.7-ppm treatment. The lowest four measurements were all in the 50% shade cloth treatment, and
ranged from 0.095 mg (±2.839, df=22.04, CL=-5.791-5.982) in the 96-ml: 12.3-ppm treatment, to 0.795 mg (±2.839, df=22.04, CL=-5.092-6.682) in the 96-ml: 40.0-ppm treatment.

**Below ground biomass (BGBM)**

There were no significant interactions that influenced below ground biomass totals. The large amount of residual variance in this model (1028.9, SD=32.23) likely influenced the lack of interaction, as it represents a large amount of variation between individual plants and the intercept value. Differences in shade treatment explained the greatest amount of variation in the model (SS=19809.5, NumDf=2, DenDF=22.578, MS=9904.7, F=2.806), however, the variance explained was not significant (p=0.0816). Examination of lsmip graphs support that finding, as BGBM was substantially greater in the no shade cloth treatments than the high and medium shade treatments. Low nitrogen and high moisture treatments also fostered increased BGBM, particularly in the low shade treatment. However, moisture treatment did not appear to strongly influence the BGBM of survivors under the conditions of this experiment, as the relationship between high and low moisture treatments remained nearly identical, and parallel, BGBM means for each shade: nitrogen treatment combination. However, as the soil nitrogen decreased, BGBM increased in the 96 ml treatment.

The greatest BGBM occurred in the 96-ml: 5.7-ppm: no-shade-cloth treatments (123.412mg±19.046mg, df=13.95, CL=82.546-164.277), which is the same treatment with the greatest leaf number, above ground biomass and survival time. Pairwise comparisons of all moisture and nutrient treatments by shade showed that the three, highest mean BGBM values were, once again, in the no shade cloth treatment. However, despite the largest mean
being substantially greater than all of the other predicted means, it was only significantly
greater than the seven lowest BGBM means. The seven low values ranged between 7.791 mg
(±29.318, df=25.41, CL=36.232-117.238, p=0.0370) in the 48-ml: 5.7-ppm: 30% shade-cloth
treatments, to 34.533 mg (±19.002, df=18.33, CL=-5.336-74.402, p=0.0035) in the 96-ml:
5.7-ppm: 50% shade-cloth treatment.

**Above ground to below ground biomass ratio (AG:BG)**

LMM analysis of the relationship between the fixed effects and the above-ground to
below-ground biomass ratio (AG:BG) found no significant influence from any single or
interacting fixed effects. Visual analysis of the AG:BG scatterplot supported that finding, but
also suggests that there may be some effect of the moisture: shade interaction at the shade
treatment levels (Figure 12). Visual analysis of the lsmip plots suggest that several complex
three-way interactions are occurring, despite the lack of significant interactions found in the
LMM and likelihood ratio tests.

The lsmip graphs portrayed low AG:BG ratio means in the 48-ml: 5.7-ppm: no-
shade-cloth treatment. In contrast, the 40.0-ppm: no-shade-cloth conditions favor high ratios
for both high and low moisture treatments. In the 30% shade cloth treatments, moisture
appears to interact very strongly with nitrogen, either promoting very high AG:BG ratios in
48ml: 5.7-ppm treatments, or very low AG:BG ratios in all three nutrient treatments in the 96
ml moisture treatment. The 50% shade-cloth treatment favored low AG:BG ratios, which
were further reduced in the 96 ml treatments.

The greatest AG:BG biomass ratio means were in the 96-ml: 40.0-ppm: no-shade-
cloth treatment at 0.2130 mg (±0.0515, df=27.91, CL=0.1097-0.3184), followed by 0.2100
mg in the 48-ml: 5.7-ppm: 30% shade-cloth treatments ($\pm 0.0360$, df=27.91, CL=0.1363-0.2837). The lowest AG:BG ratio was in the 96-ml: 12.3-ppm: 50% shade-cloth treatment, at 0.0040 mg ($\pm 0.0509$, df=27.91, CL=-0.1002-0.1082).

**Discussion**

It was not surprising that the results of this study supported the hypothesis, namely that there would be an influence of moisture, soil nitrogen and shade on the survival and growth of *Schismus arabicus* seedlings. However, the strength of the preference demonstrated for the harsh shrub interspace microhabitat features over those found in the under-canopy microhabitat of creosote bush fertility islands was somewhat unexpected. All of the mean growth responses assessed for *S. arabicus* specimens were largest in the nutrient and shade treatments designed to mimic interspace microhabitats (i.e. 5.7-ppm: no-shade-cloth). While there was a preference in growth responses for the 96 ml moisture treatment, which was set to reflect a wet year rather than set to a specific microhabitat condition. Specifically, all but one mean growth response was largest in the 96-ml: 5.7-ppm: no-shade-cloth treatment (leaf number, AGBM, and AG:BG ratio) rather than the 48-ml: 5.7-ppm: no-shade-cloth treatment which was preferred by BGBM. The survival response (number of days alive) was also greatest in the 5.7 ppm nutrient treatments.

These results indicate that not only did individuals in the 5.7 ppm nitrogen treatments survive longer than those in nitrogen treatments mimicking the near-canopy/canopy-drip-line (12.3ppm) or under-canopy (40.0 ppm) soil nitrogen habitats, but they also had larger growth responses in the more hostile microhabitats. Furthermore, the individuals which survived through the end of the experiment also demonstrated a preference for the no-shade-cloth
shade treatment which was also based on lack of shade typical in the shrub interspace. However, implications for the strong preference observed for the no-shade-cloth treatment should be somewhat tempered by the fact that the seedlings were exposed to much lower solar radiation and greater ambient shade in the Midwestern greenhouse in which the experiment took place than they would be in a shrub interspace in a desert in the American Southwest. The grow lights which augmented the available natural light were not sufficient to mimic solar conditions in the Desert Flame site.

Both the statistical analysis and visual analysis of lsmip plots for survival time indicated a significant three-way interaction between the fixed effects. However, two aspects of the visual analysis suggest that nitrogen level was the primary driver of survival time, and that it interacted with moisture regime to some degree regardless of shade treatment. The first aspect which suggests that is the clustered and similar responses of all three shade treatments to nutrient treatment within moisture treatment, suggesting shade was not a strong driver of survival time, while nitrogen was. The second aspect was the difference in mean survival times in the 12.3 ppm nutrient treatments between the 48 ml and 96 ml moisture regimes. Those differences suggest that there was a pronounced interaction between moisture and nitrogen in the 48 ml: 12.3 ppm treatment which was not evident in the 12.3 ppm: 96 ml treatment. Both aspects suggest that soil nitrogen was the biggest influence on survival under the conditions experienced in this study, and that it was hugely influential in the early survival of the *S. arabicus* seedlings. Additionally, in this experiment, it was highly interactive with moisture in at least some nutrient treatments. That interaction was most pronounced in the 48-ml: 12.3-ppm treatment. Specifically, five of the shortest mean
survival times were in that treatment, and all five were significantly lower than their 96-ml: 12.3-ppm counterparts.

The importance of nitrogen in plant growth is not surprising, as it is a topic that has been extensively discussed in the literature. However, the degree to which the slight increases in soil nitrogen influenced the growth and survival of seedlings in this study was somewhat surprising. The main reason the result was unexpected was that the 12.3 ppm nitrogen treatment was selected based on levels of nutrients found in canopy drip line habitats at the Mojave Desert Flame site, which is well populated with *S. arabicus* individuals. However, the results of this study suggest that both the 12.3 ppm and 40.0 ppm nutrient treatments negatively influenced *S. arabicus* survival and growth measures. The mean survival times generated in this project were significantly influenced by the three-way interaction between moisture, nutrients, and shade. In the 48 ml moisture treatment, all of the specimens are in a moisture regime which was close to their minimal water requirement for growth. When they experienced the increase in nitrogen from 5.7 ppm to 12.3 ppm, the plant was forced to deal with increased nitrogen stress while not having enough moisture to process or mitigate damage. The result was shorter survival time, as observed in the 48-ml: 12.3-ppm treatment. Conversely, the specimens in the 96-ml: 12.3-ppm treatment also had a reduction in survival time as they incurred some stress due to increased nitrogen levels, but the greater water availability allowed them to mitigate the damages to some degree. In the 40.0 ppm nutrient treatments of both moisture regimes, specimens in the two lowest shade conditions (no shade cloth or 30% shade cloth) exhibited roughly equal means with their counterparts in the other moisture treatment, suggesting that shade did not provide a sufficient buffer to offset nitrogen stress through reduction in water stress, which would have
resulted in longer survival times than their 48ml treatment counterparts. The difference between the 96-ml: 40.0-ppm: 50% shade-cloth treatment and the two lower shade treatments may be that the 30% and open shade regimes were not shady enough to maintain a sufficient moisture buffer near the soil, and therefore enough moisture was able to evaporate that even the advantage provided in the higher moisture treatment was not enough to counter the damages caused by such a large concentration of nitrogen.

The degree of shade exerts its influence on the growth and survival means through its influence on soil temperature, plant temperature, plant respiration rates, rate of soil moisture retention, light exposure and other physical habitat changes related to light reduction. The three-way interaction between moisture, nutrients and shade was most readily observed through the differences in mean survival times for the 50% shade cloth treatments in the 12.3 ppm and 40.0 ppm nutrient regimes relative to the survival means for the 48-ml: 50% shade-cloth treatments in the same nutrient treatments. In those treatments, the influence of shade yielded the opposite survival response between moisture treatments in the 12.3 ppm nitrogen treatment. It is possible that the longer survival means were the result of high moisture levels in the 96 ml treatment providing enough water to reduce water stress and compensate for some of the heavy nitrogen stresses faced by the plant. However, there was not enough moisture to compensate for increased nitrogen stress caused by the high nitrogen levels in the 48 ml treatment, so the increased nitrogen stress was added to the existing moisture stress. It is also possible that in the 96-ml: 50% shade-cloth treatment, there was enough moisture to create a buffer, allowing the specimens to further reduce water stress through the maintenance of more optimal moisture conditions between water events.
Conversely, it is possible that individuals in the 48-ml: 50% shade-cloth treatments were also able to create a moisture buffer due to their high shade conditions. However, as they have access to half of the water seedlings in the 96 ml treatment do, the high shade treatment may have created just enough of a moisture buffer to establish and maintain soil moisture at a level sufficient to make more of the nitrogen available in the soil. If that did occur, the seedlings may have actually been subjected to an even more hostile nitrogen environment than their moisture buffer created, but they would not have had access to the additional water needed to compensate for the increased nitrogen stress. However, monitoring of the ambient temperature and relative humidity inside and outside of shade enclosures did not yield any significant differences between shade treatments. So, if a moisture buffer was created, it would have had to have been extremely close to the soil surface.

Those findings differed from that found in Brooks (2003), who found that increases in both water and nitrogen resulted in increased growth of Mojave Desert invasive annuals. Possible explanations for the contrasting results include different soil texture and watering regimes, which combined to influence nitrogen mineralization and availability rates. In addition, the form of nitrogen applied in each experiment, differences in the timing of fertilizer applications relative to *S. arabicus*’ life cycle, and the single, large watering event applied to the site likely created markedly different soil nitrogen and moisture conditions than those in this project. Additionally, the innumerable differences which exist between the Ames, Iowa greenhouse in which this project was conducted, and the Mojave Desert sites Brooks used may have contributed to the different results observed. One large difference between this experiment and that of Brooks (2003) was that Brooks including the presence of
competition within and among species, which may help explain the different responses of *S. arabicus* to increased nitrogen between studies.

When those results are combined with the marked preference *S. arabicus* specimens demonstrated for the interspace no-shade conditions in all growth measures and applied to the Mojave Desert Flame site, the results may suggest that under high moisture conditions, *S. arabicus* individuals may be more able to tolerate the elevated nitrogen levels present in the canopy-drip-line/near-canopy microhabitats. However, results of the survival time analysis suggest that in dryer years (48 ml treatment), individuals would have extremely short survival times in even the modest nitrogen concentrations found around the canopy drip-line. Instead, it is likely that there would be a strong preference demonstrated by individuals for the interspace microhabitat conditions. However, given the fact that some *S. arabicus* individuals have the ability to complete their life cycle from sprouting to senescence in around two weeks under harsh conditions, the shortened survival time in the moisture conditions which mimicked a typical rain year may still be sufficient for some individuals in the near-canopy/canopy-drip-line habitat to complete their life cycle despite their location in the less preferable microhabitat location.

It is generally believed that the primary shade: nutrient relationship is through the influence of shade on moisture, which in turn influences soil nitrification and mineralization. Such relationships between shade, moisture and nutrients have been well established. However, research into other aspects of the soil microflora and microfauna community suggest that in many habitats, the current understanding of the microhabitat relationships to nutrient cycling and local biological communities is incomplete or overly simplistic. Deserts are areas which are traditionally relatively poorly understood and sparsely studied. Further
complicating the assimilation of available knowledge is an increasing number of studies which are beginning to find that there are many complex interactions which exert previously unknown or under estimated influences on anything from single aspects of the environment, to major ecosystem functions which were believed to be more or less understood (i.e., influence of biological crusts on nutrient availability, the role of competition between annuals in fertility island communities, etc.).

While the results in this experiment demonstrate the presence of a strong moisture: nutrient: shade interaction which influences survival, there are many possible mechanisms through which the survival trends may have interacted to yield the results in this project. However, there is relatively little data available concerning any two of the three fixed effects, and while some two-way relationships between shade, nitrogen and moisture have been explored, they are fairly limited in number, poorly understood in desert communities, and are rarely available in relation to specific plant species. Potentially of greater importance is the fact that the experiments are usually qualitative rather than calculated to mimic field conditions. In addition, most of the studies occur in the field, which allows for inter and interspecies competition. Experiments are usually qualitative, rather than calculated to mimic field conditions.

Additionally, studies of the relationships or interactions that those fixed effects have are practically guaranteed to be further complicated by influences from native microfauna (i.e. moss, lichens, biological soil crusts) and microbial communities, which can exert large influences on nutrient availability, are very poorly understood. In many regards, this study is unique amongst studies of fertility island and plant ecology in desert habitats. While studies researching the influences of one or two selected microhabitat features on survival or growth
responses of selected desert plants are not new, very few address the research questions through multivariate studies in order to address the multiplicative nature of biological influences. To the best of the authors’ knowledge, no previous study has been attempted to identify the influences and interactions between three prominent habitat features on *Schismus arabicus* survival and growth at treatment levels based on the actual concentrations present in fertility island microhabitats of interest. Additionally, the authors were unable to find any studies near this scale which attempted to address the previously stated aim in controlled conditions and in the absence of competition.

The results of this study suggest that *S. arabicus* can compensate for a certain amount of the stress caused by increased nitrogen by reducing water stress in some way so that the total stress the plant is under does not exceed what it can survive. While the creation of a moisture buffer near the soil surface in high shade and high moisture treatments may be sufficient for individuals to survive in the 40.0 ppm nitrogen treatments typical of the under-canopy fertility island microhabitats, the average volume of precipitation which falls during a 50 day period in the Mojave Desert over the growing season is approximately half of that received over the same amount of time in the 96 ml treatment in this experiment; because of that, the moisture available to *Schismus* individuals during the typical growing season in the Mojave Desert is not sufficient for *S. arabicus* survival in in the under canopy microhabitat during a year with average or low rainfall.

