Investigating the Viability of *Agave americana* as a Potential Bioenergy Feedstock and its Relative Impact on Surrounding Wildlife

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Emily R. Kuzmick

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This thesis titled
Investigating the Viability of *Agave americana* as a Potential Bioenergy Feedstock and
its Relative Impact on Surrounding Wildlife

by

EMILY R. KUZMICK

has been approved for
the Program of Environmental Studies
and the Voinovich School of Leadership & Public Affairs by

Sarah C. Davis
Assistant Professor of the Voinovich School of Leadership and Public Affairs

Mark Weinberg
Director, Voinovich School of Leadership & Public Affairs
ABSTRACT

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Investigating the Viability of *Agave americana* as a Potential Bioenergy Feedstock and its Relative Impact on Surrounding Wildlife

Director of thesis: Sarah C. Davis

*Agave* species have the possibility for energy efficiency and high net productivity, largely due to the advantages of Crassulacean Acid Metabolism (CAM). High water use efficiency (WUE) is a primary advantage of CAM that may reduce economic and environmental costs of water intensive practices common in arid agriculture. However, little is known about the effect of *Agave* cultivation on native wildlife. This study aimed to (a) assess the productivity and WUE of native *Agave americana* as a bioenergy feedstock, and to (b) investigate the impact of *Agave* crops on native wildlife species relative to the impact of a conventional crop (cotton) in Maricopa, AZ. Potential productivity of *A. americana* subjected to four irrigation treatments for three years was estimated. Pitfall arrays were used to sample wildlife biodiversity in the *Agave* field, a site with native vegetation, and a site with conventional agriculture from June to August of 2014. Estimated annual yields of *Agave* plants that have not yet reached full maturity ranged from 1.59 – 3.45 Mg ha\(^{-1}\); WUE ranged from 2.33 – 8.65 kg ha\(^{-1}\) mm\(^{-1}\). The greatest species diversity and amphibian abundance was observed in the cotton site, while greater species richness and mammalian abundance was observed in the *Agave* site. These findings indicate *A. americana* may produce commercially viable yields, as well as support local wildlife communities, when managed appropriately in arid regions.
DEDICATION

This work is dedicated to my loving mother, who has always encouraged me to pursue my dreams — even the wild ones.
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CHAPTER 1: INTRODUCTION

The rapid depletion of non-renewable resources, the search for more efficient and productive alternatives, the desire for national energy independence, and concern for a changing climate all contribute to increased interest in renewable energy sources. Bioenergy in particular has been a growing research area: in 2007, The Renewable Fuel Standard increased the required gallons of renewable fuel to be incorporated into gasoline to 36 billion gallons by the year 2022 (U.S. EPA 2010), and in 2012, a correction notice was issued allowing an increase in the amount of alcohol blended into fuel, from 25 mg L\(^{-1}\) to 42.7 mg L\(^{-1}\) (U.S. EPA 2012). Bioenergy, a form of energy that involves the utilization of resources that are renewable as well as cleaner burning compared to fossil fuels, offers an opportunity that can increase energy independence while simultaneously reducing emissions. Advanced energy cropping systems are capable of displacing carbon from fossil fuels, which are the primary source of carbon dioxide emissions, due to their ability to sequester carbon in both above- and below-ground biomass (Sartori et al. 2006).

Despite these benefits, bioethanol has been criticized as a costly and detrimental energy practice, both to society and the environment. The production of intensively managed corn, nearly half of which is now used for ethanol production in the United States, has been the focus of such criticism (USDA 2014). Some disadvantages of corn cultivation for bioenergy purposes include exploitation of a food crop, land clearing of native ecosystems (Fargione et al. 2008), and increased emissions due to the conversion of already productive lands to new croplands (Searchinger et al. 2008). There are, however, many alternative bioenergy crops and practices under consideration that might
achieve greater environmental benefits than corn ethanol production (Fargione et al. 2008; Borland et al. 2009; Dale et al. 2010; Davis et al. 2011).

Some novel crops, such as succulent plants, may have advantages over the first-generation bioenergy feedstocks that have been developed from traditional food crops. *Agave* species in particular may be a beneficial bioenergy crop since the physiology of these plants allows for high water use efficiency (WUE), defined as the amount of net carbon dioxide uptake per unit water lost through transpiration (Nobel 2010). As *Agave* can be grown on abandoned agricultural, marginal, and water-deprived lands that are unfit for many other crops, its cultivation does not involve the displacement of lands. *Agave* species are also not typically a modern widespread food source. In addition, the structural composition of *Agave* allows for the more efficient conversion of biomass into liquid fuel than other more structurally complex feedstocks (Davis et al. 2011). Species of *Agave* thus have great potential for increasing carbon sinks, and may have a lesser impact on surrounding environments. While there has been significant cultivation of *Agave* for spirit production (*i.e.* tequila), this crop has not been produced commercially for bioenergy, and experimental trials are just beginning. This study measures production of young *Agave americana* in the first experimental field trial in the U.S.

*Agave* species grow natively in the arid region of the southwestern United States and can contribute to desert ecosystem productivity, as well as provide habitat to a variety of animals. Their natural occurrence in desert regions suggests that, when managed as a crop, there will be lower impacts on surrounding desert wildlife. Although there is strong evidence for the potential of *Agave* as a sustainable energy crop
(Somerville et al. 2010, Davis et al. 2014), little is known about the effect on wildlife the cultivation of this native crop may pose. The impact of bioenergy agriculture on biodiversity has been addressed in past studies, but usually only for specific sites, most of which are in other countries (e.g. Germany (Gevers et al. 2011) and Australia (Ribeiro et al. 2009)), or the Midwestern U.S. (Meehan et al. 2010).

Sustainable bioenergy production is best achieved with feedstocks that do not require additional land clearing, and thus the destruction of habitat (Dale et al. 2010, Fargione et al. 2009). In considering wildlife conservation, especially in desert ecosystems, there is evidence that reptiles are especially affected by agricultural activity due to their ecological and physical constraints, including their low capacity for dispersal (Ribeiro et al. 2009). A study examining lizard distributions in Tucson, Arizona emphasized that reptiles are among the least understood animals in terms of response to development (Germaine and Wakeling 2000), yet they are one of the most abundant land-dwelling vertebrate classes within desert ecosystems. Comparing species richness and diversity across diverse land uses may reveal whether conventional agricultural activities are more detrimental to reptiles, as well as to other vertebrates, compared to low-input cultivation of a native crop. Although the U.S. southwest may be an ideal location for agricultural energy development that does not compete with food agriculture (Davis et al. 2011), the impact of new agricultural systems on biodiversity deserves attention.

This project addresses wildlife conservation in agricultural ecosystems of the southwestern U.S. while simultaneously exploring a new alternative energy source with minimal land-clearing and water requirements. The study sites are located in Pinal
County, Arizona, which contains 72 currently recorded species of amphibians and reptiles (HerpNet 2012), as well as 133 desert mammals throughout the entire state (American Society of Mammalogists 2015). The objectives of the study are to determine whether *Agave americana* is a successful bioenergy crop in the region, in terms of plant productivity and in terms of resource and wildlife conservation.
CHAPTER 2: LITERATURE REVIEW

Renewable Energy Sources

There have been a variety of policy standards adopted to reinforce renewable energy progress and development across the United States. For example, the Arizona Corporation Commission ratified the Renewable Energy Standard and Tariff in 2006, calling for regulated electric utility companies to produce 15 percent of their energy from renewable resources by the year 2025, much of which is aimed towards the installation of solar panels (AZCC 2010). Likewise, the amount of wind power capacity in the United States has nearly doubled within the past five years (AWEA 2014). Due to the recent surpassing of 400 parts per million (ppm) in global atmospheric carbon dioxide levels, higher renewable energy standards are a welcome step forward. However, there is often a disconnect between what is truly “sustainable” energy. For example, the United States Environmental Protection Agency defines renewable fuels as “those derived from renewable, non-petroleum sources such as crops, animal waste, or municipal solid waste,” while alternative fuels are defined as simply any “alternatives to traditional gasoline and diesel fuels” (U.S. EPA 2013). This, along with discrepancies between parties in defining “sustainability,” can hamper the well-intentioned displacement of fossil fuels and cause further environmental degradation.

While the need for renewable energy sources to displace fossil fuels is clear, there can be unintended environmental consequences. Specifically, wildlife conservation has become an area of special concern when considering the implementation of renewable energy systems (Bies 2006). The wind, solar, and bioenergy industries in particular have
been confronted with criticisms and questions regarding whether or not their practices, while efficient in generating few net emissions, are necessarily beneficial for species diversity, habitat protection, and the overall preservation of wildlife (Barclay et al. 2007, Kuvlesky et al. 2007, Lovich and Ennen 2011).

A common dispute associated with wind energy implementation is the deaths of avian species due to the height and size of wind turbines. A study reviewing the relative impact of wind turbines on birds and bats found that tower height did not influence the number of bird fatalities, but very much impacted bat fatalities. However, bird fatalities were more likely to be correlated with the number of total turbines in a single area (Barclay et al. 2007). There is also evidence that wind turbines cause a significant number of deaths in golden eagles in the United States, while golden eagles in Scotland are more likely be affected due to habitat displacement by wind farms (Fielding et al. 2006). The infrastructure surrounding wind turbine development (e.g. roads, control facilities, transition lines) poses potentially greater threats to wildlife due to the expanse of associated development (Kuvlesky et al. 2007).