Two major implications may be drawn from the results of this study. The first (1) is that it demonstrates a preference for survival and growth of *S. arabicus* in interspace microhabitat conditions which may provide an explanation for the distribution of *S. arabicus* detailed in Schaffer et al. study (2012) independent of any competitive or allelopathic
influences. Under the conditions experienced in this study, the increased nitrogen availability under shrub canopies did not benefit, but rather hindered early *S. arabicus* seedling survival. In addition, the very wet 2010-2011 growing season may have provided sufficient moisture to create conditions in the shrub interspace at the Mojave Desert Flame study site which were similar to those experienced in the 96-ml: 5.7-ppm treatment; that treatment produced the largest growth means for every measure except BGBM. Given that, it is not only possible, but likely that unfavorable soil nitrogen conditions under the shrub canopy influenced the predominantly interspace distribution observed in the 2011 Desert Flame study by favoring the low nitrogen interspaces for both survival and growth. While the microhabitat explanation does not preclude the possibility that allelopathy or significant competition with creosote bushes was occurring, it does provide an independent explanation using the results produced through an experiment which was literally tailor made to assess that possibility, by using treatment levels calculated for that site, and seeds from the plant in question. Furthermore, the possible existence and influence of allelopathy or competition as proposed in Schaffer *et al.* (2012) is irrelevant with regards to the question of *Schismus* distribution into the shrub interspace; based on the results of this study, *S. arabicus* would end up demonstrating a strong preference for the interspace microhabitat even if allelopathy or significant competition with *L. tridentata* was totally absent given the overwhelming influence of other microhabitat conditions favoring interspace microhabitat conditions for survival and growth of *S. arabicus*.

The second major implication which that can be drawn from this study is that (2) the strong influence of no shade cloth, particularly in the 5.7 ppm nitrogen treatments, has serious implications for the role of *S. arabicus* plants in the spread of fires in creosote bush...
fertility island landscapes. *S. arabicus* plants demonstrated their greatest survival and growth responses in the 5.7 ppm: no-shade-cloth and 5.7-ppm: 30% shade-cloth treatments, which roughly simulated the interspace and near-canopy/canopy-dripline microhabitats. When the results are put into the context of the fertility island landscape, they suggest that *S. arabicus* individuals prefer growing from just below the canopy edge in one fertility island, through the previously inhospitable and baron no-mans-land known as the shrub interspace, to just below the edge of another creosote bush in a different fertility island. While the shrub interspace has historically acted as a natural firebreak since virtually nothing grew there to catch fire, it is now the preferred habitat for an aggressive, exotic invasive grass which survives longest and grows largest in that microhabitat. By growing from shrub to shrub through the interspaces, *S. arabicus* has created a continuous fuel bed, linking previously isolated Fertility Islands in a poorly fire adapted landscape. And not only are the Fertility Islands being simply linked, but they are being linked by a plant that grows biggest and survives best in the interspace, facilitating *S. arabicus* to max out its potential as a fuel spreader and fuel load during fire events.

While no studies experimentally examining three-way interactions between specific microhabitat features at multiple feature levels could be found in the literature, the general combinations of major microhabitat conditions found to promote or hinder growth in this study are largely consistent with the available *S. arabicus* growth and survival data. The preference demonstrated for open shade treatments found in this study, even given that the same soil and moisture microhabitat were created under different shade conditions in the study, supports the fact that *S. arabicus* is shade intolerant (Brooks 1998 *in: Sanches-Flores* 2007). Similarly, increased *S. arabicus* biomass in dry, open conditions, when irrigation
treatments were applied, also coincides with the high growth responses in open, high moisture conditions (Gutierrez 1992).

The preference *S. arabicus* has demonstrated for the dry, sandy, interspace soils has been well established (Halvorson 2003). However, its dominance as an interspace/open area grass is usually associated with its traits as a successful competitor and invader (Brooks 2000; Brooks 2003; Halvorson 2003; Sanches-Flores 2007) rather than its preference for interspace microhabitat conditions. Conversely, this study found that the microhabitat features assessed were of at least equal influence on *S. arabicus* distribution as possible competition with the central fertility island shrub. Furthermore, potential explanations for those results include the possibility that the number of leaves at termination is related to an unaddressed variable(s). Potentially, transition stress, damage from transplanting, or the cessation of watering at the beginning of the dry phase may be responsible. Similarly, the criteria used to distinguish living from dead plants in the survivorship measurements may have been insufficient, and many individuals which were marked as alive over many observation events actually died early due to causes unrelated to their treatment assignment.

While it is easy to see the numerous ways in which the application of the results concerning the multivariate influences of microhabitat condition on survival and growth of *S. arabicus* in this study would be useful if applied to various landscapes or populations, particularly given how disjointed and inconsistent most relevant data is between studies, the actual scope of this project is quite limited. This study only assessed survival and growth of individuals from a single Mojave Desert location, with a very limited number of treatments levels, and only considered three of the innumerable potential fixed effects. As such, the
results of this study should only be applied to the Desert Flame study site from which the seeds, soil and microhabitat feature values were collected.

However, even when it is only applied to the Mojave Desert Flame S. arabicus population, the low number of replicates and limited number of variables considered limit its utility in many contexts. In addition, the high seedling mortality which occurred during the experiment was not evenly distributed across the treatments. As such, the applicability of the many of the mean growth responses in this study should be limited to use as very general references about the potential response of growth measures to a similar treatment, or used for establishing treatments in future studies. Despite the drawbacks of reducing replicate numbers when the results were analyzed separately by survivorship groups, the fixed effects which exerted strong influences on survival, but which were not particularly useful for explaining variance in growth responses, no longer obscured the results of growth-related analyses by producing inconsistencies between tests. As a result, both the explanatory ability of the LMM and GLMM models for growth responses and the consistency of the results between analytical assessments were greatly improved.

However, the most immediate issues limiting a robust assessment of how our results fit into the literature is the lack of similar studies addressing the multiplicative nature of the microhabitat features on S. arabicus, and the low number of replicates in analysis of growth measures. Those issues seriously limit the conclusions which may be reasonably drawn for the local Schismus arabicus populations. Furthermore, several studies have found that responses of vegetation in arid environments to moisture and nitrogen are variable (Fisher et al. 1987), and the complex interactions between the three common microhabitat features of fertility island landscapes found in this study provide further evidence that the growth and
survival of a given *Schismus arabicus* individual is highly context dependent. In addition, it is already known that the distributions of *S. arabicus* in creosote bush fertility island habitats is not universal, as documented in Schafer *et al.* (2012), when *Schismus* and other invasive and native plant abundances were assessed by fertility island microhabitats at different sites and in different deserts. For those reasons, the results of this study should not be applied beyond the scope of this experiment. However, the results of this study do highlight a number of interesting directions for future research.

Given both the limitations of the current data on the ecology of Fertility Island landscapes which are dominated by *S. arabicus*, future studies aiming to address the gap in the literature with additional multivariate studies could be focused in one of five directions. The first direction of future research should be focused on (1) working towards understanding similarities and differences in microhabitat interactions with key plants across landscapes through performing similar studies in multiple sites throughout the landscape. As previously discussed, this is likely the first study to address the interactions of key microhabitat features in using the multivariate, non-field approach, and as such there is no replicate data for the same site, or similar data for other local sites with which general trends may be identified. Conversely, there is a large amount of data indicating interactions and processes may differ between sites. The second direction of future research should be (2) gathering more physical site data for the various microhabitats, particularly those related to nutrient concentrations, nutrient cycling, and microbial and microflora composition. This may prove to be key in beginning to understand the mechanisms behind the influences observed in the studies, and could allow for the incorporation those features into models. Exploring the specific microhabitat feature interactions and influences on *S. arabicus*
survival and growth are warranted, particularly given the influence of nitrogen and shade demonstrated in this study, and the increased complexity of nutrients, microbes, microflaura and moisture interactions which are just beginning to be found in desert habitats. The final direction in which future research should go is towards (4) starting to address how interactions between microhabitat conditions and *S. arabicus* survival and growth are influenced by inter-and intraspecific competition, as that is a major feature of desert landscapes, but based on this study, potentially not as influential relative to habitat conditions as it is credited to be in the current literature. In all future studies, it is necessary to work with a greater number of replicates so that the analyses of growth measures can be more robust.

And finally, (5) the relationships between the central fertility island shrub and microhabitat variable concentrations to *S. arabicus* growth should be assessed at different sites throughout its range in the southwestern United States. Those relationships are key for creating accurate fire effect models, and for being able to effectively link shade and microhabitat conditions to aerial photography or satellite imagery of a site of interest. Given the results of this study, for the goal of using aerial or satellite imagery to model *S. arabicus*, the link between soil nitrogen and shade would be of particular interest. Clearly, there are numerous factors that would have to be determined before such a model could be created, including determination of site-specific microhabitat feature baseline levels, and local plant presence and interactions. However, utilizing remote sensing to evaluate the potential effect of a fire, or other disturbance, on a *S. arabicus* populations could substantially reduce the time and money associated with large field and greenhouse studies, while allowing land managers or decision makers to identify areas of risk, high conservation potential, or to predicted outcome of a disturbance or fire event. While the results of this study identified a
number of microhabitats which could act as a starting point for such a model at the Mojave Desert Flame site, there are numerous additional microhabitat, anthropogenic and biological factors that should be addressed in future research.
Literature Cited


Figure 6. Fertility island with microhabitat zones and resource levels annotated. Three nitrogen totals taken from each of the 25 shrubs photographed for percent shade at the Mojave Desert Flame site in March 2013. Soil nitrogen data was taken from plant root simulator probes placed and analyzed for Desert flame study at the Mojave site at a depth of 5-10cm between January 18 and March 22, 2011. Nitrogen mg totals calculated by applying flow-analyzer results of nitrate in interspace soil collected at Mojave Desert Flame site in March 2013. Soil moisture description: qualitative description from observations in the literature. Dark green plant=invasive, non-native species. Light green plant=native annual vegetation.
Figure 7. Mean abundance of living native, total invasive or invasive *Schismus arabicus* winter annual individuals per plot by microhabitat during a late March 2011 spring reproductive census at a (A) Mojave and (B) Sonoran Desert site. Total invasive includes *Schismus arabicus* individuals. Microhabitat abbreviations: UC = under canopy; CD = canopy drip-line; ON = open near; OF = open far. Data from 2011 Desert Flame research.
Figure 8. Split-plot design of experiment. A: arrangement of whole plots in experiment; dark gray=high shade; light gray=medium shade; white=open shade; block 12=cheese cloth control block. C: dark green fill=high nitrogen; medium green fill=medium nitrogen; light green fill=low nitrogen; dark blue outline=high moisture; light blue outline=low moisture.
Table 1. Highest level\(^1\) statistically significant (p<0.05) single or interacting fixed effects in mixed effects models of the survival and growth responses of *Schismus arabicus* individuals to varied moisture, nutrient and shade regimes over a 50 day experimental period.

<table>
<thead>
<tr>
<th>Response</th>
<th>Variable</th>
<th>Fixed effects</th>
<th>Est.</th>
<th>St. Error</th>
<th>(z) value(^2) / (t) value(^3)</th>
<th>Est. St. Error of (z)(^2) or (t)(^3) value</th>
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</thead>
<tbody>
<tr>
<td>Survival</td>
<td>Days alive all specimens</td>
<td>48ml: 5.7ppm: No shade cloth</td>
<td>3.573</td>
<td>0.139</td>
<td>25.731</td>
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<td></td>
<td></td>
<td>96ml: 12.3ppm: 30% SC</td>
<td>-0.805</td>
<td>0.209</td>
<td>-3.847</td>
<td>&lt;0.001</td>
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<td></td>
<td></td>
<td>96ml: 40.0ppm: 30% SC</td>
<td>-0.509</td>
<td>0.206</td>
<td>-2.465</td>
<td>0.013</td>
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<tr>
<td></td>
<td></td>
<td>96ml: 12.3ppm: 50% SC</td>
<td>-0.739</td>
<td>0.212</td>
<td>-3.481</td>
<td>&lt;0.001</td>
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<td></td>
<td>96ml: 40.0ppm: 50% SC</td>
<td>0.989</td>
<td>0.221</td>
<td>4.479</td>
<td>&lt;0.001</td>
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<tr>
<td>Growth</td>
<td>Leaf # all specimens</td>
<td>48ml: 5.7ppm: No shade cloth</td>
<td>1.837</td>
<td>0.200</td>
<td>9.173</td>
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<td></td>
<td></td>
<td>96ml: 12.3ppm</td>
<td>1.325</td>
<td>0.419</td>
<td>3.164</td>
<td>0.002</td>
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<td></td>
<td>12.3ppm: 30% SC</td>
<td>1.117</td>
<td>0.566</td>
<td>1.972</td>
<td>0.049</td>
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<td>Leaf # survivors</td>
<td>48ml: 5.7ppm: No shade cloth</td>
<td>2.342</td>
<td>0.139</td>
<td>16.887</td>
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<td></td>
<td></td>
<td>96ml</td>
<td>0.443</td>
<td>0.178</td>
<td>2.494</td>
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<td></td>
<td></td>
<td>12.3ppm</td>
<td>-0.458</td>
<td>0.192</td>
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<td></td>
<td></td>
<td>50% SC</td>
<td>-1.300</td>
<td>0.279</td>
<td>-4.654</td>
<td>&lt;0.001</td>
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<tr>
<td></td>
<td>Leaf # non survivors</td>
<td>48ml: 5.7ppm: No shade cloth</td>
<td>5.774(^{-1})</td>
<td>5.774(^{-1})</td>
<td>0.000</td>
<td>1.000</td>
</tr>
<tr>
<td></td>
<td>AG: BGBM survivors</td>
<td>48ml: 5.7ppm: No shade cloth</td>
<td>5.709</td>
<td>1.170</td>
<td>27.823</td>
<td>&lt;0.001</td>
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<td>-3.242</td>
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<td></td>
<td>96ml: 50% SC</td>
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<td>-4.088</td>
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<td></td>
<td></td>
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<td></td>
<td>40.0ppm: 50% SC</td>
<td>7.993</td>
<td>3.841</td>
<td>2.081</td>
<td>0.049</td>
</tr>
<tr>
<td></td>
<td>BGBM survivors</td>
<td>48ml: 5.7ppm: No shade cloth</td>
<td>-0.114</td>
<td>0.043</td>
<td>-2.669</td>
<td>0.013</td>
</tr>
</tbody>
</table>

\(^1\) Interactions between more fixed effects render interactions between fewer fixed effects irrelevant. For that reason, only model results which were significant (p<0.05) and had the greatest number of interacting fixed effects for the given test are presented.

\(^2\) Results of generalized linear mixed effect model.

\(^3\) Results of linear mixed effect model.

Bolded, the model intercept to which all other estimate values are in reference.

Est, estimate. The estimated mean based on observation data of the variable in question relative to the model intercept.

Fixed effects: Moisture, total deionized water received over the course of the 50 day experimental phase; Shade, SC (shade cloth); Nutrients, ppm soil nitrogen (NH\(_4\)NO\(_3\)).

Variable: Leaf number, number of leaves at death or termination (end of 50 day experimental phase); Survivors, specimens which survived until terminated at day 50; Non survivors, died before day 50; AG, above ground biomass (mg); BGBM, below ground biomass (mg).
Table 2. Selected results of likelihood ratio tests with model variance explanatory values of interest for single or interactions between fixed effects in generalized linear or linear mixed effects models of *Schismus arabicus* responses to varied moisture, shade and nitrogen regimes.

<table>
<thead>
<tr>
<th>Response Variable</th>
<th>Interaction or Fixed Effect</th>
<th>DF / DenDF</th>
<th>Sum of Squares</th>
<th>Mean Square</th>
<th>F value</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Survival</td>
<td>Days alive all</td>
<td>Moisture</td>
<td>1</td>
<td>73.73</td>
<td>73.730</td>
<td>73.730</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Nutrient</td>
<td>2</td>
<td>389.93</td>
<td>194.966</td>
<td>194.966</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Moisture: Nutrient</td>
<td>2</td>
<td>99.13</td>
<td>49.565</td>
<td>49.565</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Nutrient: Shade</td>
<td>4</td>
<td>30.35</td>
<td>7.588</td>
<td>7.588</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Moisture: Nutrient: Shade</td>
<td>4</td>
<td>76.54</td>
<td>19.136</td>
<td>19.136</td>
</tr>
<tr>
<td>Growth</td>
<td>Leaf number all</td>
<td>Moisture: Nutrient</td>
<td>2</td>
<td>10.556</td>
<td>5.278</td>
<td>5.278</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Nutrient: Shade</td>
<td>2</td>
<td>2.083</td>
<td>1.041</td>
<td>1.041</td>
</tr>
<tr>
<td>Leaf number</td>
<td>survivors</td>
<td>Moisture</td>
<td>1</td>
<td>1.214</td>
<td>1.214</td>
<td>1.214</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Nutrient</td>
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<td>1.433</td>
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<tr>
<td></td>
<td></td>
<td>Shade</td>
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<td>100.942</td>
<td>50.471</td>
<td>50.471</td>
</tr>
<tr>
<td>AGBM</td>
<td>survivors</td>
<td>Shade</td>
<td>2</td>
<td>338.97</td>
<td>169.485</td>
<td>15.048</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>21.609</td>
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</tr>
</tbody>
</table>

DF, numerical degrees of freedom; DenDF, denominator degrees of freedom.
All, calculated using data from all specimens regardless of when they died; Survivor, lived through entire experimental growth phase (50 days).
Moisture, total deionized water received over the course of the experiment growth phase; Nutrient, soil nitrogen (NH$_4$NO$_3$), Shade: % shade cloth.
Figure 9. The influence of shade treatment on survival time (days) of *Schismus arabicus* individuals by soil nitrogen and moisture treatment means. Maximum survival days at termination = 50. Moisture: total deionized water received over the course of the growth phase of the experiment. Shade: SC = shade cloth. Soil-N Treatment: ppm soil nitrogen (NH\text{$_4$}NO$_3$).
Figure 10. The influence of soil nitrogen treatment on the number of leaves at death or termination of all Schismus arabicus specimens when shade and moisture treatments were averaged by nutrient treatment level. Maximum survival days at termination = 50 days. Moisture: total deionized water received over the course of the 50 day growth phase of the experiment. Shade: SC = shade cloth. Nutrient: ppm soil nitrogen (NH₄NO₃).
Figure 11. The influence of shade treatment on the final number of leaves at termination for *Schismus arabicus* individuals which survived to project termination on day 50. Mean leaf numbers for shade and moisture treatments were averaged by nutrient treatment. Moisture: total deionized water received over the course of the 50-day growth phase of the experiment. Shade: SC = shade cloth. Nutrient: ppm soil nitrogen (NH$_4$NO$_3$).
Figure 12. Relationship between above ground and below ground biomass of *Schismus arabicus* individuals which survived to project termination at day 50 of the experimental growth phase by shade treatment. Blue = no shade cloth; Pink = 30% shade cloth; Green = 50% shade cloth.
CHAPTER 3.

METHODS FOR GERMINATION OF AN INVASIVE DESERT ANNUAL, 
*SCHISMUS ARABICUS*, FROM SEED BANK SOIL

A paper to be submitted to Seed Science Research

Sarah E. Emeterio, Erika L. Mudrak and Kirk A. Moloney

Abstract

The seedling emergence method can be modified to target the germination of a specific plant species from seed bank soil in greenhouse conditions if the environmental conditions required for germination are known. *Schismus arabicus* is an invasive desert annual which has proven difficult to germinate from seedbank soil in greenhouse conditions. Its germination response has been tied to light, moisture and temperature conditions in the locations where it germinates in the wild, but for various studies which may occur outside of the range in which it grows, the distance between the experimental site and where it naturally grows, in addition to large seed processing and handling times make using seeds gathered from the wild growth sites extremely inefficient, or even impossible for many studies. The purpose of this paper is to create a set of simple, and time effective methods for germinating a large number of *S. arabicus* seedlings from collected seed bank soils. Soils were subjected to a combination of three shade levels (high, low or none), five moisture levels (3ml, 6ml, 9ml, 12ml, or 15ml/watering event), and two watering periodicities (every day or every other day). Within periodicities, the relationship between total seedlings and time to first seedling was determined using generalized linear mixed effect analysis. Total seedling emergence was highest in the light shade-9 ml/every other day moisture treatment. Time to emergence of first seedling was significantly longer in low moisture treatments, with the longest mean time
of 21.8 days in the 3 ml/every two days-open shade treatment. The shortest time to
emergence was 6.33 days in the 15 ml/every day-high shade treatment. Within moisture
treatments receiving the same total moisture, the total number of seedlings was significantly
lower than all other treatments in the open shade-low moisture (84ml)-every other day
watering treatment at 4.9 seedlings. Results indicate that an optimal moisture threshold must
be met and maintained for high abundances of seedling emergence. The influences of shade
and watering periodicities were limited to their role in reaching and maintaining the optimal
soil moisture level. As long as the optimal soil moisture level is reached, there is a range of
moisture and shade treatments that will generate approximately the same high level of
germination. The simplest, cheapest and most time effective method for germinating *S.
*arabicus* from seed bank soil was to apply 9, 12 or 15 ml of water per 40 g of soil every other
day in open shade conditions.

**Introduction**

**Background and Objectives**

The seedling-emergence method is commonly used to estimate the composition of
seed bank soils (Brown 1992; Ter Heerd et al. 1996; Ter Heerd Schutter and Bakker 1999).
In this method, samples of seed bank soil are spread out in a greenhouse, subjected to a
watering regime, and the identity and abundance of the different seedlings which emerge is
determined (Ter Heerdt et al. 1996; Ter Heerdt, Schutter and Bakker 1999). As the goal of
such studies is usually to germinate as many individuals of as many species as possible, it is
essential that the greenhouse and watering conditions meet the germination requirements of
as many of the species as possible (Ter Heerdt Schutter and Bakker 1999). In addition to its
traditional use for estimating seed bank composition, the seedling-emergence method also allows for observations of plant development after emergence, and can be tailored for studies interested in the early growth and development of various species. It can be further modified to explicitly test other objectives as well, such as (1) identification of ideal environmental and water requirements for germination of a specific species, or to (2) cultivate a large number of seedlings of a single species for use in a subsequent study.

The collection and processing of seeds for many species is logistically challenging due to small windows of time when mature seeds can be collected, or the distance between field and laboratory sites. Seed collection and processing may also be extremely labor intensive due to source location or seed size. In addition, seeds of many plants, such as many desert annuals, have dry storage requirements, which add additional time between the collection of seeds from parent plants and when they can successfully germinate in the lab. For those and other reasons, the ability to germinate a targeted species from seed bank soil is one way to greatly reduce the time and uncertainty involved with seed collection for some species.

Germination requirements (i.e. light intensity, day length, temperature, soil moisture, etc.) often differ between species, and the requirements of many species are entirely or partially unknown (Dyer 1995; Ter Heerdt, Schutter and Bakker 1999). As there are often a complex suite of conditions required for germination, identifying the specific conditions necessary for maximum seedling emergence of the target species is necessary for the seedling emergence method to be successful and reliably used in this manner. One group of plants that have proved difficult to germinate from seed bank soil in unpublished pilot studies, and through personal communications with others working on similar studies are
invasive desert winter annuals, including *Schismus arabicus* (Nees) (Pers. comm. Hadas Prada, Pers. comm. Lauren Sullivan). This is likely due to complex germination regulation strategies employed by those plants in order to prevent all seeds in the soil from germinating after a single sufficient rainfall (Gremer and Venable 2014; Gutterman 1994, 1996a, 1996b). However, despite the difficulty of cultivating this group of plants outside of their wild range, their extreme influence on the distribution of native annuals, resources, and fire regimes in the Sonoran and Mojave Deserts of the Southwestern United States warrants further investigation of the growth conditions required for those species (Brooks 2000; Brooks et al. 2004; Esque et al. 2010; Levine et al. 2003; Melgoza, Nowak and Tausch 1990). As a greater understanding of the ecology of these plants may be necessary for accurate distribution and fire effects modeling, and effective conservation actions (Levine *et al.* 2003, Williamson and Fitter 1996), developing a set of simple, inexpensive, low-labor methods, which yield larger numbers of the target species seedlings would help make species-specific studies more feasible.

The objective of this study was to develop a set of simple, time efficient, and effective methods for germinating one invasive desert annual, *S. arabicus* (Nees), from seed bank soil in order to facilitate further studies of a key invasive desert annual species away from its invaded habitat. To the best of the authors’ knowledge, no papers have been published on methods for *S. arabicus* germination from seed bank soil. Three specific research questions were addressed in this study: (1) what watering and shade regime combinations resulted in the greatest abundance of *S. arabicus* seedling emergence from seed bank soil under greenhouse conditions? (2) Are there differences in the timing of seedling emergence due to differences in watering and shade regime combinations? And, (3) does the
periodicity of watering events (every day, or every other day) influence the abundance or timing of *S. arabicus* seedling emergence? Based largely on the results of informal pilot studies, the hypothesis was that the greatest germination would occur in high shade, high moisture treatments watered on alternant days.

**Target Species**

*S. arabicus* is an invasive winter annual dominant in the Mojave and Sonoran Deserts of the Southwestern United States (Brooks 2000; Esque et al. 2010; Levine et al. 2003) that is native to the Middle Eastern and Mediterranean regions (GISD 2005; Gutterman 1996a, 1996b). *S. arabicus* reproduces solely by seeds (Brooks 2000), with most germination occurring in late fall to early winter following sufficient rainfall of approximately one cm (Gutterman 1996a, 1996b). The tiny size of the caryopses (seeds) allows for wind dispersal (Gutterman 1996a), but makes processing them for cultivation difficult.

Information available on *S. arabicus* germination in laboratory conditions is largely due to the work of Yitzchak Gutterman. Gutterman found that the germinability of *S. arabicus* seeds was influenced by the day length during its maturation (1996a), which helps regulate germination so that the entire *S. arabicus* seedbank does not germinate following a single sufficient rain event. An after-ripening period of high temperatures was also found necessary for germination, the purpose of which is likely to prevent seeds from germinating when conditions are too hot and dry for seedling survival (Gutterman 1996b). Further studies found that seeds that matured on plants during long-days (further into the spring) demonstrated higher levels and more rapid germination than their shorter-day counterparts (Gutterman 1996a). Temperature and light during wetting also influenced germination, with
increased time to the start of germination as temperatures decreased from 20º C. For temperatures at or below 20º C, high light conditions suppressed germination, while dark conditions resulted in increased germination. At higher temperatures (25º C and 30º C), the percentages of seeds that germinated were low regardless of light condition (Gutterman 1996a). The highest percentage of germination overall was found at 15º C in dark conditions (Gutterman 1996a, 1996b).

**Methods**

In this study, the abundance and timing of seedling emergence are the response variables of interest. While seedling emergence is the completion of germination, rather than the actual germination process, the terms germination and seedling emergence are used interchangeably here.

**Experimental Design and Variables**

I conducted a germination experiment between January 21 (day 0) and February 18, 2014 (day 28) in the Iowa State University Greenhouse in Ames, Iowa. The experiment utilized a split-split plot design, in which shade treatment was applied to main plots, watering schedule was applied to two subplots within each main plot, and moisture treatment (i.e. ml per watering event) was randomly applied to sub-subplots within each subplot (Figure 13). Nine replicates of each treatment combination were used. All 27 blocks (9 per shade treatment) were arranged on the same greenhouse bench (Figure 13). Each main plot was surrounded by a shade enclosure, comprised of four- 4” tall posts to which 50% shade cloth (high shade), cheesecloth (light shade), or nothing (open shade) was affixed. The shade cloth
and cheesecloth completely covered the top and all four sides of the enclosures. Within each block there were two subplots, one of which was watered daily, while the other was watered every other day. Within each subplot, each of the five cells of the 1020 seedling tray insert was randomly assigned one of the five moisture treatments levels. The watering treatments were 3, 6, 9, 12 and 15 ml deionized water per watering event.

During analysis, the subplots were considered separately due to the differences in total moisture received by each moisture treatment over the course of the entire project. In subplots assigned to the daily watering schedule, cells underwent 28 watering events. In the every other day watering schedule, cells underwent 14 watering events. Cells were watered with deionized water with a needleless syringe daily between 2 pm and 4 pm throughout the course of the experiment. During this time, all newly emerged seedlings were counted and removed daily, and the total number of *S. arabicus* seedlings were recorded for each cell by day. Care was taken to remove as much of the seedling root as possible in order to reduce any fertilization effects through decomposition of root material. However, minimizing disturbance to the soil surface during seedling extraction was given priority over extraction of all of the root material.

**Soil Collection**

The seedbank soil used in this experiment was collected from sites of known *S. arabicus* growth in the Mojave and Sonoran Deserts. The Mojave site was located on Fort Irwin National Training Center, north of Barstow California (35° 9’ 21” N, 116° 53’ 6” W). The site was on an east facing bajada with “coarse-loamy, mixed, superactive, hyperthermic, Typic Haplicalcis” soils. The soils are characterized by low to very low runoff, which are
“somewhat excessively drained” or “well drained” (USDA 2005). The mean annual rainfall between 1973 and 2006 was 147 mm, which fell mostly during the winter and spring. The 30 year mean annual temperature was 17.7º C (Data for Goldstone Echo 2, 22 km N of the Mojave study site, Western Regional Climate Center, www.wrcc.dri.edu, in: Schafer et al. 2013).

The Sonoran site was located within the Barry M. Goldwater Range, south of Gila Bend, Arizona (32º 41’ 49” N, 112º 50’ 22” W). Soils are “coarse-loamy, mixed, superactive, hyperthermic, Typic Haplicalcids” with low runoff which are “somewhat excessively drained” (USGS 2005). The mean annual precipitation between 1992 and 2010 was 153 mm, falling predominantly during a summer and a winter wet season. Mean annual temperatures for the same time period were 22.7º C (Data for Gila Bend, AZ, -29 km NNW of the site, Western Regional Climate Center, www.wrcc.dri.edu, in: Schafer et al. 2012).

An extremely wet 2010-2011 growing season (153.9 mm between October 1 and March 31) in the Mojave produced a large yield of S. arabicus individuals. As the fertility of S. arabicus offspring has been linked to environmental conditions during the mother plant’s life (Gutterman 1996a, 2001), the spring 2011 adults were not only abundant, but likely produced high-quality seeds. While the large number of fertile seeds entered the seedbank, the relatively dry fall of 2011 and drought during the spring 2012 growing season (16.0 mm October 1-March 31) provided insufficient moisture for a large portion of the seeds to germinate. The precipitation during the following growing season was also low, with only 32.1 mm falling between October 1, 2012 and March 31, 2013, when the soil was collected.

The Sonoran site also had a dry 2012-2013 growing season, receiving approximately half the rainfall between October 1 and March 31 (42.0 mm) that it did the previous growing
season (82.0 mm). For the purposes of this experiment, this means that the seed bank soils collected at both sites in late March 2013 likely had an extensive and viable *S. arabicus* component that had already experienced the over-summering period necessary for germination.