Solar energy, which has been commended for its exceptional potential for displacing fossil fuels as a non-emitting alternative energy form, has also been associated with adverse impacts on wildlife habitat. Potential consequences range from habitat fragmentation to prevention of gene flow to altering microclimates, all of which raise concerns for utility-scale solar energy development (USSED) (Lovich and Ennen 2011). These possible issues are especially pronounced in the U.S. desert southwest, where fenced-off facilities limit various sensitive species’ territories (e.g. Gopherus agassizii,
the desert tortoise) and intense temperatures are exacerbated, causing incineration of small arthropod species. This, however, quantifies as “low impact” for wildlife and habitat in comparison to other anthropogenic activities (e.g. a mere six deaths per year in bird populations were recorded as a result of solar facilities in a 2011 study) (Turney and Fthenakis 2011). Although such minor impacts could be justified in comparison to more destructive non-renewable energy systems, failure to acknowledge these negative effects upon wildlife and the environment could easily delay further alternative energy development based on a lack of impact assessments.

While solar and wind power have been in the forefront of renewable energy development for the past decade, the bioenergy industry has been gaining substantial momentum as research has expanded to investigate a broad range of bioenergy feedstocks that may be suited to different environmental conditions. Corn, the leading crop in the United States, has been the poster child of the bioenergy sector for years, which has not exactly given bioethanol the greenest reputation: land-use change associated with the production of corn for bioenergy often causes the destruction of native ecosystems and actually increases greenhouse gas emissions (Fargione et al. 2008), while irrigation necessary for corn cultivation in arid and semi-arid regions depletes natural water resources that would otherwise be attributed to riparian corridors or watersheds (Lemly et al. 2000), as over 90% of consumptive water use in water-scarce areas across the globe is attributed to agricultural irrigation (Richter 2014). Irrigation in arid regions can be especially detrimental to native wildlife populations and wetlands due to subsurface irrigation drainage, which is common in places where the rate of evaporation exceeds that
of precipitation. Subsurface irrigation drainage results in the build-up of salts, trace elements, and alkalinity below the surface of the soil, which then drains into waterways, causing contamination (Lemly 1993). Specifically, groundwater recharge associated with land-use change in the United States southwest has been found to be detrimental to water quality, as the conversion of rangelands to agricultural lands in this region results in the downward displacement of nutrients, thus potentially contaminating groundwater (Scanlon et al. 2005). In considering wildlife, subsurface irrigation drainage has caused death and deformities in thousands of waterfowl, inflicted harm upon migrating birds due to irrigation in arid flyway zones, and contributed to selenium contamination of every animal group in the San Joaquin Valley of California (Lemly 1993).

Choosing appropriate management practices is a crucial step in achieving a sustainable and successful bioenergy system (Davis et al. 2013). For example, replacing traditional row crops with grasslands, opting for strip harvesting (i.e. harvesting small portions of agriculture at different time intervals rather than harvesting all crops at once), and leaving biomass residue to aid in the maintenance and establishment of wildlife communities have recently been acknowledged as adequate, or even essential, mitigation practices. The Chariton Valley Project in Iowa is one study location currently examining the benefits that mitigation factors might have upon surrounding fauna (Bies 2006). Many of these strategies and proposed research projects are just beginning to become an integral part of the bioenergy industry, and as global carbon levels continue to rise, the pressure to produce environmentally sound energy supersedes that of economic feasibility.
CAM for Bioenergy

Choosing bioenergy feedstocks that avoid land and resource degradation, competition with the food industry, and provide possible compatibility with surrounding ecosystems have the ability to serve as carbon sinks and reduced emitters. For example, plant species that utilize Crassulacean Acid Metabolism (CAM) as a photosynthetic pathway — typically desert succulents (*e.g.* *Agave* spp.) — have a theoretical potential biomass yield 147% greater than *C₄* species (*i.e.* grasses, *e.g.* corn, miscanthus, sorghum) (Davis et al. 2014). This is due to the unique physiology of CAM, different from the other photosynthetic pathways of *C₃* and *C₄*, and having evolved repeatedly in response to limited water availabilities (Borland et al. 2011).

CAM has four separate phases of activity that contribute to the low water requirements of CAM species: Phase I involves carbon fixation by PEPC (phosphoenolpyruvate carboxylase), resulting in the accumulation of carbon during the dark period, or night, which is stored as malic acid in the vacuole. Once the dark period ceases (*i.e.* dawn), Phase II begins, and there is a shift from PEPC to rubisco (ribulose biphosphate carboxylase/oxygenase) activity. As temperatures rise during the day, Phase III begins with stomatal closure to prevent water loss, and the decarboxylation of malic acid allows CO₂ to accumulate at the rubisco activation site. Finally, phase IV concludes the cycle with the onset of cooler evening temperatures, when stomata reopen and allow atmospheric CO₂ to diffuse into the leaf while rubisco is still active (Borland et al. 2011, Davis et al. 2014).
Aside from stomatal closure during the hotter daytime hours, CAM plants also maintain high WUE due to their succulence, which allows for the holding of considerable amounts of water for extended periods of time, as well as for hydraulic isolation from dehydrated soil (Davis et al. 2014). Measuring malic acid accumulation (quantifying titratable acidity) and carbon assimilation (quantifying leaf gas exchange over 24 hours) are two ways one can estimate the relative productivity of CAM species. Biomass harvests are, of course, another way to estimate productivity. Minimal water input and increased biomass yields are both desirable traits in the agricultural sector, making CAM plants inherently promising bioenergy candidates, since their exceptional productivity allows for the accumulation of biomass throughout harsh conditions. However, as the bioenergy industry is relatively new in itself, much research on CAM plants for bioenergy is still needed (Escamilla-Treviño 2012, Davis and Long 2014, Yang et al. 2015).

CAM species at the forefront of modern bioenergy research are from the genera *Opuntia* and *Agave*. There is current research geared towards the species *Opuntia ficus-indica* (the prickly pear) due to its promising biomass yields, its ability to thrive in arid and semi-arid conditions, and its wide-spread presence in both natural and agro-ecosystems throughout the globe today (Borland et al. 2009). According to a study examining the species *Opuntia ficus-indica*’s growth, it was found that soluble sugars, starch content, and productivity increased in response to elevated CO$_2$ (Nobel and Cui 1994). An experimental trial has been established in southern Nevada in an attempt to assess the productivity of *Opuntia* for bioenergy purposes, since productivity resultant of
commercial fodder and forage ranges from 47 – 50 Mg ha\(^{-1}\) y\(^{-1}\) (Nobel 1996), and its high WUE and drought-tolerance make for a favorable bioenergy feedstock (Cushman et al. 2015). However, \textit{Opuntia} spp. have also been vilified for its potentially invasive qualities, making its establishment as an energy crop questionable (Novoa et al. 2014).

\textit{Agave} has also received recent attention in the bioenergy sector due to its native abundance in arid regions and its ability to thrive in marginal and abandoned agricultural lands in conditions unsuitable for other commercial energy and food crops (Davis et al. 2011). Consequently, the competition for land and resultant direct and indirect land-use change can be reduced if \textit{Agave} is adopted as a cropping system, over more intensive, conventional systems. This facet is particularly valuable since the bioenergy industry has been heavily criticized for its exploitation of undisturbed ecosystems and the alteration of natural lands (\textit{e.g.} grasslands, rainforests, savannas, etc.) to accommodate the production of food crop-based bioenergy feedstocks (\textit{e.g.} corn, sugarcane). Land clearing can release 17 to 420 times more CO\(_2\) than projected annual reductions in greenhouse gases, thereby generating a “biofuel carbon debt” (Fargione et al. 2008). Although the incorporation of biofuels into the energy market aims to eventually repay this carbon debt (provided management and production practices generate less greenhouse gas emissions than those produced from fossil fuels), the payback time varies with species, location, and management practice (Fargione et al. 2008, Davis et al. 2013). However, cultivating biofuel feedstocks on degraded and abandoned agricultural lands could help successfully mitigate greenhouse gas emissions associated with global climate change more immediately, as well as minimize habitat destruction. Risks associated with the
exploitation of biologically diverse and culturally important lands persist with any new resource production system, and research that defines this risk is essential.

Agave Species Potential

*Agave* species are excellent candidates for researching potential environmental benefits and risks associated with bioenergy production in arid regions because they are native to rain-deprived regions in the United States southwest and Mexico. Although currently grown for spirit production, the species *Agave tequilana* has proven to be a successful plantation crop in areas experiencing limited rainfall where many species are incapable of thriving. *Agave sisalana* and *Agave fourcroydes* are grown commercially for fiber, including in South Africa and Australia, the latter of which has supported *Agave* agriculture since before 1915 (Ravenscroft et al. 2009, Holtum et al. 2011). Having been an established crop for so long in a water-deprived region further proves the resilience of these species, as well as their potential to be grown for alternative purposes. Not only does this increase potential land availability for a new agricultural commodity, but this also carries the possibility of increased job creation in areas where agriculture is not yet established due to less than ideal climatic conditions.