Seedbank soil was collected from inter-shrub areas that contained several living and dead *S. arabicus* individuals. The top 1-2 cm of soil under and around these plants was collected. Given the maternal effects previously discussed, the possibility that seedlings from seeds which originated in different microhabitats of the fertility island may respond differently to the experimental variables was addressed by collecting additional soil using a nearest-neighbor approach, working from *S. arabicus* patch to *S. arabicus* patch from the inter-shrub space towards the shrub stem. The seed bank soil was stored in the dark at ambient room temperatures until the project began.

**Sample Preparation**

In January 2014, the Sonoran and Mojave seedbank soils were combined in a 50:50 ratio by volume. Observations during pilot studies suggested that the finer texture of Sonoran site soil became nearly impervious to water in the high moisture treatments after four to five watering events, while the coarser texture of the Mojave seedbank soil was so permeable that water added rapidly percolated through the soil in high moisture conditions. However, when the soils were mixed, the moisture was absorbed and retained by the soil in all but the highest moisture treatments, which still lost water out of the bottom of the cells towards the end of the projects. For this reason, the soils were combined for this study.
Replicates were prepared in the cells of standard 1020 propagation trays, the same type which are commonly used for plugs of decorative flowers for home landscaping. Four layers of paper towel, cut to the dimensions of the bottom of the cell, were placed in each cell. Two tablespoons of large-grain vermiculite were added to each cell to help maintain soil moisture between watering events. On top of the vermiculite, four layers of cheesecloth (~1.25in x 2.5in each) were added to prevent soil from running into the vermiculite, while still allowing for water drainage. Forty grams of the mixed seed bank soil was added last, forming a soil layer approximately one cm deep in the cell.

Daily temperatures and relative humidity were monitored under the shade enclosures of each main plot along the bench to establish baseline general microhabitat condition differences between shade treatments. Measurements were taken approximately one inch above the soil surface in the enclosures at the start of germination counts each day using a digital psychrometer. At the end of the project, the percent of photosynthetically active radiation (PAR) reduction experienced inside the shade enclosures compared to that available one inch above the enclosures was calculated. PAR sources were both solar and generated by growth lamps on adjacent benches. Microhabitat condition results were analyzed with linear mixed effect models in R (R Core Team 2014) using the package \texttt{lme4} (Bates et al. 2014). Means for each shade condition were calculated using the package \texttt{lsmeans} (Lenth 2014). Models set temperature, relative humidity and PAR percent reduction as response variables, shade as the fixed effect, and incorporated random error due to differences between main plots and the main plot location on the block location on the bench by writing blocks and zones as random effects.
**Statistical Analysis**

All three research questions were addressed using generalized linear mixed effect analysis of the relationships in question. The analysis was performed using R (R Core Team 2014), *lme4* (Bates et al. 2014) and *lsmeans* (Lenth 2014). As count data was used, models were written using a Poisson family distribution. All results were in the form of the mean predicted response (i.e. total number of seedlings emerged, or day of first seedling emergence) per cell in a given treatment.

First, I address which shade and moisture treatment combinations yielded the greatest number of *S. arabicus* seedlings within the watering schedules, analyzing the relationship between shade and moisture treatment and the total *S. arabicus* germination per cell response. Second, I address the timing of first emergence as it differed between moisture treatments within the same watering schedule by analyzing the relationship between shade and moisture treatments and the day of first *S. arabicus* seedling emergence. For both of these questions, the fixed effects were moisture and shade treatments (with an interaction term), while random effects entered were main plot and subplot nested within main plot.

Finally, I address if the watering schedule influenced the abundance or timing of *S. arabicus* seedling emergence. In this analysis, selected data from the daily and alternant day watering schedules were combined. Results from cells receiving a total of 84 ml of water (3 ml/watering-event in the daily watering schedule, and 6 ml/watering-event in the alternate day watering schedule) or 168 ml of water (6 ml/watering-event in the daily watering schedule, and 12 ml/watering-event in the alternate day watering schedule) were compared within total moistures, between watering schedules. The fixed effects in this model were shade and watering schedule (with an interaction term), with the random effects of plot and
subplot nested within plot. The influences from single or interacting fixed effects were compared to the intercept treatment, which was the 15 ml/daily-watering/high-shade treatment.

Results

Bench Environmental Monitoring

Estimated means for the relative humidity were not significantly different between the 30 or 50% shade treatments (p=1.0000). The mean relative humidity of both were significantly higher than that of the bench (p=0.0001) and the open shade treatment (p=0.0072 and p=0.0082 for the high and light shade treatments, respectively). Means for the open shade treatment (30.5%, SE=1.712, df=6.55, CL=32.74-40.93) and bench conditions (26.8%, SE=1.719, df=6.67, CL=2.67-30.88) did not differ (p=0.1615).

The mean predicted enclosure temperatures were all significantly different from the ambient bench temperature of 13.8°C (p<0.01). The highest mean temperatures were in the light shade enclosures (16.5° C, SE=0.455, df=4.84, CL=15.35-17.71), which differed significantly (p=0.0077) from the open shade enclosures (15.2° C, SE=0.456, df=4.84, CL=13.99-16.35). The high shade mean of 16.0° C (SE=0.455, df=4.84, CL=14.82-17.18) did not differ from either the light or open shade treatments.

Mean PAR above the enclosures was 228.5 units (med.=240.5, Q1=165.2, Q3=274.0). The differences in mean percent reduction were significant for all shade treatment comparisons (p≤0.0001). The greatest reduction was in the high shade treatment (78.36%, SE=2.256, df=10.49, CL=73.36-83.35), followed by an 18.8% reduction in the light
shade enclosures (SE=2.725, df=19.6, CL=13.06-24.45) and the 2.36% reduction in PAR in the open enclosures (SE=2.26, df=10.47, CL=-2.54-7.45).

**Water Schedule 1: watered daily**

Results of the generalized linear mixed effect model (GLMM) indicated that seedling abundance was not significantly influenced by shade treatment, or moisture-shade treatment interactions within the daily watering schedule. However, moisture did exert a significant influence on the total number in the 3 ml (0.6034, SE=0.1552, z=3.888, p=0.0001) and 6 ml (0.4758, SE=0.1589, z=2.995, p=0.0027) moisture treatments. Overall, moisture had a large influence on total seedling emergence, accounting for up to 61.76% of the variance between treatments (SS=61.79, df=4, F=15.45).

Results indicate that all emergence totals clustered into a high emergence or a low emergence group. Regardless of shade, the two lowest moisture treatments promoted a large number of seedlings, while the two highest moisture treatments generated low emergence totals (Figure 14). The middle moisture treatment fell into the high production group in the light and open shade treatments, but clustered with the low emergence group in the high shade treatment (Figure 14). Furthermore, when comparisons of totals between moisture treatments within the high or low emergence groups were made, no significant differences were identified. This suggests that there was no increase in seedling number even if more water was provided in a treatment than the amount which was being received by the lowest moisture treatment in the high production group. However, there were significant differences between mean seedling emergence numbers when those in the high production group were compared to those in the low production group. In terms of the mean number of seedlings
estimated per sample, the highest estimate was for moisture treatment 4 (12 ml/day) in the open shade treatment at 15.0 seedlings (SE = 1.562, CL = 12.232-18.399). The lowest estimate was 5.9 seedlings in the 6 ml/day moisture treatment in the high shade treatment (SE=0.879, CL 4.427-7.922). The fact that the 9 ml treatment switched from the low to the high emergence group as the level of shade decreased indicates that for treatments watered daily, the 9 ml moisture treatment generated soil moistures at the upper limit of an optimal moisture threshold for *S. arabicus* germination.

The GLMM results examining the timing of seedling emergence indicated that moisture treatment explained the most variance in the model (SS=37.363, df=4, F=9.3407), however the effect was only significantly different for the lowest moisture treatment (0.3878, SE=0.1716, z=2.260, p=0.0238). The low explanatory power of shade in the model (SS=5.936, df=2, F=2.9679) was evident in pairwise comparisons of the predicted days of first emergence within each moisture level between shade treatments. Within each individual moisture treatment, there were no significant differences in the day of first seedling emergence between shade treatments. Overall, estimated days of emergence were fairly close, ranging from 6.33 days in the 15 ml/day:high-shade treatment (SE=0.8389, CL=4.89-8.21), to the latest emergence of 11.67 days in the 3 ml/day:open treatment (SE=1.1386, CL=9.64-14.13). In general, emergence of the first seedling occurred sooner in high moisture, shaded treatments, and slightly later in low moisture, less shaded treatments. There were no significant differences in time to first emergence within the high shade treatment. In the light shade treatment, the three earliest emergence times (15 ml and 9 ml [6.78 days, SE=0.8678, CL=5.27-8.71]; 12 ml [7.11 days, SE=0.8889, CL=5.57-9.09]) were significantly sooner than the latest time of 11.44 days (SE=1.1279, CL=9.43-13.88) in the 3
ml/day treatment. Similarly, in the open shade treatment, only the longest (11.67 days, SE=1.139, LCL=9.64-14.13) and shortest (6.67 days, SE=0.8607, CL=5.18-8.59) times to emergence were significantly different (3 ml and 12 ml treatments, respectively).

In summary, for the shade and moisture conditions considered in this section, the highest abundances of seedlings were promoted by low to medium water additions (3-9 ml/day) in open and light shade conditions, and by 3 ml-6 ml of water per day in high shade conditions. However, while the lower moisture treatments promoted high seedling abundances, the time to first emergence was slightly longer than it was for high moisture treatments. The slight increase in time to first emergence for low moisture treatments was more marked in the light and open shade conditions, suggesting a certain level of soil moisture had to be reached before any germination could occur. The reduced germination at high moisture levels, despite a more rapid commencement of germination, strongly suggests that 12 and 15 ml treatments applied every day reduces the total germination of *Schismus arabicus* seedlings from seed bank soil under the conditions used in this project.

**Watering Schedule 2: watered every other day**

The seedling abundance results of samples watered every other day indicated several significant moisture (3 ml and 6 ml treatments), shade (open), and moisture-shade interactions (3 ml-light shade, 6 ml-open shade, and 3 ml-open shade treatments). The two-way interactions between moisture and shade treatments make it difficult to determine how a specific independent variable, or the interaction between independent variables, influences the response variable. Instead, the results must be interpreted on a case-by-case basis.
However, it should be noted that all of the significant interactions were for low moisture treatments, and drier shade treatments.

All four of the highest mean emergence totals were found in the light shade treatment, for the 9 ml (15.4 seedlings, SE=1.676, CL=12.5-19.1), 12 ml (14.7 seedlings, SE=1.618, CL=11.8-18.2), 6 ml (13.2 seedlings, SE=1.508, CL=10.6-16.6) and 3 ml (12.7 seedlings, SE=1.65, CL=10.1-15.9) treatments, none of which were significantly different from each other (Figure 15). The lowest three predicted totals were markedly lower than all of the other totals, and were found in the high and open shade treatments. In the high shade treatment, the lowest germination was in the 3 ml treatment (4.7 seedlings, SE=0.782, CL=3.4-6.5). The total was significantly lower than the estimates for the other four moisture treatments within the shade treatment (p<0.0001-p=0.0367), which ranged from 8.04 in the 15 ml treatment (SE=1.087, CL=6.2-10.5) to 11.3 for 6 ml treatment (SE=1.356, CL=8.9-14.3). The other two lowest estimates were in the two low moisture treatments (3 ml [3.8, SE=0.691, CL=2.6-5.4] and 6 ml/event [4.87, SE=0.800, CL=3.5-6.7], respectively). Both values were significantly lower than the estimates for the other three moisture levels in the open treatment (p-values ≤0.0001).

The timing of first emergence was strongly influenced by moisture (SS=238.491, df=4, F=59.6228) in the 3 ml treatments (0.872, z-value=5.855, p=4.76e-9) (Figure 15). In all three shade treatments, the longest predicted times to first seedling emergence were in the 3 ml moisture treatments. The single longest time was in the open shade treatment, at 21.85 days (SE=1.620, CL=18.890-25.265), followed by the high shade (16.968 days, SE=1.416, CL=14.408-19.983), light shade (16.747 days, SE=1.406, CL=14.207-19.743) and the 6 ml-open shade treatments (16.079 days, SE=13.596-19.016). None of the highest four values
were significantly different from each other, while all were significantly longer than the predicted time to first emergence in the other nine treatments (p-values ≤ 0.0001 to 0.0061). The shortest time to generation was 6.43 days for the 15 ml-light shade treatment (SE = 0.855, CL = 4.957-8.347), which was not significantly different from any but the four longest times.

In summary, the moisture treatment, shade treatment and their interaction was important in treatments receiving water every other day. The low number of seedlings in the 3 ml-open and 6 ml-open treatments, paired with the long delay before the first seedling emergence, suggests that the reduction of soil moisture between watering events was too great for the soil to reach and maintain the required soil moisture levels for high *Schismus* germination from seed bank soil. The overall high abundance of seedlings in the light shade treatment for the four lowest moisture treatments suggests that the light shade treatment in the alternate days watering schedule provided conditions for sufficient moisture retention between watering events for the low and moderate moisture treatments to reach and maintain optimal soil moisture for high germination rates. This may be due to the higher humidity and slightly shaded conditions promoting the maintenance of sufficient soil moisture for germination even when watering events occurred every other day. The light shade conditions also allowed for sufficient moisture reduction so that the high moisture treatments could remain within the optimal range of soil moisture for germination.

**Comparing watering schedules**

Pairwise comparisons of the mean total seedling emergence between the two watering event periodicities (every day, or every other day) within a moisture treatment (84 ml or 168 ml total), indicated only one significant difference. With a mean value of 4.9 seedlings
(SE=0.799, CL=3.5-6.7), the mean total seedling emergence for cells watered every other day was significantly lower in the open shade treatment than in any other 84 ml or 168 ml treatment (p≤0.0001-0.0004). No other seedling abundance estimates were significant, within or between moisture and shade levels. The mean totals for all other treatments were between 10.5 seedlings in the 84 ml-periodicity one-high shade treatment (SE=1.284, CL=8.3-13.3), and 14.9 seedlings in the 84 ml-periodicity one-open shade treatment (SE=1.623, CL=12.0-18.4) (Figure 15). This suggests that for the two moisture levels selected, the only difference in abundance due to periodicity was in the 84 ml-open shade-periodicity two treatment combination. That is not surprising as the only significant interaction was between the 84 ml-open shade-periodicity two treatments (-0.6159, SE=0.2926, z=-2.105, p=0.0353).

Pairwise comparisons of the time to first seedling emergence between periodicities by shade yielded only one significant difference within the 84 ml moisture treatments. In the open shade treatment, the mean day of first seedling emergence (16.07 days, SE=1.387, CL=13.571-19.035) was significantly later in periodicity two than it was in periodicity one (10.22 days, SE=1.108, CL=8.263-12.638) (p=0.0011). The two shortest times to first emergence were in the 168 ml-high shade treatment, at 7.55 days for periodicity one (SE=0.9162, CL=5.957-9.583) and 8.76 days for periodicity two (SE=1.017, CL=6.978-11.000); both times were significantly shorter than the longest time to emergence (16.07 days). In the 168 ml treatments, the day of first seedling emergence ranged from 5.77 days (SE=0.801, CL=4.402-7.582) in the high shade - periodicity two treatments, to 9.11 days (SE=1.006, CL=7.338-11.313) in open shade - periodicity one treatments. None of the predictions in 168 ml treatments were significantly different. In summary, periodicity was only a strong influence on the abundance and timing of seedling emergence in the driest
conditions, namely at the lowest moisture treatment, when the water was administered every other day, and the shade conditions favored drying conditions. Under those conditions, the soil likely fell below the required threshold for soil moisture required for germination between watering events, delaying the start of germination and resulting in a reduced total in the number of seedlings emerging.

**Discussion**

For the soil preparation methods and environmental settings experienced in this study, it is apparent that there were two moisture thresholds above which seed bank soil needed to be in order to produce germination. Regarding the high moisture threshold, if moisture treatments were sufficient for the seedbank soil to reach and maintain a sufficient level of soil moisture or greater the sample would experience high levels of seedling emergence. In contrast, treatments which received water additions insufficient to reach the soil moisture threshold produced significantly lower numbers of seedlings emerged. The low moisture threshold was the minimal soil moisture which had to be maintained for any decent amount of germination; treatments which did not reach and maintain soil moisture levels exceeding that level would have extremely low, if any, seedling emergence. While the lower threshold was reached by all treatments which were watered daily the upper threshold existed between 9 and 12 ml/day in the open and light shade treatments, and between 6 and 9ml in the high shade treatment. In the periodicity two treatments, the lower threshold was between 3 and 6 ml/alternate-days in the high shade treatment, and between 6 and 9ml/alternate day in the open treatment. The upper threshold for high germination was between 12 and 15 ml/alternate days in the light shade treatment.
In contrast to the problem of surpassing the upper threshold of soil moisture for high germination, demonstrated in the daily watering schedule treatments, the more common problem in the alternate days watering schedule treatments was reaching or remaining above the lower soil moisture threshold for high germination. The 3 ml/alternate day treatment was too low for the replicates to reach the minimum moisture threshold for high germination in the high and open shade treatments. In the open shade treatment, the 6 ml/alternate day treatment was similarly unable to either reach or maintain the required soil moisture for a sufficient amount of time for germination.

It is also apparent that there was an optimal range of soil moisture for high germination between the upper and lower thresholds. Of the variables considered in this study, moisture was the primary driver of germination, with shade and watering periodicity only influential in so much as they aided or hindered the maintenance of soil moisture between the required and optimal thresholds. Towards that end, the shade, moisture treatment and periodicity of watering events required to reach and maintain soil moistures within the range that promotes high germination varied.

The optimal range for germination resulted in the germination of approximately 70-100% of seeds germinating (proportion of seedlings relative to the highest total number of seedlings in any one treatment (n=141)). In treatments watered daily, the optimal range was achieved in the 3, 6, and 9 ml treatments under light and open shade conditions. In treatments watered every other day, the optimal range was achieved in the 3, 6, 9 and 12 ml treatments under light shade conditions, and in the 9, 12 and 15 ml treatments in the open shade conditions. While not significantly higher than most treatments in the optimal germination
range, it is worthy to note that the highest number of seedlings were in the periodicity one, light shade conditions, and in the periodicity one open shade conditions.

As species-specific soil moisture thresholds for germination have been observed for many plants, it is not surprising to find them in this study. One study found that the optimal soil water potential for *S. arabicus* germination was -0.050 MPa or above. The same study found that the soil moisture potential had to be maintained for a minimum of 52 hours before germination could start, but only low germination rates occurred unless exposure time increase to 58-72 hours of sufficient soil moisture (Meidan 1990). While the specific water potential of the soil used in this study was not measured, the existence of a certain amount of time required above the minimal soil moisture threshold for germination is observed in this study. Another study found that *S. arabicus* germination from seedbank soil was greatest at 80 ml of water total, spread out in 7 mm applications twice a week, compared to 10-40 mm, and 180-340 mm treatments (Vidiella and Armesto 1989).

In conditions that promoted drying between watering events (open shade, alternate day watering frequency, low moisture treatments in the alternate day watering schedule), higher moisture levels were required to reach the minimal threshold for germination, which translated into longer times to first seedling emergence. This did not occur in periodicity one, but was evident in the 3 and 6 ml treatments in periodicity two. In conditions favorable for retention of soil moisture between watering events (high shade, periodicity one watering frequency, high moisture treatments in periodicity one), lower moisture treatments were necessary to prevent soil moisture from going above the upper soil moisture threshold for germination. In periodicity one, the 15 and 12 ml/day treatments passed above the upper threshold in all shade conditions, as did the 9 ml treatment in the high shade treatments. In
the periodicity two treatments, only the 15 ml treatment passed above the upper threshold in the light and heavy shade treatments. There are several possible explanations for the reduced number of seedlings in treatments once they passed above the upper soil moisture threshold. One possibility is that high moisture additions may have flushed a portion of the seeds and soil through the cheesecloth separating the seed bank soil from the vermiculite, reducing the total number of seeds that could potentially germinate. Other possibilities include soil moisture too high for survival interacting with germination cues in *Schismus* seeds and preventing the initiation of germination, or high soil moistures drowning germinating seeds before they emerged as seedlings.

The results have several key methodological implications. The first is that as long as the optimal soil moisture level is reached, there is a range of moisture and shade treatments that will generate approximately the same high level of *S. arabicus* germination. The highest abundances of germination may be reached through the application of low to medium moisture applications every other day in light shade conditions (6, 9, or 12ml of water per 40g soil every other day), or through low, daily applications of water (3, 6, or 9ml of water /40g soil/day) in open shade conditions. As both methods produce approximately the same, highest numbers of seedlings, the decision of which is preferable can be based on the preference of time and resource allotment over the course of the experiment. If space for shade enclosures, or the time or cost of constructing shade enclosures is restrictive, daily watering in open conditions can replace shaded treatments. However, if labor during the course of the germination process is the primary limitation, watering every other day and utilizing a light shade enclosure cuts maintenance time in half.
If the quantity of seedbank soil is not prohibitive, or only a modest number of
seedlings are needed, the simplest, least expensive and most time effective method for
germinating *S. arabicus* from seed bank soil is to apply 9, 12 or 15 ml of water per 40 g of
soil every other day in open shade conditions for slightly, but not significantly, lower
numbers germinating. That method would allow for high germination with the minimum set-
up time, the minimum space requirement, and the lowest amount of time spent watering.
Literature Cited


Figure 13. Experimental set-up of main plots on greenhouse bench.
Figure 14. Cumulative percent of *Schismus arabicus* seedling emergence for each moisture and shade combinations in periodicity one (watered daily). Total percent based on highest germination for any treatment (n=141). High shade=50% shade cloth; light shade=cheesecloth; No shade=Open. Seedlings germinated from mixed Mojave-Sonoran Deserts seedbank soils (50:50 by volume) in the Iowa State University ecology greenhouse in Ames, IA, between Jan. 21 (day 0) and Feb.18 (day 28), 2014.
Figure 15. Cumulative percent of *Schismus arabicus* seedling emergence for each moisture and shade combinations in periodicity two (watered every-other day). Total percent based on highest germination for any treatment (n=141). High shade=50% shade cloth; Light shade=cheesecloth; No shade=Open. Seedlings germinated from mixed Mojave-Sonoran Deserts seedbank soils (50:50 by volume) in the Iowa State University ecology greenhouse in Ames, IA, between Jan. 21 (day 0) and Feb.18 (day 28), 2014.
CHAPTER 4. GENERAL CONCLUSIONS

Conclusions

The general objective of the thesis was to explore how an invasive desert annual, *Schismus arabicus*, germinated, grew and survived in response to various environmental conditions. It was unexpected that the over the course of both projects, the ability of multivariate analysis to both simplify our understanding of a system by highlighting a single main influence, and its ability to increase the depth of our understanding of a system by identifying complex interactions between multiple variables and variable levels, would be experienced. In the germination methods study, it was found that there was an optimal soil moisture threshold that must be reached and maintained in order for high numbers of seedlings to emerge. Towards that end, it was discovered that shade and watering periodicity only influence germination insomuch as they facilitate the loss or retention of soil moisture.

In the growth and survival study, the use of multivariate studies led to an increased understanding of the complex shade, moisture and soil-nitrogen interactions that influence the growth and survival of *S. arabicus* in greenhouse conditions. A large number of strong, context-dependent interactions were identified when just three microhabitat variables were manipulated, and it is likely many more significant interactions would be found if additional variables were included. The study results also highlighted the importance of working at the local scale, as the results reached in one situation or location may not be the same as those identified at a different site. Future studies are needed to identify additional interactions and significant microhabitat influences.
A promising direction for future studies is utilizing shade data, and the shade/soil-nitrogen interactions in the creosote bush fertility island habitat to explore remote sensing based models of *S. arabicus* responses to various fire or disturbance events. Towards that end, additional studies are needed that explore how varied climate, competitive or anthropogenic conditions impact microhabitat influences on *S. arabicus* growth and survival. More site-specific and detailed interaction and response data will likely prove valuable for modeling *S. arabicus* distribution following fire or disturbance event scenarios.
REFERENCES


APPENDIX A. ANNOTATED R CODE, CHAPTER 2

#########################################################################
#Annotated R code for analysis of thesis chapter 2: "SURVIVORSHIP AND GROWTH OF
AN INVASIVE DESERT ANNUAL, SCHISMUS ARABICUS, UNDER VARIED
SHADE, SOIL MOISTURE AND SOIL-NITROGEN REGIMES"
#########################################################################

#Question 1: Are the influences of shade, moisture and soil-N the same for all survival and
growth measures?

#Question 2: Which combination of shade, moisture and soil-N resulted in the greatest and
lowest survival and growth responses in S. arabicus?

#########################################################################
For question 1: influences on survival time
#########################################################################

#Read data into R from Excel CSV file
Grow=read.csv("Master_Ch2Q1ToQ3ResponseTotalsShade1,2,3_26Oct2014.csv")

#Change categorical data from integers to factors
Grow$IDCode=as.factor(Grow$IDCode)
Grow$Block=as.factor(Grow$Block)
Grow$Subblock=as.factor(Grow$Subblock)
Grow$Cell=as.factor(Grow$Cell)
Grow$TransDay=as.factor(Grow$TransDay)
Grow$Shade=as.factor(Grow$Shade)
Grow$TrtCombo=as.factor(Grow$TrtCombo)
Grow$Moisture=as.factor(Grow$Moisture)
Grow$Nutrient=as.factor(Grow$Nutrient)
Grow$SurvToEnd=as.factor(Grow$SurvToEnd)

#Load packages I will need to do glmers, to get means, and to make lsmip graphs
library(lme4)
library(lsmeans)
library(lattice)

#Run generalized linear mixed effect model
Surv.glme=glmer(DaysAlive~
Moisture*Nutrient*Shade+(1|Block)+(1|Subblock:Block)+(1|TransDay), data=Grow,
family=poisson)

#Get summary of influences and intercept data
summary(Surv.glme)

#Check how much variation is due to each IV or combination of IV (test of likelihood ratio test)
anova(Surv.glme)

#Since there are significant three way interactions, need to graph the growth response for each IV at a time, with the growth response totals averaged (held constant) for the other two IVs. For visual analysis of three-way interactions:

#Calculate the mean response per treatment based on the GLMM model requirements above
means=lsmeans(Surv.glme, ~Shade*Moisture*Nutrient)

#Graph the influence of soil-N
lsmip(means, Nutrient~Shade|Moisture, type="response", ylab="Survival Time (Days)", xlab="Shade Treatment")

#Graph the influence of moisture
lsmip(means, Moisture~Shade|Nutrient, type="response", ylab="Survival Time (Days)", xlab="Shade Treatment")

#Graph the influence of shade
lsmip(means, Shade~Nutrient|Moisture, type="response", ylab="Survival Time (Days)", xlab="Soil-N Treatment")

#################
#For question 2: treatment combinations promoting shortest & longest survival times

#Reread data into R from Excel CSV file
Grow=read.csv("Master_Ch2Q1ToQ3ResponseTotalsShade1,2,3_26Oct2014.csv")

#Change categorical data from integers to factors
Grow$IDCode=as.factor(Grow$IDCode)
Grow$Block=as.factor(Grow$Block)
Grow$Subblock=as.factor(Grow$Subblock)
Grow$Cell=as.factor(Grow$Cell)
Grow$TransDay=as.factor(Grow$TransDay)
Grow$Shade=as.factor(Grow$Shade)
Grow$TrtCombo=as.factor(Grow$TrtCombo)
Grow$Moisture=as.factor(Grow$Moisture)
Grow$Nutrient=as.factor(Grow$Nutrient)
Grow$SurvToEnd=as.factor(Grow$SurvToEnd)

#Run generalized linear mixed effect model
Surv.glme=glmer(DaysAlive~Moisture*Nutrient*Shade+(1|Block)+(1|Subblock:Block)+(1|TransDay), data=Grow, family=poisson)

#Get predicted mean response values for each treatment combination
means=lsmeans(Surv.glme, ~Shade*Moisture*Nutrient)
means
summary(means, type="response")

#Do pairwise comparisons to test to statistically significant differences in means
#(look at unadjusted first due to the large number of families being compared, n=18)
means=lsmeans(Surv.glme, pairwise=~Shade*Moisture*Nutrient)
means
summary(means, type="response")

#The four longest survival times were in nitrogen treatment 3, so check for significant
#differences within nitrogen treatment 3 with adjustment 6 families
means=lsmeans(Surv.glme, pairwise=~Shade*Moisture|Nutrient)
means
summary(means, type="response")

###########################################################################
###########################################################################
#Question 1: influences on number of leaves at time of termination

#Read data into R from Excel CSV file
Grow=read.csv("Master_Ch2Q1ToQ3ResponseTotalsShade1,2,3_26Oct2014.csv")

#Change categorical data from integers to factors
Grow$IDCode=as.factor(Grow$IDCode)
Grow$Block=as.factor(Grow$Block)
Grow$Subblock=as.factor(Grow$Subblock)
Grow$Cell=as.factor(Grow$Cell)
Grow$TransDay=as.factor(Grow$TransDay)
Grow$Shade=as.factor(Grow$Shade)
Grow$TrtCombo=as.factor(Grow$TrtCombo)
Grow$Moisture=as.factor(Grow$Moisture)
Grow$Nutrient=as.factor(Grow$Nutrient)
Grow$SurvToEnd=as.factor(Grow$SurvToEnd)
Grow$LeafNum=as.integer(Grow$LeafNum)
Grow$DaysAlive=as.integer(Grow$DaysAlive)

#check structure of data
str(Grow)
#Load packages I will need to do glmers, to get means and to graph
library(lme4)
library(lsmeans)
library(lattice)

#Model for all plants (survivors and those that died during the experiment)
LeafNum.glme=glmer(LeafNum~Moisture*Nutrient*Shade+(1|Block)+(1|Subblock:Block)+(1|TransDay), data=Grow, family=poisson)

#Model results
summary(LeafNum.glme)

#Check how much variation is due to each IV or combination of IV (test of likelihood ratio test)
anova(LeafNum.glme)

#influence of soil-N
lsmip(means, Nutrient~Shade|Moisture, type="response", ylab="Leaves at Termination (#)", xlab="Shade Treatment")

#influence of moisture
lsmip(means, Moisture~Shade|Nutrient, type="response", ylab="Leaves at Termination (#)", xlab="Shade Treatment")

#influence of shade
lsmip(means, Shade~Nutrient|Moisture, type="response", ylab="Leaves at Termination (#)", xlab="Soil-N Treatment")

#Plot days leaf number by days alive to see if it is better to separate plants that died before the end from those that survived to the end
attach(Grow)
plot(DaysAlive, LeafNum, xlim=c(0,50), ylim=c(0,25), xlab="Days Alive (#)", ylab="Leaves at Termination (#)", pch=20, cex=1)

#################################
#Comparing leaf number for survivors only
#Read in data
Grow=read.csv("Master_Ch2Q1ToQ3ResponseTotalsShade1,2,3_26Oct2014.csv")