There is evidence that *Agave* spp. can be grown in a number of different soil types, so that most free-draining soils should be adequate for cultivation (Holtum et al. 2011). Identifying crops that can be cultivated on existing plantations where many other crops cannot be grown is a rare occurrence and one that can be incorporated into renewable energy initiatives: there have been trends examined in *Agave* cultivation on abandoned plantations, which reveal their ability to thrive in these marginal or degraded
lands, while other crops might have inconsistent production trends, depending on annual and seasonal climatic conditions (e.g. high temperatures, lack of rainfall) (Davis and Long 2014). While there is a definite need for refining cultivation, harvesting, and production techniques, there is commercial potential for Agave as a bioenergy crop in these regions.

Like other CAM species, Agave has high WUE, and thus low water requirements. This combination of minimal land and irrigation needs makes Agave a potentially low-impact crop, specifically in regions facing drought and other resource deficiencies. Structurally, Agave spp. are desirable energy candidates since they tend to have relatively low lignin content but high levels of soluble carbohydrates, both of which facilitate the conversion process of plant biomass into liquid fuel (Davis 2011). Lower lignin levels allow for the more efficient deconstruction of fibers, and abundant carbohydrates offer readily available sugars to be converted into bioethanol. These structural characteristics are rooted in the physiology of CAM: low transpiration causes low xylem tension, thus requiring less lignin to support the xylem (Davis et al. 2011). Soluble carbohydrates are accumulated as a result of aiding PEPC-mediated carboxylation, producing low molecular weight sugars, such as glucose and sucrose (Borland et al. 2009). The thick, epicuticular wax layer found on the outside of Agave leaves allows for resistance to climatic and other potential environmental inflictions, while the morphological characteristics of Agave protect aboveground biomass from herbivorous predation (e.g. long pointed and barbed leaves) (Borland et al. 2009). These traits make Agave a hardy and resilient crop that can withstand a variety of harsh conditions.
While these morphological, structural, and physiological traits are all promising assets to the bioenergy industry, actual estimated yields are what will determine the success of integrating Agave as a commercial feedstock into the bioenergy industry. Previous studies indicate that Agave species may have a greater net productivity than many other bioenergy species (Somerville et al. 2010; Davis et al. 2011; Davis et al. 2014); however, this depends upon location and climate, which influence land and water requirements. Typically, productivity is expressed in terms of biomass per hectare (1 ha = 10,000 m²) and is based upon either total dry mass — because water fluctuations due to changes in seasonal or instantaneous rainfall can influence wet, or fresh, mass — or aboveground (shoot) mass, since belowground (root) mass generally contributes to only 8 – 12% of total plant biomass, compared to 30 – 50% for C₃ and C₄ plants (Nobel 2010). Theoretical yields based upon data from previously published literature found that Agave fourcroydes, Agave tequilana, and Agave sisalana yield 8.5 Mg ha⁻¹ y⁻¹, 22 Mg ha⁻¹ y⁻¹, and 12 Mg ha⁻¹ y⁻¹ harvestable biomass, resulting in an estimated annual ethanol product of 3.3 kL ha⁻¹, 9.7 kL ha⁻¹, and 4.7 kL ha⁻¹, respectively (Davis et al. 2014).

Some species have recently been viewed as having higher potential in the bioenergy sector, despite little consideration in the past for this functional purpose. One such species is Agave americana, otherwise known as the “century plant,” attributed to its relatively lengthy lifespan before reaching full maturity and flowering. Also referred to as a “maguey” and originating as far back as the 15th and 16th centuries, this species is among those whose sap is used to produce pulque and aguamiel, which is used in common beverages today (e.g. alcohol, energy drinks) (Nobel 2010). Other species have
been used historically and presently for fiber (e.g. *A. fourcroydes*, *A. sisalana*) and tequila production (e.g. *A. tequilana*). By contrast, *A. americana* has been used in the past for birth control, as it contains considerable amounts of sapogenins, steroids that can be converted rather easily into progesterone and estrogen (Nobel 2010). Today, it is a common ornamental species, due to its generally broad and sometimes variegated (*i.e.* lined) leaves, thickened base, and ornate flowers upon inflorescence (Nobel 2010). This species thrives well in climates ranging from Mediterranean, arid desert, semi-arid desert, to southern temperate, and is tolerant of urban development, drought, and variation in soil type (Gentry 1982). It is native to the United States southwest and Mexico and is potentially cold tolerant when compared to other species — *A. tequilana* in particular is relatively intolerant of cold temperatures (Nobel et al. 1998).

These morphological and ecological aspects, as well as industrial successes, might indicate that *A. americana* can serve as an effective bioenergy crop. Anatomically, broader leaves could lead to increased productivity by aiding in increased photosynthetically active radiation (PAR) as a result of increased surface area for light intake. Functionally, *A. americana* has recently proven to be a successful crop in the impoverished South African region of Great Karoo, where it has promoted the creation of jobs and both small-scale and commercial industries in textiles, fibers, and paper products, with the possibility for zero-waste management (Boguslavsky et al. 2007). These attributes make *A. americana* a prime candidate for a modern bioenergy feedstock in marginal or degraded lands within arid or semi-arid climates.
The exceptional drought tolerance and high WUE of *A. americana* begs the question of whether or not the species requires additional water input in order to produce a commercially viable yield for bioenergy purposes. Assessing whether or not irrigation is required to successfully cultivate these crops in arid regions would eliminate concern for subsurface irrigation drainage and the displacement of natural water resources in drought-stricken regions. Furthermore, the consideration of a crop native to the United States southwest offers potential wildlife benefits, as native fauna may be adapted to the native flora belonging to a region.

**Environmental Impact**

Renewable energy development is faced with the difficult task of displacing fossil fuels, an industry upon which our country has depended for development. Consequently, it is challenging to propose a shift in the energy industry from non-renewable to renewable sources. In framing renewable energy development as a response to rapidly increasing rates of atmospheric CO$_2$ levels — the years 1957 – 1999 resulted in an average annual increase of 1.4 ppm, while the years 2000 – 2010 resulted in an average annual increase in 2.1 ppm — and the resultant increasing temperatures and decreasing rainfall in regions already experiencing higher temperatures and drought rates, the public is much more likely to agree with this shift in the energy market. Fortunately, there is evidence that CAM plants in particular are expected to thrive amidst these climatic changes. CAM biomass tends to increase with atmospheric CO$_2$ levels, and as these plants have evolved to withstand extremely high temperatures and long periods of drought, their success in desert regions is expected to continue (Nobel 2010). In
considering the most practical energy form for places such as the Sonoran Desert region, CAM bioenergy feedstocks (*e.g.* *A. americana*) might do well in the modern energy sector.

For decades, arid regions have supported agricultural production that requires irrigation far beyond levels of natural rainfall. However, bioenergy has not been the renewable energy form of focus in arid regions as of late: the copious amounts of solar radiation have put solar energy at the forefront of renewable energy development in the United States southwest. While the widespread integration of this energy practice has proven successful in many locations, the idea of combining both solar energy and CAM bioenergy offers an interesting outlook on how to maximize energy potential in these areas deficient of land and water resources. A study examining the possibility of integrating these two energy forms within a single landscape found that the water required for cleaning solar panels is similar to the amount required to irrigate viable yields of *Agave* (Ravi et al. 2014). This is just one example of how the combination of alternative sources could produce sufficient amounts of renewable energy while taking into consideration rising emissions and environmental impact in sensitive regions.

While there are many instances where biodiversity is valued solely for the conservation or aesthetics of an environment, there is evidence that biodiversity may be just as important for ecosystem functioning as other biotic factors (Frainer et al. 2014), and that species diversity may have a dynamic relationship with ecosystem productivity (Cardinale et al. 2000). There is indication that biodiversity within an agro-ecosystem, specifically ‘associated biodiversity’ (defined as the organisms that colonize within a site
based upon its structure and management), not only has the ability to support sustainability, but also the ability to provide ecological services, including nutrient cycling, pest control, and the conservation of natural resources (e.g. water and soil) (Altieri 1999). Additionally, certain non-prey species have been proven to be sufficient indicators of their surrounding environment, particularly in transitional agricultural systems in the southwestern United States (Dean 2005). Therefore, wildlife impact assessments are essential to the success of sustainable and healthy agricultural systems.

In a study that reviewed sustainability criteria within the bioenergy industry, 137 experts rated criteria for bioenergy systems in terms of relevance, practicality, reliability, and importance: environmental criteria were rated highest in terms of both importance and relevance; these were followed by economic, then social criteria, the last of which includes employment generation (Buccholz et al. 2009). While experts in this field are concerned with the economic success of the industry, the majority still considered the biotic features of a landscape the most important factor for sustainability, as no economic or societal benefits can come from an energy system that has failed due to an environmental system that cannot support it (Buccholz et al. 2009). Likewise, the persistence of native wildlife in an energy production system would be an indicator of environmental compatibility.

Conversely, agricultural ecosystems play a great role in the functional diversity of wildlife. Specifically regarding Agave species, bats are perhaps the most important animals with which they are associated. Often considered keystone species, bats play an important role in desert ecosystems as pollinators for Agave, as well as for Stenocereus
thurberi (organ pipe cacti) and Carnegiea gigantea (saguaro cacti), the pollination of which sustains a multitude of species within desert regions (Pennisi et al. 2004). The relationship between bats and Agave flowers is particularly interesting since the nectar of these flowers evolved solely for bat pollination: the nectar contains two amino acids that are of no use to the plant itself, but are essential to the species Leptonycteris nivalis and Choeronycteris mexicana (the Mexican long-nosed bat and the Mexican long-tongued bat) for building muscle tissue and for providing growth stimulants in the milk of lactating mothers (Pennisi et al 2004, Arita and Wilson 1987). Agave plants benefit from bat pollination by facilitating seed production, the ratio of which drops as low as 1:3000 in the absence of bats (Howell 1980). This mutualistic relationship is just one example of how surrounding wildlife benefits from the presence of Agave species in desert regions.

Many other animals utilize Agave as well: hummingbirds serve as additional pollinating agents, as do wild and honey bees, and birds ranging from doves, pigeons, and wrens, to ravens and Mexican parrots have also been observed to visit inflorescences, while plants are often foraged upon by a variety of rodents (e.g. ground squirrels, pack rats) (Gentry 1982).

Other species contributing to the surrounding, or associated, biodiversity of Agave plants are likely to be affected by its management. Although the only two federally classified endangered lizard species in the state of Arizona are Heloderma suspectum, the Gila monster, and Phrynosoma mcallii, the flat-tailed horned lizard, reptiles in general are a particularly sensitive class of animals due to their dependence on temperature for activity, including reproductive and metabolic function. Specifically, lizards have critical
thermal maximums and minimums that serve as thresholds for mobility: reported critical thermal minimums range from 1.4 °C (e.g. *Eumeces faciatus*, the five-lined skink) to 13.9 °C (e.g. *Crotaphytus collaris*, the collared lizard) (Spellerberg 1973). This reliance upon temperature makes the issue of climate change that much more relevant for reptilian species, and physiological models have been derived in an effort to predict extinctions in lizard species (e.g. *Sceloporus* spp.) due to projections in increased temperatures, and thus restricted activity times (Sinervo et al. 2011). Desert lizard assemblages are also structured by ecological constraints (e.g. limitations from development, land-use change), which result in restrictions in physiology, morphology, and guild structure (Winemiller and Pianka 1990).

Rainfall has also proven to be an important climatic factor for both reptilian and amphibian classes alike. A study examining the effect of dehydration on the thermal preferences of populations of *Notechis scutatus*, the Western tiger snake, residing within a wetland environment and a semi-arid environment found that wetland snakes had a slightly lower preferred body temperature than semi-arid snakes, while dehydrated snakes from both environments selected a lower preferred body temperature as well (Ladyman and Bradshaw 2003). Meanwhile, amphibians are known to rely upon water intake for functioning: a 14-year study of amphibian metacommunities and populations discovered that the demographics of most amphibian species were influenced by seasonal precipitation, while differences in hydroperiod influenced amphibian diversity and abundance (Brodman 2008).
Whereas mammals are endotherms and rely upon external temperature for regulating and maintaining their body temperature at a physiologically active level, there is evidence that rainfall indirectly influences the reproductive capabilities of a Sonoran Desert species, *Dipodomys merriami* (Merriam’s kangaroo rat), due to the importance of green vegetation in kangaroo rat reproduction (Van de Graaff and Balda 1973). Temperature was found to influence hibernation factors and successes in the same species (Yousef and Dill 1971). As climate change projections involve predictions of increased temperatures and decreased amounts of rainfall in areas already experiencing high degrees of heat and drought, understanding the implications of these variations and planning for anthropogenic activities in a manner that mitigates these changes in the most environmentally responsible manner would promote wildlife conservation.

Having knowledge of surrounding species’ biotic requirements, behavioral tendencies, and life histories could provide valuable information in how to effectively manage agricultural or energy activities, without negatively impacting wildlife. Integrating alien terrestrial plant species into an otherwise native ecosystem has been found to incur the greatest ecological impacts upon ecosystem services in comparison to the integration of other non-native organisms. This suggests that selecting for native flora where possible could aid in avoiding these costs (Vilà et al. 2010). In support of this, an Australian study comparing marsupial and reptilian abundance amongst patches of native and non-native replanted vegetation found that both classes of animals were less likely to occur in patches of land that lacked native flora (Cunningham et al. 2007).
Alternatively, irrigation has been shown to have an impact on native wildlife as well, even in areas not as drought-stricken as the Sonoran Desert. For example, wetlands have continued to degrade as a result of agricultural irrigation, despite federal protection (Lemly et al. 2000). Of course, water use is a much more prevalent issue in water-deprived regions: as recently as April of 2015, California became the first state to implement mandatory restrictions on water use in response to drought and alarming levels of decreased snowpack (Megerian et al. 2015).

Wildlife impact evaluations surrounding bioenergy systems have been considered in a variety of regions, although few have focused solely upon the southwestern United States. A study examining the impact of bioenergy activities upon bird species in the Midwestern U.S. found species diversity to decrease significantly when low-impact high-diversity crops were replaced with high-impact low-diversity crops, indicating that crop choice is just as important in supporting biodiversity as management (Meehan et al. 2010). An additional study focused upon the herbaceous species Panicum virgatum (switchgrass) and evaluated the associated environmental consequences when produced for bioenergy purposes: both crop choice and management practices were deemed important in considering wildlife conservation, particularly if the chosen crop and management provide cover suitable for wildlife (McLaughlin and Walsh 1997).

However, there have been studies done, primarily in tropical regions, that argue management technique as the primary perpetrator of wildlife degradation: fuelwood harvesting in the Brazilian Atlantic Forest has been proven to seriously disturb ecosystems and hamper biodiversity conservation (Specht et al. 2015), while the
promotion of landscape heterogeneity in palm oil production is a proposed management practice that could greatly conserve biodiversity (Azhar et al. 2015). Yet a study focused upon bioenergy production within California’s agricultural systems found that crop choice encompasses management practices as well, as certain crops require more irrigation or land use, resulting in trade-offs between resource conservation and ecosystem displacement (Stoms et al. 2012). Therefore, choosing bioenergy feedstocks and placing them appropriately within landscapes to optimize ecological ecosystem services remains a challenge that research for sustainable bioenergy must continue to investigate (U.S. DOE 2014).

Environmental impact within agro-ecosystems involves the evaluation of multiple biotic and abiotic factors. The integration of a native crop that may not require irrigation, such as Agave americana, into a sensitive desert ecosystem like the southwestern U.S., could offer an environmentally responsible alternative form of renewable energy that has the potential to conserve natural resources (e.g. land, water), as well as wildlife.
CHAPTER 3: METHODOLOGY

Objectives

The objectives of this study are as follows:

1) Measure the effect of irrigation on *Agave americana* biomass production.
2) Determine whether irrigation is required to produce a commercially viable yield of *Agave americana* by evaluating water use efficiency (WUE).
3) Monitor and compare the abundance, richness, and diversity of wildlife in sites with *Agave* agriculture, native vegetation, and conventional agriculture.

Study Sites

The study sites were located in Maricopa, Arizona and represented three different land managements: a site where trial crops of *A. americana* were established in 2012, a site that had been developed for conventional agricultural crops (*i.e.* cotton, a leading commercial crop in Arizona), and a site with native vegetation and no known history of agriculture (Fig. 1). Today, the city of Maricopa experiences an average annual rainfall of approximately 200 mm (U.S. Climate Data 2015).
Figure 1. Aerial views of each study site.  
**Left:** An aerial view of the *Agave* site at the University of Arizona Maricopa Agricultural Center; **Center:** An aerial view of the native Sonoran Desert site in Maricopa, AZ; **Right:** An aerial view of the conventional agricultural site, adjacent to the *Agave* field. (Photo credit: Google Earth)

The *Agave* site was located at the University of Arizona Maricopa Agricultural Center and subdivided into replicated plots with four different irrigation treatments: control (rainfall only), 200 mm (52.8 gal m\(^{-2}\) y\(^{-1}\); 200,112 L ha\(^{-1}\) y\(^{-1}\)) irrigation, 400 mm (105.6 gal m\(^{-2}\) y\(^{-1}\); 400,224 L ha\(^{-1}\) y\(^{-1}\)) irrigation, and 800 mm (211.2 gal m\(^{-2}\) y\(^{-1}\); 800,448 L ha\(^{-1}\) y\(^{-1}\)) irrigation annually. To achieve these accelerating treatment levels, 100 mm (35,640 gallons) were applied twice, every other month, and monthly throughout the growing season (March – October) for the 200 mm, 400 mm, and 800 mm treatments, respectively. Starting in year 2, the entire field was treated annually with a N:P\(_2\)O\(_5\):K\(_2\)O fertilizer with a ratio of 40:80:80. These applications were consistent across all irrigation treatments and were derived from agricultural recommendations based upon commercial plantation management in the tequila industry. There were four 15 m x 15 m *A. americana* plots in each irrigation treatment. Other agricultural plots that are planted
with various crops throughout the year surrounded the *Agave* field to the north, east, and west, with a residential housing development to the immediate south of the field.

The conventional agricultural site, where sixteen acres of cotton were planted in early 2014, was a ~81 m² field also located at the University of Arizona Maricopa Agricultural Center, northwest of the *Agave* field. The field received a total of approximately 140 mm (5.5 inches) irrigation throughout establishment during the months of March and April, with set irrigation treatments beginning in May (Table 1).