#Change categorical variables from integers to factors
Grow$IDCode=as.factor(Grow$IDCode)
Grow$Block=as.factor(Grow$Block)
Grow$Subblock=as.factor(Grow$Subblock)
Grow$Cell=as.factor(Grow$Cell)
Grow$TransDay=as.factor(Grow$TransDay)
Grow$Shade=as.factor(Grow$Shade)
Grow$TrtCombo=as.factor(Grow$TrtCombo)
Grow$Moisture=as.factor(Grow$Moisture)
Grow$Nutrient=as.factor(Grow$Nutrient)
Grow$SurvToEnd=as.factor(Grow$SurvToEnd)
Grow$LeafNum=as.integer(Grow$LeafNum)
Grow$DaysAlive=as.integer(Grow$DaysAlive)

#Subset data to only include individuals that survived to day 50
subset(Grow, SurvToEnd=="1")

#Run the GLMM model on the subsetted data
LeafNum.glme=glmer(LeafNum~Moisture*Nutrient*Shade+(1|Block)+(1|Subblock:Block)+(1|TransDay), data=subset(Grow, SurvToEnd=="1"), family=poisson)
summary(LeafNum.glme)
anova(LeafNum.glme)

#Get means from subsetted data so that the lsmip graphs can be generated
means=lsmeans(LeafNum.glme, pairwise~Nutrient*Moisture|Shade)

#Graphs for subsetted data:
#influence of soil-N
lsmip(means, Nutrient~Shade|Moisture, type="response", ylab="Leaves at Termination (#)", xlab="Shade Treatment")

#influence of moisture
lsmip(means, Moisture~Shade|Nutrient, type="response", ylab="Leaves at Termination (#)", xlab="Shade Treatment")

#influence of shade
lsmip(means, Shade~Nutrient|Moisture, type="response", ylab="Leaves at Termination (#)", xlab="Soil-N Treatment")

#Evaluating the influence of fixed effects on plants terminated before the project ended
Grow=read.csv("Master_Ch2Q1ToQ3ResponseTotalsShade1,2,3_26Oct2014.csv")

#Change categorical variables from integers to factors
Grow$IDCode=as.factor(Grow$IDCode)
Grow$Block=as.factor(Grow$Block)
Grow$Subblock=as.factor(Grow$Subblock)
Grow$Cell=as.factor(Grow$Cell)
Grow$TransDay=as.factor(Grow$TransDay)
# Subset data to only include individuals that survived to day 50
subset(Grow, SurvToEnd=="2")

# Run the GLM model on the subsetted data
LeafNum.glme=glmer(LeafNum~
  Moisture*Nutrient*Shade+(1|Block)+(1|Subblock:Block)+(1|TransDay), data=subset(Grow,
  SurvToEnd=="2"), family=poisson)
summary(LeafNum.glme)
anova(LeafNum.glme)

# Question 2: combinations promoting shortest and longest survival times

# NOTE: Given the results of the question 1 analysis, only totals for plants
# that survived through the end of the project were calculated

# Read data into R from Excel CSV file
Grow=read.csv("Master_Ch2Q1ToQ3ResponseTotalsShade1,2,3_26Oct2014.csv")

# Change categorical data from integers to factors
Grow$IDCode=as.factor(Grow$IDCode)
Grow$Block=as.factor(Grow$Block)
Grow$Subblock=as.factor(Grow$Subblock)
Grow$Cell=as.factor(Grow$Cell)
Grow$TransDay=as.factor(Grow$TransDay)
Grow$Shade=as.factor(Grow$Shade)
Grow$TrtCombo=as.factor(Grow$TrtCombo)
Grow$Moisture=as.factor(Grow$Moisture)
Grow$Nutrient=as.factor(Grow$Nutrient)
Grow$SurvToEnd=as.factor(Grow$SurvToEnd)

# Load packages I will need to do glmers, to get means and to graph
library(lme4)
library(lsmeans)
library(lattice)

# Subsetting data for survivors only
subset(Grow, SurvToEnd=="1")
# Run generalized linear mixed effect model
LeafNum.glme = glmer(LeafNum ~ Moisture*Nutrient*Shade + (1|Block) + (1|Subblock:Block) + (1|TransDay), data = subset(Grow, SurvToEnd == "1"), family = poisson)

# Get predicted mean response values for each treatment
means = lsmeans(LeafNum.glme, ~Shade*Moisture*Nutrient)
means
summary(means, type = "response")

# Do pairwise comparisons to test to statistically sig. difs in means (unadjusted due)
means = lsmeans(LeafNum.glme, pairwise ~ Shade*Moisture*Nutrient)
means
summary(means, type = "response")

# Four longest survival times in nutrient 3, so check for sig difs within nut 3
# to avoid adjustment for 18 families
means = lsmeans(LeafNum.glme, pairwise ~ Shade*Moisture|Nutrient)
means
summary(means, type = "response")

# Anova found shade to be a large explanatory factor for survivor leaf number, so pairwise given shade
means = lsmeans(LeafNum.glme, pairwise ~ Nutrient*Moisture|Shade)
means
summary(means, type = "response")

# Question 1: influences on above ground biomass

# Note: the model has to be changed from a generalized linear to just linear mixed effects model from all previous model since the data is ~continuous data instead of count data

# Read in data
Grow = read.csv("Master_SurvAndGrowCh2_4Nov2014.csv")

# Check structure of data
str(Grow)

# Change categorical data from integers to factors
Grow$IDCode = as.factor(Grow$IDCode)
Grow$Block=as.factor(Grow$Block)
Grow$Subblock=as.factor(Grow$Subblock)
Grow$Cell=as.factor(Grow$Cell)
Grow$TransDay=as.factor(Grow$TransDay)
Grow$Shade=as.factor(Grow$Shade)
Grow$TrtCombo=as.factor(Grow$TrtCombo)
Grow$Moisture=as.factor(Grow$Moisture)
Grow$Nutrient=as.factor(Grow$Nutrient)
Grow$SurvToEnd=as.factor(Grow$SurvToEnd)

#Load packages I will need to do lmers, to get means and to graph
library(lme4)
library(lsmeans)
library(lattice)
library(lmerTest)

#subset data
subset(Grow, SurvToEnd=="1")

#Run generalized linear mixed effect model
agbm.lme=lmer(AGBM~
  Moisture*Nutrient*Shade+(1|Block)+(1|Subblock:Block)+(1|TransDay),
data=subset(Grow, SurvToEnd=="1")
)

#Get summary of influences and intercept data
summary(agbm.lme)

#Check how much variation is due to each IV or combination of IV (likelihood ratio test)
anova(agbm.lme)

#Since there are significant three way interactions, need to graph the
growth response for each IV at a time, with the growth response totals
#averaged (held constant) for the other two IVs
means=lsmeans(agbm.lme, ~Shade*Moisture*Nutrient)
means
summary(means, type="response")

#influence of soil-N
lsmip(means, Nutrient~Shade|Moisture, type="response", ylab="Above Ground Biomass (mg)", xlab="Shade Treatment")

#influence of moisture
lsmip(means, Moisture~Shade|Nutrient, type="response", ylab="Above Ground Biomass (mg)", xlab="Shade Treatment")

#influence of shade
lsmip(means, Shade~Nutrient|Moisture, type="response", ylab="Above Ground Biomass (mg)", xlab="Soil-N Treatment")

########################################################################
#Question 2: greatest and lowest AGBM estimates
#read in data
Grow=read.csv("Master_SurvAndGrowCh2_4Nov2014.csv")

#Check structure of data
str(Grow)

#Change categorical data from integers to factors
Grow$IDCode=as.factor(Grow$IDCode)
Grow$Block=as.factor(Grow$Block)
Grow$Subblock=as.factor(Grow$Subblock)
Grow$Cell=as.factor(Grow$Cell)
Grow$TransDay=as.factor(Grow$TransDay)
Grow$Shade=as.factor(Grow$Shade)
Grow$TrtCombo=as.factor(Grow$TrtCombo)
Grow$Moisture=as.factor(Grow$Moisture)
Grow$Nutrient=as.factor(Grow$Nutrient)
Grow$SurvToEnd=as.factor(Grow$SurvToEnd)

#Load packages I will need to do lmers, to get means and to graph
library(lme4)
library(lsmeans)
library(lattice)
library(lmerTest)

#subset data
subset(Grow, SurvToEnd=="1")

#Run generalized linear mixed effect model
agbm.lme=lmer(AGBM~Moisture*Nutrient*Shade+(1|Block)+(1|Subblock:Block)+(1|TransDay), data=subset(Grow, SurvToEnd=="1"))

#Get the means predicted by the model
means=lsmeans(agbm.lme, ~Shade*Moisture*Nutrient)
means
summary(means, type="response")

#Since shade appears to be the greatest influence, doing pairwise by that
means=lsmeans(agbm.lme, pairwise~Moisture*Nutrient|Shade)
means
summary(means, type="response")

#########################################################################
#########################################################################
#Question 1: influences on below ground biomass (BGBM)

#read in data
Grow=read.csv("Master_SurvAndGrowCh2_4Nov2014.csv")

#Check structure of data
str(Grow)

#Change categorical data from integers to factors
Grow$IDCode=as.factor(Grow$IDCode)
Grow$Block=as.factor(Grow$Block)
Grow$Subblock=as.factor(Grow$Subblock)
Grow$Cell=as.factor(Grow$Cell)
Grow$TransDay=as.factor(Grow$TransDay)
Grow$Shade=as.factor(Grow$Shade)
Grow$TrtCombo=as.factor(Grow$TrtCombo)
Grow$Moisture=as.factor(Grow$Moisture)
Grow$Nutrient=as.factor(Grow$Nutrient)
Grow$SurvToEnd=as.factor(Grow$SurvToEnd)

#Load packages I will need to do glmers, to get means and to graph
library(lme4)
library(lsmeans)
library(lattice)
library(lmerTest)

#subset data
subset(Grow, SurvToEnd=="1")

#Run linear mixed effect model
bgbm.lme=lmer(BGBM~Moisture*Nutrient*Shade+(1|Block)+(1|Subblock:Block)+(1|TransDay), data=subset(Grow, SurvToEnd=="1"))

#Get summary of influences and intercept data
summary(bgbm.lme)

#Check how much variation is due to each IV or combination of IV (test of likelihood ratio test)
anova(bgbm.lme)

#Since there are significant three way interactions, need to graph the
growth response for each IV at a time, with the growth response totals
averaged (held constant) for the other two IVs
means = lsmeans(bgbm.lme, ~Shade*Moisture*Nutrient)

#influence of soil-N
lsnip(means, Nutrient~Shade|Moisture, type="response", ylab="Below Ground Biomass (mg)", xlab="Shade Treatment")

#influence of moisture
lsnip(means, Moisture~Shade|Nutrient, type="response", ylab="Below Ground Biomass (mg)", xlab="Shade Treatment")

#influence of shade
lsnip(means, Shade~Nutrient|Moisture, type="response", ylab="Below Ground Biomass (mg)", xlab="Soil-N Treatment")

#Question 2: greatest and lowest BGBM estimates
#read in data
Grow = read.csv("Master_SurvAndGrowCh2_4Nov2014.csv")

#Check structure of data
str(Grow)

#Change categorical data from integers to factors
Grow$IDCode = as.factor(Grow$IDCode)
Grow$Block = as.factor(Grow$Block)
Grow$Subblock = as.factor(Grow$Subblock)
Grow$Cell = as.factor(Grow$Cell)
Grow$TransDay = as.factor(Grow$TransDay)
Grow$Shade = as.factor(Grow$Shade)
Grow$TrtCombo = as.factor(Grow$TrtCombo)
Grow$Moisture = as.factor(Grow$Moisture)
Grow$Nutrient = as.factor(Grow$Nutrient)
Grow$SurvToEnd = as.factor(Grow$SurvToEnd)

#Load packages I will need to do lmers, to get means and to graph
library(lme4)
library(lsmeans)
library(lattice)
library(lmerTest)

#subset data
subset(Grow, SurvToEnd == "1")
# Run generalized linear mixed effect model
bgbm.lme=lmer(BGBM~ Moisture*Nutrient*Shade+(1|Block)+(1|Subblock:Block)+(1|TransDay), data=subset(Grow, SurvToEnd=="1"))

# Get the means predicted by the model
means=lsmeans(bgbm.lme, ~Shade*Moisture*Nutrient)
means
summary(means, type="response")

# Since shade appeared to be the greatest influence in question 1,
# I am going to start with pairwise comparisons within shade treatments
means=lsmeans(bgbm.lme, pairwise~Moisture*Nutrient|Shade)
means
summary(means, type="response")

# Because the highest value is not significantly different from much lower values, I am going to try all pairwise comparisons unadjusted
means=lsmeans(bgbm.lme, pairwise~Shade*Moisture*Nutrient, adjust="none")
means
summary(means, type="response")

###########################################################################
###########################################################################
# Question 1: influences on above ground: below ground biomass ratio

# Read data into R from Excel CSV file
Grow=read.csv("Master_BiomassSurvivedToEnd_3Nov2014.csv")

# Change categorical data from integers to factors
Grow$IDCode=as.factor(Grow$IDCode)
Grow$TransDay=as.factor(Grow$TransDay)
Grow$Shade=as.factor(Grow$Shade)
Grow$TrtCombo=as.factor(Grow$TrtCombo)
Grow$Moisture=as.factor(Grow$Moisture)
Grow$Nutrient=as.factor(Grow$Nutrient)

# Subset to remove cheesecloth block
subset(Grow, Shade!="4")

# Run linear mixed effect model
agbgratio.lme=lmer(AGBGRatio~ Moisture*Nutrient*Shade+(1|TransDay),
data=subset(Grow, Shade!="4"))

# Get summary of influences and intercept data
summary(agbgratio.lme)

#Check how much variation is due to each IV or combination of IV (test of likelihood ratio
test)
anova(agbgratio.lme)

#need to graph the growth response for each IV at a time, with the growth response totals
#averaged (held constant) for the other two IVs to look for interactions
means=lsmeans(agbgratio.lme, ~Shade*Moisture*Nutrient)

#influence of soil-N
lsmip(means, Nutrient~Shade|Moisture, type="response", ylab="AG:BG Ratio",
xlab="Shade Treatment")

#influence of moisture
lsmip(means, Moisture~Shade|Nutrient, type="response", ylab="AGBG Ratio", xlab="Shade
Treatment")

#influence of shade
lsmip(means, Shade~Nutrient|Moisture, type="response", ylab="AGBG Ratio", xlab="Soil-
N Treatment")

#Question 2: greatest and least AG:BG ratios

#Read data into R from Excel CSV file
Grow=read.csv("Master_BiomassSurvivedToEnd_3Nov2014.csv")

#Change categorical data from integers to factors
Grow$IDCode=as.factor(Grow$IDCode)
Grow$TransDay=as.factor(Grow$TransDay)
Grow$Shade=as.factor(Grow$Shade)
Grow$TrtCombo=as.factor(Grow$TrtCombo)
Grow$Moisture=as.factor(Grow$Moisture)
Grow$Nutrient=as.factor(Grow$Nutrient)

#Subset to remove cheesecloth block
subset(Grow, Shade!="4")

#Run linear mixed effect model
agbgratio.lme=lmer(AGBGRatio~ Moisture*Nutrient*Shade+(1|TransDay),
data=subset(Grow, Shade!="4")

#Get predicted mean response values for each treatment
means=lsmeans(agbgratio.lme, ~Shade*Moisture*Nutrient)
means
summary(means, type="response")