Table 1

*Summer 2014 Irrigation Schedule for the Observed Cotton Field*

<table>
<thead>
<tr>
<th>Month</th>
<th>Irrigation (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>May</td>
<td>38.1 total</td>
</tr>
<tr>
<td>June</td>
<td>63.5 total</td>
</tr>
<tr>
<td>July</td>
<td>50.8/week</td>
</tr>
<tr>
<td>August</td>
<td>38.1/week</td>
</tr>
</tbody>
</table>

The entire field was fertilized with 59.7 kilograms per hectare of liquid CH₃N₂O-NH₄NO₃-N fertilizer three times throughout the growing season, in May, June, and July 2014. Various agricultural fields also surrounded the cotton site with the USDA Arid-Land Agricultural Research Center facility in close proximity directly southeast.

The native site was located approximately 40 km southwest of the agricultural center near the Gila River Indian Reservation. This site was characterized by relatively undisturbed habitat with vegetation typical of the Sonoran Desert biotic province —
dominantly legume trees, including mesquite trees (genus *Prosopis*) and palo verde trees (genus *Parkinsonia*), and columnar cacti, including saguaro cacti (*Carnegiea gigantean*) and barrel cacti (genera *Ferocactus* and *Echinocactus*) — and no known history of agriculture.

**Measuring Plant Productivity**

The physiological response of *A. americana* to the different irrigation treatments was measured by use of a Li-Cor LI-6400XT Portable Photosynthesis and Fluorescence System (Li-Cor; Lincoln Nebraska). This infrared gas analyzer was used to record photosynthetic rates for plants from each irrigation treatment over a time course starting before sunset and extending until after dawn. These measurements were made three times throughout the year (March, June, and November) to assess differences in carbon assimilation across treatments. Total net carbon assimilation was estimated by integrating the measurements of gas exchange over time (typically a 15-hour period). Patterns of gas exchange were also compared to determine if timing of peak photosynthetic rates and the phases of CAM differed across the four different irrigation treatments.

Subsamples of above- and below-ground biomass from the sixteen *A. americana* plots were harvested and weighed after the second and third growing season (mid-January 2014, early February 2015) to evaluate plot-level differences in biomass across irrigation treatments. Plants were then dried in an oven for two weeks at 65 °C to obtain dry weights, which accounted for water loss. Net WUE was estimated as total dry biomass per unit of water input (including both precipitation and irrigation).
The statistical program R was used to perform one-way ANOVAs and Tukey’s HSD (Honest Significant Differences) tests to determine whether there were significant differences in carbon uptake or biomass among treatments at each time point. Survival and reproduction rates of plants were also compared across irrigation treatments. The mean percentage of surviving plants in the four plots was used to estimate survivorships. The mean number and condition of offshoots produced by each plant was also determined by plot to quantify the percent of mortality in each irrigation treatment and predict offshoot survivorships.

Measuring Wildlife Impact

Wildlife species diversity in the Agave, native, and conventional agricultural sites was assessed from late June through early August of 2014. Three pitfall arrays, each containing four pitfall traps (5-gallon plastic buckets flush with the ground) with 7.5-meter spacing (connected with lengths of aluminum screening secured in the ground with 76-mm long wooden stakes), were assembled prior to sampling in three random 15 m x 15 m plots at each site. Y-design pitfall arrays were constructed at the Agave and native sites, and a linear-design pitfall array was used in the conventional agriculture site to better fit with the row cropping of cotton (Fig. 2).
Figure 2. Pitfall array designs. Consisting of four pitfall traps (A) and three 7.5-meter drift fences (B). **Left:** A y-array design (placed at the *Agave* and native sites); **Right:** A linear array design (placed at the cotton site).

Traps were checked twice daily — trapped specimens were removed from the traps, identified, recorded, marked for recapture, and released. Timed 15-minute walking transects were used for visual observations and species identification; specimens encountered during walking transects were identified and recorded by species and location. Visual observations and climatic conditions were recorded at each site. Two HOBO data-loggers that contained a copper model to imitate reptilian morphology and thus thermal preferences were placed at each site to measure operative temperatures ($T_e$) *(i.e. the expected body temperature for a non-thermoregulating organism)*, to be compared across sites and observed in relation with animal capture times and determine patterns in animal activity and preferred operative temperature.

Visual representations were used to illustrate class-level composition of species captured and species similarities across sites; hierarchical cluster analyses were used to
demonstrate the relationship of reptile, amphibian, and mammal species captured across sites; species richness and abundance were summarized at the class-level by site. Additionally, species diversity for each class of animals captured at each site was calculated using the Shannon-Weaver Diversity Index. Heterogeneity in species composition among the three land-use classes were resolved using a Pearson’s Chi-Square Test of Significance ($\alpha = 0.05$). Rank-abundance curves were generated based on accumulation data of unique individuals captured (excluding recaptures) to determine relative class abundance and capture rate in each site over time.

Temperature and rainfall data recorded by The Arizona Meteorological Network (AZMET) (University of Arizona 2014) were used to determine minimum, maximum, and average temperatures and average rainfall for the months during which sampling took place. Rainfall data recorded between June and August 2014 were used to compare amphibian captures to rainfall events. Temperatures recorded by the HOBO data-loggers with copper models were used to determine whether there were significant differences in operative temperatures between sites, between operative and AZMET-logged, or ambient, temperatures, as well as to examine a possible relationship between hourly operative temperature and animal activity. These tests were used to make inferences about the role of biodiversity in agro-ecosystems, the factors that may influence the amplitude of biodiversity across sites, and the degree of functional diversity between site factors (e.g. water availability, plant biomass) and animal species within each site.
CHAPTER 4: SYNTHESIS OF RESULTS

Plant Productivity

In March of 2014, peak photosynthetic rates reached 34 CO$_2$ µmol m$^{-2}$s$^{-1}$ in the 800 mm y$^{-1}$ irrigation treatment around 7:45 PM, although there were little differences in rates of carbon assimilation between the control and high irrigation treatments: plants in the control treatment plots displayed similar peak photosynthetic rates to that of the high irrigation treatment shortly after sunset (~31 CO$_2$ µmol m$^{-2}$s$^{-1}$) (Fig. 3).

![Figure 3. Overnight net CO$_2$ assimilation of A. americana between all irrigation treatments in March 2014.](image)

In June 2014, there were greater differences in photosynthetic rates among irrigation treatments than in March. Additionally, peak rates occurred later in the night than in March — the 800 mm y$^{-1}$ treatment peaked around 11:40 PM at 31 CO$_2$ µmol m$^{-2}$s$^{-1}$, while the control treatment peaked around 3:30 AM at 26 CO$_2$ µmol m$^{-2}$s$^{-1}$ (Fig. 4).
Figure 4. Overnight net CO$_2$ assimilation of A. americana between all irrigation treatments in June 2014.

November 2014 measurements show earlier activity, with plants in the 800 mm y$^{-1}$ irrigation treatment showing activity as early as 5:30 PM and peaking around 9:30 PM at 22 CO$_2$ µmol m$^{-2}$s$^{-1}$. Plants in the control treatment peaked earlier than in previous months, around midnight, with rates of about 19 CO$_2$ µmol m$^{-2}$s$^{-1}$ (Fig. 5).
Figure 5. Overnight net CO₂ assimilation of *A. americana* between all irrigation treatments in November 2014 (24-hour time scale).

A one-way ANOVA was used to compare nightly CO₂ uptake across irrigation treatments and determine significant differences ($\alpha = 0.05$) for each time point (in March, June, and November). Significant differences were observed in June and November only (Table 2).
Table 2

One-way ANOVA Test for Significance of Irrigation Treatment on CO$_2$ Uptake – March, June, and November 2014.

<table>
<thead>
<tr>
<th>Month</th>
<th>Variable</th>
<th>Df</th>
<th>Sum Sq</th>
<th>Mean Sq</th>
<th>F value</th>
<th>Pr(&gt;F)</th>
</tr>
</thead>
<tbody>
<tr>
<td>March</td>
<td>Irrigation</td>
<td>3</td>
<td>124</td>
<td>41.4</td>
<td>0.694</td>
<td>&gt; 0.05</td>
</tr>
<tr>
<td></td>
<td>Residuals</td>
<td>205</td>
<td>12200</td>
<td>59.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>June</td>
<td>Irrigation</td>
<td>3</td>
<td>3760</td>
<td>1250</td>
<td>12.1</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td></td>
<td>Residuals</td>
<td>188</td>
<td>19500</td>
<td>104</td>
<td></td>
<td></td>
</tr>
<tr>
<td>November</td>
<td>Irrigation</td>
<td>3</td>
<td>1030</td>
<td>345</td>
<td>5.14</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td></td>
<td>Residuals</td>
<td>392</td>
<td>26300</td>
<td>67.0</td>
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</tr>
</tbody>
</table>

Total net photosynthetic rates were compared across irrigation treatments at each time point as well (Fig. 6).
Figure 6. Total net photosynthetic rates across treatments.
Compared at each time point throughout the growing season.

Tukey’s HSD tests between treatments in June indicated significant differences ($\alpha = 0.05$) in CO$_2$ uptake between the 800 mm y$^{-1}$ and 200 mm y$^{-1}$ treatments ($P < 0.05$), between the control and 200 mm y$^{-1}$ treatments ($P < 0.05$), and between the control and both of the high irrigation treatments (i.e. 400 mm y$^{-1}$ and 800 mm y$^{-1}$) ($P < 0.001$).

These are reflected in the percent differences in total net photosynthetic rates between treatments: the control net photosynthetic rate is 33.4% less than the 200 mm y$^{-1}$, 43.1% less than the 400 mm y$^{-1}$, and 59.9% less than the 800 mm y$^{-1}$ treatments.

Tukey’s HSD tests between treatments in November revealed significant differences ($\alpha = 0.05$) in CO$_2$ uptake between the 800 mm y$^{-1}$ treatment and the two
lowest irrigation treatments (800 vs. 200: $P < 0.05$, 800 vs. ctrl: $P < 0.001$). These are reflected in the differences in total net photosynthetic rates between treatments, although with an opposite trend compared to that of the June measurements: the 200 mm $y^{-1}$ treatment had a total net photo rate 1.43 times that of the 800 mm $y^{-1}$ treatment, while the control treatment’s rate was 1.25 times that of the 800 mm $y^{-1}$ treatment.

A sample of plants from replicate plots of each irrigation treatment was harvested in January 2014 and early February of 2015 to estimate growth responses to varying irrigation treatments after two and three years. In 2014, the control treatment resulted in 54% lower mean biomass than the 800 mm $y^{-1}$ treatment, with estimated yields ranging from 3.18 Mg ha$^{-1}$ in the control treatment to 6.90 Mg ha$^{-1}$ in the high irrigation treatment after 2 years of growth, and estimated annualized yields ranging from 1.59 Mg ha$^{-1}$ in the control treatment to 3.45 Mg ha$^{-1}$ in the high irrigation treatment (Fig. 7).
Figure 7. Estimated annual yields of A. americana experiencing varying irrigation applications (based on two years of growth, January 2014).

A one-way ANOVA was used to compare biomass after two years’ of growth amongst irrigation treatments, and determined there are significant differences in biomass across treatments ($P < 0.001$) (Table 3). Tukey’s HSD tests for significant differences ($\alpha = 0.05$) indicated that the only non-significant differences were found in comparing biomass amongst the high irrigation treatments (800 mm y$^{-1}$ and 400 mm y$^{-1}$) ($P > 0.05$).

Table 3

One-way ANOVA Test for Significance of Irrigation Treatment on Total Biomass – January 2014.

<table>
<thead>
<tr>
<th></th>
<th>Df</th>
<th>Sum Sq</th>
<th>Mean Sq</th>
<th>F value</th>
<th>Pr(&gt;F)</th>
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<tr>
<td>Irrigation</td>
<td>3</td>
<td>5.54</td>
<td>1.85</td>
<td>26.5</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Residuals</td>
<td>12</td>
<td>0.834</td>
<td>0.0695</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Belowground biomass from the control, 200 mm y\(^{-1}\), 400 mm y\(^{-1}\), and 800 mm y\(^{-1}\) treatments contributed to 12.0%, 8.15%, 10.6%, and 9.17% of the total harvested biomass, respectively. WUE calculated using these estimated yields ranged from 5.75 kg ha\(^{-1}\) mm\(^{-1}\) in the highest irrigation treatment to 7.96 kg ha\(^{-1}\) mm\(^{-1}\) in the control treatment in 2014.

In February 2015, a greater sample of plants (n = 129) was harvested in order to estimate growth responses to varying irrigation treatments after three years of growth. This was due to greater mortality in year 3 from infestation of the plots by *Scyphophorus acupunctatus*, the *Agave* snout weevil. This harvest involved the removal and weighing of all dead *A. americana* aboveground biomass in one plot per irrigation block. Plot-level mortality rates were 21.2%, 31.2%, 42.5%, and 79.4% for the control, 200 mm y\(^{-1}\), 400 mm y\(^{-1}\), and 800 mm y\(^{-1}\) treatments, respectively (Fig. 8). There was a significant effect of irrigation on the susceptibility of plants to the *Agave* snout weevil.
Figure 8. Average dead or dying mother plants. Respective of each irrigation treatment, after 3 years of growth and infestation by the *Agave* snout weevil.

A one-way ANOVA revealed significant differences ($\alpha = 0.05$) in mother plant mortality rates between irrigation treatments ($P < 0.05$) (Table 4), although Tukey’s HSD tests revealed significant differences ($\alpha = 0.05$) in mortality between control and 800 mm $y^{-1}$ irrigation treatments only ($P < 0.05$).

Table 4

One-way ANOVA Test for Significance of Irrigation Treatment on Mother Plant Mortality – February 2015.

<table>
<thead>
<tr>
<th></th>
<th>Df</th>
<th>Sum Sq</th>
<th>Mean Sq</th>
<th>F value</th>
<th>Pr(&gt;F)</th>
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<tr>
<td>Irrigation</td>
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<td>1180</td>
<td>394</td>
<td>4.72</td>
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<tr>
<td>Residuals</td>
<td>12</td>
<td>1000</td>
<td>83.4</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
An average of the total dead or dying offshoots (pups) associated with the harvested mother plants in each irrigation plot were counted to ascertain offshoot mortality: 32.2% of the pups in the 800 mm y\(^{-1}\) irrigation plots were dead or dying, while 40.6%, 17.6%, and 12.2% of the pups in the 400 mm y\(^{-1}\), 200 mm y\(^{-1}\), and control irrigation plots were dead or dying, respectively (Fig. 9). The mean number of pups counted in 2014 indicated an average of 7.33 pups produced per plant in the 800 mm y\(^{-1}\) treatment plots, which suggests the numbers counted in the 800 mm y\(^{-1}\) treatment plots in 2015 (~3.85 pups/plant) did not include some dead pups that were removed from the field.

*Figure 9.* Proportion of average dead or dying pups to average total pups. Respective of each irrigation treatment, after 3 years of growth and infestation by the *Agave* snout weevil.
A one-way ANOVA revealed non-significant differences ($\alpha = 0.05$) in living pups between treatments ($P > 0.05$), and significant differences in pup mortality rates between irrigation treatments ($P < 0.01$) (Table 5). Tukey’s HSD tests revealed significant differences ($\alpha = 0.05$) in pup mortality between the control and 400 mm y$^{-1}$ treatments ($P < 0.05$) and the 200 mm y$^{-1}$ and 400 mm y$^{-1}$ treatments ($P < 0.05$).

Table 5

One-way ANOVA Tests for Significance of Irrigation Treatment on Living Pups and Pup Mortality – February 2015.

<table>
<thead>
<tr>
<th>Condition of Pups</th>
<th>Variable</th>
<th>Df</th>
<th>Sum Sq</th>
<th>Mean Sq</th>
<th>F value</th>
<th>Pr(&gt;F)</th>
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<tbody>
<tr>
<td>Living</td>
<td>Irrigation</td>
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<td>1.06</td>
<td>0.353</td>
<td>0.596</td>
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<td></td>
<td>Residuals</td>
<td>12</td>
<td>7.12</td>
<td>0.592</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dead/Dying</td>
<td>Irrigation</td>
<td>3</td>
<td>3.31</td>
<td>1.10</td>
<td>6.12</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td></td>
<td>Residuals</td>
<td>12</td>
<td>2.16</td>
<td>0.180</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The control treatment resulted in 25.8% lower mean biomass than the 800 mm y$^{-1}$ treatment, with estimated yields ranging from 5.19 Mg ha$^{-1}$ in the control treatment to 8.45 Mg ha$^{-1}$ in the 200 mm y$^{-1}$ irrigation treatment after 3 years of growth and estimated annual yields ranging from 1.73 Mg ha$^{-1}$ in the control treatment to 2.82 Mg ha$^{-1}$ in the 200 mm y$^{-1}$ irrigation treatment (Fig. 10).
Figure 10. Estimated annual yields of *A. americana* experiencing varying irrigation applications (based on three years of growth, February 2015).

A one-way ANOVA was used to compare biomass after three years of growth amongst irrigation treatments to determine significant differences ($\alpha = 0.05$) in biomass across irrigation treatments. Differences were non-significant between treatments ($P > 0.05$) (Table 6).

Table 6

*One-way ANOVA Test for Significance of Irrigation Treatment on Total Biomass – February 2015.*

<table>
<thead>
<tr>
<th></th>
<th>Df</th>
<th>Sum Sq</th>
<th>Mean Sq</th>
<th>F value</th>
<th>Pr(&gt;F)</th>
</tr>
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<tbody>
<tr>
<td>Irrigation</td>
<td>3</td>
<td>1.29</td>
<td>0.431</td>
<td>0.996</td>
<td>&gt; 0.05</td>
</tr>
<tr>
<td>Residuals</td>
<td>5</td>
<td>2.16</td>
<td>0.433</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Belowground biomass for the 2015 harvest was difficult to obtain fully due to snout weevil infestation and thus degraded root biomass. However, samples collected from all irrigation treatments revealed that belowground biomass from the control, 200 mm y$^{-1}$, 400 mm y$^{-1}$, and 800 mm y$^{-1}$ treatments contributed to only 1.86%, 1.16%, 1.01%, and 1.48% of the total harvested biomass, respectively. Calculated WUE after three years’ growth ranged from 2.33 kg ha$^{-1}$ mm$^{-1}$ in the highest irrigation treatment to 8.65 kg ha$^{-1}$ mm$^{-1}$ in the control treatment.