#Do pairwise comparisons to test to statistically sig. difs in means (unadjusted)
means=lsmeans(agbgratio.lme, pairwise~Shade*Moisture*Nutrient)
means
summary(means, type="response")
APPENDIX B. ANNOTATED R CODE, CHAPTER 3

#########################################################################
### Annotated R code for thesis project 2 (Chapter 3):
###  "Methods for germination of an invasive desert annual, Schismus arabicus,
###   from seed bank soil"
###
### NOTE: For research questions 1 and 2, H2OPeriodicities 1 and 2
###   are being considered separately due to the different total amount
###   of water used for each treatment (i.e. 3ml/day in H2OPeriodicity
###   1 received twice as much water overall than 3ml every other day
###   in H2OPeriodicity 2)
#########################################################################

### Baseline bench and enclosure microhabitat info

###Relative humidity
temprh=read.csv("Data_Temp&RH1_5Apr2014.csv")
str(temprh)
temprh$Date=as.factor(temprh$Date)
temprh$Time=as.factor(temprh$Time)
temprh$Block=as.factor(temprh$Block)
temprh$Zone=as.factor(temprh$Zone)
temprh$Shade=as.factor(temprh$Shade)
str(temprh)

library(lme4)

temprh.lmer=lmer(RH~Shade+(1|Zone)+(1|Block:Zone),data=temprh)
summary(temprh.lmer)
anova(temprh.lmer)

library(lsmeans)
library(pbkrtest)
means=lsmeans(temprh.lmer, pairwise~Shade)
means

###Temperature

temprh=read.csv("Data_P2Temp&RH1_5Apr2014.csv")
str(temprh)

library(lme4)

temprh.lmer=lmer(RH~Shade+(1|Zone)+(1|Block:Zone),data=temprh)
summary(temprh.lmer)
anova(temprh.lmer)

library(lsmeans)
library(pbkrtest)
means=lsmeans(temprh.lmer, pairwise~Shade)
means

temprh
library(lme4)  
temprh.lmer=lmer(Temp~Shade+(1|Zone)+(1|Block:Zone),data=temprh)  
summary(temprh.lmer)  
anova(temprh.lmer)  
library(lsmeans)  
library(pbkrtest)  
means=lsmeans(temprh.lmer, pairwise~Shade,)  
means

###Photosynthetically active radiation (PAR)  
Light=read.csv("Master_PARProject2Pilot_10Apr2014.csv")  
str(Light)  
Light$Zone=as.factor(Light$Zone)  
Light$Block=as.factor(Light$Block)  
Light$Subblock..Position=as.factor(Light$Subblock..Position)  
Light$Shade=as.factor(Light$Shade)  
str(Light)  
library(lme4)  
Light.lmer=lmer(X.PAR_reduction~Shade+(1|Zone)+(1|Block:Zone),data=Light)  
summary(Light.lmer)  
anova(Light.lmer)  
library(lsmeans)  
library(pbkrtest)  
means=lsmeans(Light.lmer, pairwise~Shade,)  
means

###########################################################################  
### Question 1: Which What watering and shade regime combinations resulted in  
###             the greatest amounts of Schismus arabicus seedling emergence  
###             from seed bank soil under greenhouse conditions?

###########################################################################  
### Question 1: To answer question 1 for the H2OPeriodicity 1 replicates #######

### Step 1: Read the seedling emergence data from Excel CSV spreadsheet into R:  

### Step 2: Changes the treatment level columns into nominal instead of  
### integer values:
Germ$Block=as.factor(Germ$Block)
Germ$Subblock=as.factor(Germ$Subblock)
Germ$Shade=as.factor(Germ$Shade)
Germ$Moisture=as.factor(Germ$Moisture)
Germ$H2OPeriodicity=as.factor(Germ$H2OPeriodicity)

### Step 3: Checks that the structure of the columns I just changed to nominal categories are now nominal (Say factor instead of int (for integer) next to them:
str(Germ)

### Step 4: Open the package needed to do generalized linear mixed effect models:
library(lme4)

### Step 5: Subset data so that only data where H2OPeriodicity =1 are used:
subset(Germ, H2OPeriodicity=="1")

### Step 6: Run a model of using the subsetted data.
### Model is a generalized linear mixed effect model, and is fit by maximum likelihood (glmerMod on the total germ data)
### What the code is "saying": "Run a generalized linear mixed effect model, which is named Germ.glme, on the data subsetted to only include H2OPeriodicity 1, where the total estimated germination per replicate is predicted by the multiplicative interaction of the moisture and shade treatments to which the replicate was subjected, and the additive random effects of block, and the subblock nested within the block to which it was assigned are taken into account."
Germ.glme=glmer(Total~ Moisture*Shade+(1|Block)+(1|Subblock:Block), data=subset(Germ, H2OPeriodicity=="1"), family=poisson)

### Step 7: Get the summary data for the model run with the code above (the object named Germ.glme)
summary(Germ.glme)
anova(Germ.glme)

### Step 8: Run the means (expected total germ values) for glmer model run on the subsetted data above:
means=lsmeans(Germ.glme, ~Moisture*Shade)

#To examine influences one periodicity at a time
lsmip(means, Moisture~Shade, type="response", ylab="Predicted seedling emergence (mean ")", xlab="Shade treatment")
lsmip(means, Shade~Moisture, type="response", ylab="Predicted seedling emergence (mean ")", xlab="Moisture treatment")

### Step 9: Get the model results in table form:
means
summary(means, type="response")

### Step 10: Determine treatment with greatest and lowest estimated emergence
### by looking at the rate (rate=estimated total seedling emergence for each
### moisture-shade treatment combination)

### Step 11: Check if the greatest and lowest emergence estimates are
### significantly different (p<0.05) from the other treatment estimates; use
### the pairwise comparisons without adjustment generated by the code below.
### NOTE: Pairwise comparisons gives you every treatment combo
### run against each other, and with adjust=none, R does not do Tukey
### adjustment and gives you raw values
### 1. Uses a 0.05 confidence interval cutoff, which means that
### the probability that the real pattern is not there but the
### data says it is 5%
### 2. Since I am doing 15 tests (5 moisture x 3 shade combos), if a
### 5% chance of error is acceptable, then I would expect a few
### false negatives/positives
### 3. To address that problem, correct it with Bonferroni (~Tukey)
### -Tukey divides the alpha (0.05) by the total number of tests
### which increases the requirements for significant differences
### -Tukey is the model used in p-value adjustment in R
### 4. Doing pairwise unadjusted lets you key into treatments that are
### likely different so you can ID subgroups to analyze at lower test
### numbers so Tukey is not adjusting for a huge number of tests
means=lsmeans(Germ.glme, pairwise~Moisture*Shade, adjust="none")
means
summary(means, type="response")

### looking for sig. difs in emergence for a given moisture treatment between
### different shade treatments
means.shade=lsmeans(Germ.glme, pairwise~Shade|Moisture)
means.shade
summary(means.shade, type="response")

###########################################################################
#
# To answer question 1 for H2O Periodicity 2 replicates
#
### NOTE: Since I am using the same data file as I did in the H2O Periodicity 2
### replicates (above), the main difference in analysis is switching H2O
### Periodicity 1 to H2O Periodicity 2 throughout the code.

### Step 1: Read the seedling emergence data from Excel CSV spreadsheet into R:
### Step 2: Changes the treatment level columns into nominal instead of integer values:

```r
Germ$Block = as.factor(Germ$Block)
Germ$Subblock = as.factor(Germ$Subblock)
Germ$Shade = as.factor(Germ$Shade)
Germ$Moisture = as.factor(Germ$Moisture)
Germ$H2OPeriodicity = as.factor(Germ$H2OPeriodicity)
```

### Step 3: Checks that the structure of the columns I just changed to nominal categories are now nominal (Say factor instead of int (for integer) next to them):

```r
str(Germ)
```

### Step 4: Open the package needed to do generalized linear mixed effect models:

```r
library(lme4)
```

### Step 5: Subset data so that only data where H2OPeriodicity = 1 are used:

```r
subset(Germ, H2OPeriodicity == "2")
```

### Step 6: Run a model of using the subsetted data.

Model is a generalized linear mixed effect model, and is fit by maximum likelihood (glmerModon the total germ data)

What the code is "saying": "Run a generalized linear mixed effect model, which is named Germ.glme, on the data subsetted to only include H2OPeriodicity 1, where the total estimated germination per replicate is predicted by the multiplicative interaction of the moisture and shade treatments to which the replicate was subjected, and the additive random effects of block, and the subblock nested within the block to which it was assigned are taken into account."

```r
Germ.glme = glmer(Total ~ Moisture*Shade + (1|Block) + (1|Subblock:Block), data = subset(Germ, H2OPeriodicity == "2"), family = poisson)
```

### Step 7: Get the summary data for the model run with the code above (the object named Germ.glme)

```r
summary(Germ.glme)
```

### Step 7b: Run an ANOVA of the model to determine how much of the variance is explained by each fixed effect (or interaction between different fixed effects)

```r
anova(Germ.glme)
```

### Step 8: Run the means (expected total germ values) for glmer model run on the subsetted data above: (graphs after this step)

```r
means = lsmeans(Germ.glme, ~ Moisture*Shade)
```
### Step 9: Get the model results in table form:

```r
means
summary(means, type="response")
```

### Step 10: Determine treatment with greatest and lowest estimated emergence

#### by looking at the rate (rate=estimated total seedling emergence for each

#### moisture-shade treatment combination)

### Step 11: Check if the greatest and lowest emergence estimates are

#### significantly different (p<0.05) from the other treatment estimates; use

#### the pairwise comparisons without adjustment generated by the code below.

```r
means=lsmeans(Germ.glme, pairwise~Moisture*Shade, adjust="none")
means
summary(means, type="response")
```

### Step 11b: to see if the differences between pairwise comparisons of the

### highest and lowest emergence total estimates remain significantly

### different of not when the Tukey adjustments are made

```r
means=lsmeans(Germ.glme, pairwise~Moisture*Shade)
means
summary(means, type="response")
```

### Step 12: Since the relationship between moisture and emergence totals

### appears vary by shade, looking at the pairwise comparisons between

### moisture treatments within shade treatments may be useful

```r
means.moisture=lsmeans(Germ.glme, pairwise~Moisture|Shade)
means.moisture
summary(means.moisture, type="response")
```

### Step 12b: Looks like the shade influence may be even more evident if I

### look at the data from a single moisture at a time compared to itself

### under a different shade treatment

```r
means.shade=lsmeans(Germ.glme, pairwise~Shade|Moisture)
means.shade
summary(means.shade, type="response")
```
#to get graphs of both periodicities by periodicity:

### Read the seedling emergence data from Excel CSV spreadsheet into R:

### Changes the treatment level columns into nominal instead of integer values:
Germ$Block=as.factor(Germ$Block)
Germ$Subblock=as.factor(Germ$Subblock)
Germ$Shade=as.factor(Germ$Shade)
Germ$Moisture=as.factor(Germ$Moisture)
Germ$H2OPeriodicity=as.factor(Germ$H2OPeriodicity)
Germ$Total=as.integer(Germ$Total)

Germ.glme=glmer(Total~ Moisture*Shade*H2OPeriodicity+(1|Block)+(1|Subblock:Block),
data=Germ, family=poisson)

### Run the means (expected total germ values) for glmer model
### run on the subsetted data above:
means=lsmeans(Germ.glme, ~Moisture*Shade*H2OPeriodicity)

### to make the graphs (both periodicities at once)
library(lattice)
lsnip(Germ.glme, Moisture~Shade|H2OPeriodicity, type="response", ylab="Mean Total Seedling Emergence (\#)", xlab="Shade Treatment")
lsnip(Germ.glme, Shade~Moisture|H2OPeriodicity, type="response", ylab="Mean Total Seedling Emergence (\#)", xlab="Moisture Treatment")

### to graph one at a time, use this in each of the above sections
lsnip(means, Moisture~Shade, type="response")
lsnip(means, Shade~Moisture, type="response")

#to graph both total germ by moisture by shade for each periodicity (next to each other) - correct code
lsnip(Germ.glme, Moisture~Shade|H2OPeriodicity, type="response", ylab="Mean Seedling Emergence (\#)", xlab="Shade Treatment")


### Question 2: Are there differences in the timing of seedling emergence
# due to differences in watering and shade regime combinations?
### Part 1a. Differences in timing of first seedling emergence (H2OPeriodicity 1)

#Read in data
Germ=read.csv("Ch3Q2TimingFirstSeedling_31Oct2014.csv")

#format variables from integers into factors
Germ$Block=as.factor(Germ$Block)
Germ$Subblock=as.factor(Germ$Subblock)
Germ$Shade=as.factor(Germ$Shade)
Germ$Moisture=as.factor(Germ$Moisture)
Germ$H2OPeriodicity=as.factor(Germ$H2OPerio)

#Check structure of the data
str(Germ)

#Load needed packages
library(lme4)
library(lsmeans)

#Subset data so that only data where H2OPeriodicity =1 are used:
subset(Germ, H2OPeriodicity=="1")

#Run a model of using the subsetted data; Model is a generalized linear
# mixed effect model, and is fit by maximum likelihood (glmerMod on the
# total germ data)
Germ.glme=glmer(DayFirstSE~ Moisture*Shade+(1|Block)+(1|Subblock:Block),
data=subset(Germ, H2OPeriodicity=="1"), family=poisson)

#Get summary data of the model
summary(Germ.glme)

#Run anova of the influences to determine how much variance is accounted
# for by each fixed effect
anova(Germ.glme)

#Run the means (expected total germ values) for glmer model
means=lsmeans(Germ.glme, ~Moisture*Shade)

#Get the model results in table form:
means
summary(means, type="response")

#Determine treatment with earliest and latest estimated emergence date
# (look at the rates, rates=estimated mean day of first emergence for
# each treatment)
# Run pairwise comparisons to check if rates (estimated day of first emergence) # are significantly different from each other
means=lsmeans(Germ.glme, pairwise~Moisture*Shade)

# Get results of pairwise comparisons
means
summary(means, type="response")

# looking for sig. difs in emergence for a given moisture treatment between # different shade treatments to reduce families adjusted for in pairwise
means.shade=lsmeans(Germ.glme, pairwise~Shade|Moisture)

# Get results of pairwise between moistures within shade
means.shade
summary(means.shade, type="response")

# look for sig. difs in emergence date between moistures within shade treatments
means.moisture=lsmeans(Germ.glme, pairwise~Moisture|Shade)

# Get the results
means.moisture
summary(means.moisture, type="response")

# graph the results for H2OPeriodicity 1
lsmip(means, Moisture~Shade, type="response", ylab="Predicted seedling emergence (mean #)", xlab="Shade treatment")

lsmip(means, Shade~Moisture, type="response", ylab="Predicted seedling emergence (mean #)", xlab="Moisture treatment")

lsmip(means, Moisture~Shade, type="response")

lsmip(means, Shade~Moisture, type="response")

#########################################################################
### Part 1b. Differences in timing of first seedling emergence (H2OPeriodicity 2)

# Read in data
Germ=read.csv("Ch3Q2TimingFirstSeedling_31Oct2014.csv")

# format variables from integers into factors
Germ$Block=as.factor(Germ$Block)
Germ$Subblock=as.factor(Germ$Subblock)
Germ$Shade=as.factor(Germ$Shade)
Germ$Moisture=as.factor(Germ$Moisture)
Germ$H2OPeriodicity=as.factor(Germ$H2OPerio)

# Check structure of the data
str(Germ)

# Load needed packages
library(lme4)
library(lsmeans)

# Subset data so that only data where H2OPeriodicity =1 are used:
subset(Germ, H2OPeriodicity=="2")

# Run a model of using the subsetted data; Model is a generalized linear
# mixed effect model, and is fit by maximum likelihood (glmerMod on the
# total germ data)
Germ.glme=glmer(DayFirstSE~ Moisture*Shade+(1|Block)+(1|Subblock:Block),
data=subset(Germ, H2OPeriodicity=="2"), family=poisson)

# Get summary data of the model
summary(Germ.glme)
summary(Germ.glme, type="response")
# Run anova of the influences to determine how much variance is accounted
# for by each fixed effect
anova(Germ.glme)

# Run the means (expected total germ values) for glmer model
means=lsmeans(Germ.glme, ~Moisture*Shade)

# Get the model results in table form:
means
summary(means, type="response")

# Determine treatment with earliest and latest estimated emergence date
# (look at the rates, rates=estimated mean day of first emergence for
# each treatment)

# Run pairwise comparisons to check if rates (estimated day of first emergence)
# are significantly different from each other
means=lsmeans(Germ.glme, pairwise~Moisture*Shade)

# Get results of pairwise comparisons
means
summary(means, type="response")

# Looking for sig. difs in emergence for a given moisture treatment between
# different shade treatments to reduce families adjusted for in pairwise
means.shade=lsmeans(Germ.glme, pairwise~Shade|Moisture)

#Get results of pairwise between moistures within shade
means.shade
summary(means.shade, type="response")

#look for sig. difs in emergence date between moistures within shade treatments
means.moisture=lsmeans(Germ.glme, pairwise~Moisture|Shade)

#Get the results
means.moisture
summary(means.moisture, type="response")

#########################################################################
#Graphing both periodicities stacked on top of each other:
#Read in data
Germ=read.csv("Ch3Q2TimingFirstSeedling_31Oct2014.csv")

#format variables from integers into factors
Germ$Block=as.factor(Germ$Block)
Germ$Subblock=as.factor(Germ$Subblock)
Germ$Shade=as.factor(Germ$Shade)
Germ$Moisture=as.factor(Germ$Moisture)
Germ$H2OPeriodicity=as.factor(Germ$H2OPeriodicity)

#Load needed packages
library(lme4)
library(lsmeans)

#Run a model of using the subsetted data; Model is a generalized linear
# mixed effect model, and is fit by maximum likelihood (glmerMod on the
# total germ data)
Germ.glme=glmer(DayFirstSE~
Moisture*Shade*H2OPeriodicity+(1|Block)+(1|Subblock:Block), data=Germ,
family=poisson)

#Run the means & graph them (expected total germ values) for glmer model
means=lsmeans(Germ.glme, ~Moisture*Shade|H2OPeriodicity)

#graph the results for H2OPeriodicity 1
lsmip(means, Moisture~Shade|H2OPeriodicity, type="response", ylab="First Seedling
Emergence (Day)", xlab="Shade Treatment")

lsmip(means, Shade~Moisture|H2OPeriodicity, type="response", ylab="First Seedling
Emergence (Day)", xlab="Moisture Treatment")

lsmip(means, Moisture~Shade, type="response")

lsmip(means, Shade~Moisture, type="response")

###################################################################
###########
###################################################################
#### Research question 3: Does the periodicity of watering (every day, or
#### every other day) influence the abundance or timing of S. arabicus
#### seedlings from seed bank soil?
####
#### NOTE: for this question, only treatments that experienced the same total
#### moisture were compared (3ml/day to 6ml every other day; 6ml/day to 12ml
#### every other day)

###################################################################
#### Part 1: differences in abundance by H2O Periodicity

#### Step 1: read in the data
Germ=read.csv("Master_GermForRQ3Abundance_26Oct2014.csv")

#### Step 2: change treatment label columns from integer to nominal values
Germ$Block=as.factor(Germ$Block)
Germ$Subblock=as.factor(Germ$Subblock)
### Step 3a: load the package needed to run the GLMM models
library(lme4)
library(lsmeans)
library(lattice)

### Step 3b: Run a generalized linear mixed effect model to get estimated total seedling emergence abundances
Germ.glme=glmer(Total~ Moisture*Shade*H2OPeriodicity+(1|Block)+(1|Subblock:Block), data=Germ, family=poisson)
summary(Germ.glme)
anova(Germ.glme)

#Graphing results
library(lattice)
lsmip(Germ.glme, H2OPeriodicity~Shade|Moisture, type="response", ylab="Mean Total Seedling Emergence (#)", xlab="Shade Treatment")

#Getting the mean result values from model
means=lsmeans(Germ.glme, ~Moisture*Shade*H2OPeriodicity)
means
summary(means, type="response")

#comparing means for significant differences
means=lsmeans(Germ.glme, pairwise~Moisture*Shade*H2OPeriodicity)
means
summary(means, type="response")

#comparing means for H2OPeriodicity and shade combinations within moisture trts
means=lsmeans(Germ.glme, pairwise~H2OPeriodicity*Shade|Moisture)
means
summary(means, type="response")

#Recalculating the means and graphing the H2O periodicity *Shade interaction holding moisture constant
means=lsmeans(Germ.glme, ~H2OPeriodicity*Shade*Moisture)
lsmip(means, H2OPeriodicity~Shade|Moisture, type="response", ylab="Mean seedling emergence (#)", xlab="Shade treatment")

### Step 4b: Examine the differences in estimated total emergence solely due to H2OPeriodicity for each of the Moisture/Shade treatment combinations
means.period=lsmeans(Germ.glme, pairwise~H2OPeriodicity|Moisture*Shade)
means.period = lsmeans(Germ.glme, pairwise ~ H2OPeriodicity * Moisture * Shade)

means.period

summary(means.period, type = "response")

library(lattice)
lsmip(Germ.glme, Moisture ~ Shade | H2OPeriodicity, type = "response", ylim = c(1, 3),
ylab = "Mean Seedling Emergence (#)", xlab = "Shade Treatment")

### Step 4c: Get the results of that test

means.period

### Step 4d: Get the estimates in their exponentiated form so that the rate is
### the actual estimated mean emergence number per treatment

summary(means.period, type = "response")

############################
# Graphing abundance by periodicity

Germ = read.csv("Master_GermForRCh3Q3Part1_25Oct2014.csv")

Germ$Block = as.factor(Germ$Block)
Germ$Subblock = as.factor(Germ$Subblock)
Germ$Shade = as.factor(Germ$Shade)
Germ$Moisture = as.factor(Germ$Moisture)
Germ$H2OPeriodicity = as.factor(Germ$H2OPeriodicity)

Germ.glme = glmer(Total ~ Moisture * Shade * H2OPeriodicity + (1 | Block) + (1 | Subblock: Block),
data = Germ, family = poisson)

library(lattice)
lsmip(Germ.glme, H2OPeriodicity ~ Shade | Moisture, ylim = c(1, 3))

means = lsmeans(Germ.glme, ~ Moisture * Shade * H2OPeriodicity)
lsmip(means, H2OPeriodicity ~ Shade | Moisture, type = "response", ylab = "Mean seedling emergence (#)", xlab = "Shade treatment")

################################################## Part 2: Differences in timing due to H2O Periodicity ####################################################
# Read in data and check structure
Germ = read.csv("GermForRQ3DayFirstSeedling_1Nov2014.csv")
str(Germ)

# format variables from integers into factors
Germ$Block = as.factor(Germ$Block)
Germ$Subblock = as.factor(Germ$Subblock)
Germ$Shade=as.factor(Germ$Shade)
Germ$Moisture=as.factor(Germ$Moisture)
Germ$H2OPeriodicity=as.factor(Germ$H2OPerio)

#Check structure of the data
str(Germ)

#Load needed packages
library(lme4)
library(lsmeans)

#Subset data so that only data where H2OPeriodicity =1 are used:
subset(Germ, Moisture=="84ml")

#Run a model of using the subsetted data; Model is a generalized linear
# mixed effect model, and is fit by maximum likelihood (glmerModel on the
# total germ data)
Germ.glme=glmer(Day~H2OPeriodicity*Shade+(1|Block)+(1|Subblock:Block),
data=subset(Germ, Moisture=="84ml"), family=poisson)
#Get summary data of the model
summary(Germ.glme)

#Run anova of the influences to determine how much variance is accounted
# for by each fixed effect
anova(Germ.glme)

#Run model of both 84 and 168ml data, and get means
Germ.glme=glmer(Day~H2OPeriodicity*Shade*Moisture+(1|Block)+(1|Subblock:Block),
data=Germ, family=poisson)
means=lsmeans(Germ.glme, ~H2OPeriodicity*Shade*Moisture)
means
summary(means, type="response")

#Determine treatment with earliest and latest estimated emergence date
# (look at the rates, rates=estimated mean day of first emergence for
# each treatment)

#Run pairwise comparisons to check if rates (estimated day of first emergence)
# are significantly different from each other
means=lsmeans(Germ.glme, pairwise~H2OPeriodicity*Shade)

#Get results of pairwise comparisons
means
summary(means, type="response")

#looking for sig. difs in emergence for a given moisture treatment between
# different shade treatments to reduce families adjusted for in pairwise means.shade=lsmeans(Germ.glme, pairwise~H2OPeriodicity|Shade)

#Get results of pairwise between moistures within shade means.shade summary(means.shade, type="response")

#look for sig. difs in emergence date between moistures within shade treatments means.moisture=lsmeans(Germ.glme, pairwise~H2O|Shade)

#Get the results means.moisture summary(means.moisture, type="response")

###########################################################################
#########
#graph the results for H2OPeriodicity 1 lsmip(means, Moisture~Shade, type="response", ylab="Predicted seedling emergence (mean #)", xlab="Shade treatment")

lsmip(means, Shade~Moisture, type="response", ylab="Predicted seedling emergence (mean #)", xlab="Moisture treatment")

lsmip(means, Moisture~Shade, type="response")
lsmip(means, Shade~Moisture, type="response")

#############
#Graph all timing results
#Read in data
Germ=read.csv("GermForRQ3DayFirstSeedling_1Nov2014.csv")
str(Germ)

#format variables from integers into factors Germ$Block=as.factor(Germ$Block) Germ$Subblock=as.factor(Germ$Subblock) Germ$Shade=as.factor(Germ$Shade) Germ$Moisture=as.factor(Germ$Moisture) Germ$H2OPeriodicity=as.factor(Germ$H2OPerio)

#Run model and get means Germ.glme=glmer(Day~H2OPeriodicity*Shade*Moisture+(1|Block)+(1|Subblock:Block), data=Germ, family=poisson)
means=lsmeans(Germ.glme, ~H2OPeriodicity*Shade*Moisture)

#Graph means
lsmip(means, H2OPeriodicity~Shade|Moisture, type="response", ylab="First Seedling Emergence (Day)", xlab="Shade Treatment")

lsmip(means, Shade~H2OPeriodicity|Moisture, type="response", ylab="First Seedling Emergence (Day)", xlab="Watering Periodicity")

# H2OPeriodicity 2 results (168ml treatments)

#Read in data and check structure
Germ=read.csv("GermForRQ3DayFirstSeedling_1Nov2014.csv")
str(Germ)

#format variables from integers into factors
Germ$Block=as.factor(Germ$Block)
Germ$Subblock=as.factor(Germ$Subblock)
Germ$Shade=as.factor(Germ$Shade)
Germ$Moisture=as.factor(Germ$Moisture)
Germ$H2OPeriodicity=as.factor(Germ$H2OPerio)

#Check structure of the data
str(Germ)

#Load needed packages
library(lme4)
library(lsmeans)

#Subset data so that only data where H2OPeriodicity =1 are used:
subset(Germ, Moisture=="168ml")

#Run a model of using the subsetted data; Model is a generalized linear mixed effect model, and is fit by maximum likelihood (glmerModon the total germ data)
Germ.glme=glmer(Day~H2OPeriodicity*Shade+(1|Block)+(1|Subblock:Block),
data=subset(Germ, Moisture=="168ml"), family=poisson)

#Get summary data of the model
summary(Germ.glme)

#Run anova of the influences to determine how much variance is accounted for by each fixed effect
anova(Germ.glme)

#Run the means (expected total germ values) for glmer model
means=lsmeans(Germ.glme, ~H2OPeriodicity*Shade)

#Get the model results in table form:
means
summary(means, type="response")

#Determine treatment with earliest and latest estimated emergence date
# (look at the rates, rates=estimated mean day of first emergence for
# each treatment)

#Run pairwise comparisons to check if rates (estimated day of first emergence)
# are significantly different from each other
means=lsmeans(Germ.glme, pairwise~H2OPeriodicity*Shade)

#Get results of pairwise comparisons
means
summary(means, type="response")

#looking for sig. difs in emergence for a given moisture treatment between
# different shade treatments to reduce families adjusted for in pairwise
means.shade=lsmeans(Germ.glme, pairwise~H2OPeriodicity|Shade)

#Get results of pairwise between moistures within shade
means.shade
summary(means.shade, type="response")

#look for sig. difs in emergence date between moistures within shade treatments
means.moisture=lsmeans(Germ.glme, pairwise~H2O|Shade)

#Get the results
means.moisture
summary(means.moisture, type="response")

########################################################################
#Graphing the results for both periodicities
Germ=read.csv("GermForRQ3DayFirstSeedling_1Nov2014.csv")
Germ$Block=as.factor(Germ$Block)
Germ$Subblock=as.factor(Germ$Subblock)
Germ$Shade=as.factor(Germ$Shade)
Germ$Moisture=as.factor(Germ$Moisture)
Germ$H2OPeriodicity=as.factor(Germ$H2OPerio)
Germ.glme=glmer(Day~H2OPeriodicity*Shade*Moisture+(1|Block)+(1|Subblock:Block),
data=Germ, family=poisson)
means=lsmeans(Germ.glme, ~H2OPeriodicity*Shade*Moisture)
lsmip(means, H2OPeriodicity~Shade|Moisture,type="response", ylab="First Seedling
Emergence (Day)", xlab="Shade Treatment")
lsmip(means, Moisture~Shade|H2OPeriodicity,type="response", ylab="First Seedling Emergence (Day)", xlab="Moisture Treatment")

#graph the results for H2OPeriodicity 1 only
Germ=read.csv("Master_GermForRCh3Q3Part1_25Oct2014.csv")
Germ$Block=as.factor(Germ$Block)
Germ$Subblock=as.factor(Germ$Subblock)
Germ$Shade=as.factor(Germ$Shade)
Germ$Moisture=as.factor(Germ$Moisture)
Germ$H2OPeriodicity=as.factor(Germ$H2OPeriodicity)
library(lattice)
Germ.glme=glmer(Total~ Moisture*Shade*H2OPeriodicity+(1|Block)+(1|Subblock:Block),
data=Germ, family=poisson)
lsmip(Germ.glme, H2OPeriodicity~Shade|Moisture, ylab="Predicted seedling emergence (mean #)", xlab="Shade treatment")

means=lsmeans(Germ.glme, ~Moisture*Shade*H2OPeriodicity)
lsmip(means, H2OPeriodicity~Shade|Moisture,type="response", ylab="Mean Total Seedling Emergence (#)", xlab="Watering Periodicity")
lsmip(means, Moisture~Shade, type="response", ylab="Mean Total Seedling Emergence (#)", xlab="Watering Periodicity")
lsmip(means, Shade~Moisture, type="response", ylab="Mean Total Seedling Emergence (#)", xlab="Moisture Treatment")
lsmip(means, Moisture~Shade, type="response")
lsmip(means, Shade~Moisture, type="response")