Wildlife Impact

There were 30, 21, and 78 total captures over the sampling period in the Agave, cotton, and native sites, respectively. However, the total number of species by class captured in each site over the sampling period are as follows: one reptile species, one amphibian species, and three mammal species at the Agave site; three reptile species, three amphibian species, and two mammal species at the cotton site; and five reptile species, one amphibian species, and two mammal species at the native site (Fig. 11).
Figure 11. Species composition captured at each of the study sites.

Hierarchical cluster analyses were used to display species relationships based on capture location (Fig. 12, 13, 14).
Figure 12. Hierarchical cluster analyses of reptile species captured. Displaying relation between species (top) and amongst sites (bottom).
Figure 13. Hierarchical cluster analyses of amphibian species captured. Displaying relation between species (top) and amongst sites (bottom).
Similarities in captured species were compared across sites: the Agave site contained only one unique species, while the cotton and native sites yielded two and four unique species, respectively. Only one species, *Chaetodipus baileyi* (Bailey’s pocket mouse), was captured at all three sites (Fig. 15).
Figure 15. Comparison of species similarities between sites.

The *Agave* site yielded the greatest number of different mammal species, while the greatest number of amphibian and reptile species were found at the cotton and native sites, respectively; however, more total captures occurred in the *Agave* site than in the cotton site (Table 7).
Species observed during walking transects throughout the sampling period in the native site include: *Callisaurus draconoides* (zebra-tailed lizard), *Dipsosaurus dorsalis* (desert iguana), *Sceloporus magister* (desert spiny lizard), *Aspidoscelis tigris* (tiger whiptail), *Pituophis catenifer* (gopher snake), *Xerospermophilus tereticaudus* (round-tailed ground squirrel), *Campylorhynchus brunneicapillus* (cactus wren), *Callipepla gambelii* (Gambel’s quail), *Dipodomys merriami* (Merriam’s kangaroo rat), *Zenaida macroura* (mourning dove), *Gryllus pennsylvanicus* (field crickets), ground beetles, and a titmouse species.

Species observed during walking transects throughout the sampling period in the *Agave* site include: *Agelenopsis spp.* (grass spider), round-tailed ground squirrels, mourning doves, field crickets, ground beetles, and a sand piper species.
Species observed during walking transects throughout the sampling period in the cotton site include: *Geococcyx californianus* (greater roadrunner), *Sylvilagus audubonii* (desert cottontail), Gambel’s quail, mourning doves, field crickets, and ground beetles.

Shannon-Weaver Diversity Indices quantified species richness and abundance for each site: the native, cotton, and *Agave* sites represented the greatest reptilian, amphibian, and mammalian diversity, respectively, although the cotton site exhibited the highest diversity amongst all classes. Pearson’s Chi-Square Tests were used to determine whether there were significant differences ($\alpha = 0.05$) in classes of animals captured across sites, revealing significant differences in reptiles ($P < 0.001$), amphibians ($P < 0.01$), and mammals ($P < 0.001$) (Table 8).

### Table 8

*Shannon-Weaver Diversity Indices for Classes Captured at Each Site (Top) and Results from Pearson’s Chi-Square Tests of Significant Differences in Classes Captured across Sites, Depicting Chi-Square Values ($X^2$) (Bottom).*

<table>
<thead>
<tr>
<th>Site</th>
<th>Reptiles</th>
<th>Amphibians</th>
<th>Mammals</th>
<th>All Classes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Native</td>
<td>1.53</td>
<td>0.000</td>
<td>0.279</td>
<td>1.79</td>
</tr>
<tr>
<td>Cotton</td>
<td>1.10</td>
<td>1.06</td>
<td>0.683</td>
<td>1.93</td>
</tr>
<tr>
<td><em>Agave</em></td>
<td>0.000</td>
<td>0.000</td>
<td>2.36</td>
<td>1.47</td>
</tr>
<tr>
<td>$X^2$</td>
<td>54.3 ***</td>
<td>12.2 **</td>
<td>29.2 ***</td>
<td></td>
</tr>
</tbody>
</table>

Signif. codes: ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
Rank-abundance curves indicate that the capture rate increased over time at each site for reptilian and mammalian classes, while the cotton site was the only site with an increasing amphibian capture rate over the sampling period (Figures 16, 17, 18).

*Figure 16. Accumulation curve for Agave site captures.*
Figure 17. Accumulation curve for cotton site captures.

Figure 18. Accumulation curve for native site captures.

Climate data collected by AZMET showed that Maricopa’s highest temperatures of 2014 occurred in June (max. 42.8 °C, avg. 31.7 °C) and July (max. 44.5 °C, avg. 32.9 °C).
°C), while the second highest average rainfall of the year occurred in July (54.1 mm) (Fig. 19).

Figure 19. Averaged climate data and the sampling period for 2014 in Maricopa, AZ. (AZMET 2014)

An ANOVA comparing operative temperature data to that of the AZMET data revealed no significant differences ($\alpha = 0.05$) between daily average MET temperatures and operative temperatures in the native site ($P > .05$), but significant differences between daily average AZMET temperatures and operative temperatures in the Agave and cotton sites ($P < .05$).

Animal capture activity occurred mostly in the early morning (or overnight), with few captures occurring in the later afternoon hours, when temperatures were much higher. The most diurnal wildlife activity occurred when ambient temperatures were
between 28 °C and 34 °C, with little activity occurring when temperatures exceeded 34 °C. Data collected from each HOBO channel were averaged to compare hourly “sun” and “shade” operative temperatures to animal activity at each site (Fig. 20, 21, 22).

*Figure 20. Average operative temperatures ($T_e$) compared with total animal observations at the native site over 24 hours.*
Figure 21. Average operative temperatures ($T_o$) compared with total animal observations at the *Agave* site over 24 hours.

Figure 22. Average operative temperatures ($T_o$) compared with total animal observations at the *cotton* site over 24 hours.
Two-way ANOVAs were used to determine whether sun or shade operative temperatures and/or animal activity are dependent on site. Results were non-significant ($\alpha = 0.05$) between animal activity and site type, but significant between sun and shade operative temperatures and site type, indicating that operative temperature differs amongst sites (Table 9).

Table 9

*Two-Way ANOVAs Testing whether Average Hourly Sun and Shade Operative Temperature ($T_e$) and Total Animal Observations are Significantly Different Across Sites.*

<table>
<thead>
<tr>
<th></th>
<th>Df</th>
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<th>Mean Sq</th>
<th>F value</th>
<th>Pr(&gt;F)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Activity ~ Site</td>
<td>2</td>
<td>116</td>
<td>58.2</td>
<td>0.604</td>
<td>&gt; 0.05</td>
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<tr>
<td>Residuals</td>
<td>72</td>
<td>6930</td>
<td>96.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sun $T_e$ ~ Site</td>
<td>2</td>
<td>4460</td>
<td>2230</td>
<td>693</td>
<td>&lt; 0.001</td>
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<tr>
<td>Residuals</td>
<td>72</td>
<td>232</td>
<td>3.22</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Shade $T_e$ ~ Site</td>
<td>2</td>
<td>3990</td>
<td>1990</td>
<td>746</td>
<td>&lt; 0.001</td>
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<tr>
<td>Residuals</td>
<td>72</td>
<td>193</td>
<td>2.67</td>
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</table>
CHAPTER 5: DISCUSSION AND SIGNIFICANCE

Overview

While increased irrigation increased plant productivity, irrigation decreased WUE. There was no difference in comparing biomass yields between the 400 mm y$^{-1}$ and 800 mm y$^{-1}$ irrigation treatments for either harvest. Water content was maintained across irrigation treatments, as the 2014 biomass harvest revealed that percent of weight lost (post-dehydration) was approximately 85.5% across all irrigation treatments, while the dried biomass from the 2015 harvest decreased by about 75.0% from the fresh biomass across all irrigation treatments. As a result, differences in biomass yield across irrigation treatments declined with time, due to a lack of difference between high irrigation treatments and pest infestation. Crops experiencing lower irrigation were not as susceptible to snout weevil infestation and continued to thrive due to lower mortality rates. Although the WUE of the *A. americana* experiencing no irrigation still exceeded that of conventional crops in the region, *Agave* yields were lower than what has been previously indicated in the literature (Davis et al. 2014, Li et al 2012, Escamilla-Treviño 2012, Holtum et al. 2011, Nobel 1991, Nobel & Valenzuela-Zapata 1987, Davis et al. 2011, Owen and Griffiths 2014). However, non-irrigated *Agave* yields after three years’ growth were similar to other low-yielding bioenergy crops when cultivated under similar conditions (Escamilla-Treviño 2012).

Although *Agave* cultivation did have an impact on native wildlife, represented by lower species diversity than the native site, it hosted greater species richness than cotton cultivation in Maricopa, Arizona. As the native site was used as a standard site for
measuring Sonoran Desert region biodiversity, the *Agave* and cotton sites showed a reduction in species diversity by comparison: the *Agave* site only shared two captured species with the native site, while the cotton site only shared three. However, the two agro-ecosystem sites shared three common species not found in the native site, which may be attributed to their more urban and managed surrounding landscapes (Fig. 23).

The *Agave* site represented the greatest species richness in both mammals and reptiles, suggesting this site may be more fit for supporting local wildlife communities. These findings are dependent on the natural life history of each animal class: amphibians rely heavily on both warmth and hydration for activity (*e.g.* reproductive success and water absorption), while mammals and reptiles rely upon land availability (*e.g.* burrowing), foraging (*e.g.* vegetation and arthropod availability), and solar radiation (*e.g.* thermoclines for metabolic function) (Rodríguez et al. 2005, Drabek 1973). In addition, a difference in residential and non-residential species could contribute to the species composition of a location, depending on the time of year and recent climatic events – amphibians, for example, often disperse upon ample precipitation, while reptiles might use a site for dispersal purposes. Additionally, some tree-dwelling reptiles (*e.g.* ornate tree lizards) and birds (*e.g.* mourning doves) are known to nest on the ground (Hogan 2015, NOAA 2015). These varying characteristics help classify residential species within a site and can help explain species presence at certain times throughout the year.

*Biofuel Potential*

The varied patterns of CO₂ assimilation at the three different points during the year provide valuable information relating the influence of temperature, water input, and
plot disturbance (i.e. pest infestation) to *A. americana* productivity, which can be seen in the estimated yields obtained from the harvested biomass. The estimated annual yields and the WUE of *A. americana* in all irrigation treatments exceeded that of conventional cropping systems established in the region, for both harvests conducted: the WUE of *A. americana* in 2014 was 4.14 – 5.73 times greater than that of cotton, and the WUE of *A. americana* in 2015 was 1.67 – 6.22 times greater than the WUE of cotton (e.g. cotton crops cultivated on arid agricultural lands in Arizona typically yield 1.46 Mg ha\(^{-1}\) annually and require about 1,046 mm y\(^{-1}\) irrigation, with a WUE of only 1.39 kg ha\(^{-1}\) mm\(^{-1}\) (USDA 2012; University of Arizona 2001)).

It is important to note the calculated yields resultant of the 2014 and 2015 harvests were much lower than projected yields based upon the literature of *A. americana*, as well as for different *Agave* species from other arid and semi-arid locations around the globe. Theoretical ethanol yields for *A. americana* have been based on a range of 9.07 – 30.8 Mg ha\(^{-1}\) y\(^{-1}\) (Li et al. 2012), while yields exceeding 42 Mg ha\(^{-1}\) y\(^{-1}\) have been suggested for *A. americana* growing in Australia (Escamilla-Treviño 2012). However, while historical fiber yields of *A. americana* grown in Australia equated to approximately 1 ton day\(^{-1}\) (.907 Mg day\(^{-1}\)), there are no previous realized biomass yields or knowledge of how this particular species would fare when cultivated for bioenergy purposes (Holtum et al. 2011). For other *Agave* species, yields as high as 26 Mg ha\(^{-1}\) y\(^{-1}\) in parts of central Mexico (Nobel 1991), 16 Mg ha\(^{-1}\) y\(^{-1}\) in the Yucatan Peninsula (Nobel & Valenzuela-Zapata 1987), and 34 Mg ha\(^{-1}\) y\(^{-1}\) without irrigation have been predicted (Davis et al. 2011). However, these previous yields are based upon *Agave* cultivated in
more semi-arid, rather than arid, regions, and the harsh drought and heat conditions of Maricopa, Arizona likely inhibited maximum growth potential.

Infestation by the *Agave* snout weevil prevented increased yield between years 2 and 3. In 2014, the control treatment biomass was 54.0% less than that of the 800 mm y\(^{-1}\) biomass; in 2015, however, the biomass calculated from the control plots was only 25.8% lower than that of the 800 mm y\(^{-1}\) plots, and biomass from the 200 mm y\(^{-1}\) plots, the most productive for the 2015 harvest due to lower mortality rate, was 1.21 times greater than biomass from the highest irrigation treatment plots. Although yields were not as great as predicted, the low irrigation treatments still produced greater yields than those obtained from the previous year’s harvest (38.7% greater in the control treatment and 47.1% greater in the 200 mm y\(^{-1}\) treatment), and greater annual yields than those associated with cotton, the state’s leading commercial crop (15.6% greater in the control treatment and 37.3% greater in the 200 mm y\(^{-1}\) treatment). These results indicate that even *Agave* crops experiencing snout weevil degradation might serve as bioenergy candidates.

Differences in carbon uptake between irrigation treatments, specifically those observed in June 2014, suggest that drought reduces carbon uptake in *A. americana* and results in lower biomass productivity; however, crops experiencing increased degrees of drought exhibited higher WUE than well-irrigated crops. The higher and less variable photosynthetic rates measured in March of 2014 might be attributed to the cooler temperatures associated with the season and a recent rainfall event: after over 70 days of no rain, over 25 mm of rainfall occurred within the first week of March. Additionally, the average temperature for March 2014 was 17.8 °C, while the minimum recorded
temperature was 4.44 °C (AZMET 2014). According to a study examining the response of variations in night-time temperature to CO$_2$ assimilation and WUE in *A. americana*, it was found that this species relies upon low nocturnal temperatures for optimum CO$_2$ uptake – it was demonstrated that high nocturnal temperatures induced stomatal closure and increased WUE, with a tolerance threshold of 36 °C (Neales 1972).

Photosynthetic rates measured in June of 2014 were the most different between treatments, with the largest differences observed between irrigation treatments than other times of the year. As June is typically one of the hottest months in central Arizona, significantly warmer ambient temperatures can explain the delay in nocturnal photosynthetic activity in the control and low irrigation (200 mm y$^{-1}$) treatments. Significant differences in the rate of carbon uptake observed between the control and 200 mm y$^{-1}$ irrigation treatments in June might suggest that additional water input (as opposed to none) results in a significant increase in photosynthetic productivity, which is reflected in the differences in biomass between treatments: the 2014 harvest revealed that the 200 mm y$^{-1}$ treatment yielded 30.3% more aboveground biomass and 14.4% more belowground biomass than the control treatment. However, while the 2015 harvest revealed that the 200 mm y$^{-1}$ treatment yielded 38.0% more aboveground biomass than that of the control treatment, it resulted in the same amount of belowground biomass as the control treatment. This is reflected in the non-significant differences in biomass between treatments observed in the February 2015 harvest data and is likely due to root degradation, resultant of infestation by the *Agave* snout weevil.
Significant differences in carbon assimilation were also observed between the control and 800 mm y$^{-1}$ treatments in June and November. The 2014 harvest revealed that the 800 mm y$^{-1}$ treatment yielded 55.3% more aboveground biomass and 39.4% more belowground biomass than the control treatment, while the 2015 harvest revealed that the 800 mm y$^{-1}$ treatment yielded 22.5% more aboveground biomass than the control treatment, but 29% less belowground biomass than the control treatment (again, likely due to snout weevil infestation). Similarly, a study examining irrigation response in the species *A. lechuguilla* found that weekly irrigation increased shoot, or aboveground, biomass by 75% and root, or belowground, biomass by 40%, and resulted in an increased reliance on diurnal CO$_2$ uptake and decreased WUE (Nobel et al 1989). These trends in increased photosynthetic and biomass productivity as a result of increased irrigation are not surprising. However, it is interesting to note that there was no significant difference in biomass yield between the 400 mm y$^{-1}$ and 800 mm y$^{-1}$ treatments for either harvest.

Due to the decreased hours of daylight in November in central Arizona, all irrigation treatments exhibited earlier signs of carbon assimilation, although this reduction in radiation also resulted in the lowest peak photosynthetic rates of the growing season. Observations in CO$_2$ uptake in November of 2014 were very different from those in previous sampling time periods and were likely due to the *Agave* snout weevil impacts, which were most pronounced in the higher irrigation treatments. Crops in the 200 mm y$^{-1}$ treatment exhibited the highest peak photosynthetic rates at this time, while rates observed in the 800 mm y$^{-1}$ treatment plots were unusually low. The effect of the snout weevil is reflected in the high proportion of dead or dying pups in the 800 mm y$^{-1}$ and
400 mm y\textsuperscript{-1} irrigation treatments, as well as in the significant differences in mortality between the 800 mm y\textsuperscript{-1} and control treatments.

For this experiment, a larvae-targeted pesticide was implemented in an attempt to eradicate snout weevil infestation. Though unsuccessful, these results provide an opportunity to explore different methods of the removal of snout weevil from \textit{Agave} fields. A 2012 study argued the benefits to targeting adult snout weevils, rather than larvae, since the adult form is the most destructive to \textit{Agave} crops, given their ability to bore into the stem of the plant where they lay their eggs and leave bacteria (\textit{Erwinia} sp.), resulting in bacterial infection and plant rot (DBG 2010). A suggested insecticide is hydroethanolic extract from the castor oil plant, \textit{Ricinus communis}, due to the repellent effect its leaves have upon adult \textit{Agave} snout weevils (Pacheo-Sánchez et al. 2012). As this is the first experimental site for \textit{Agave} as a potential bioenergy feedstock in the United States, trialed methods and results can provide information for future endeavors examining \textit{A. americana} as an energy crop.

\textit{Effect on Wildlife}

In comparing species composition amongst sites, only one mammal species was found amongst all three sites; the native and \textit{Agave} sites shared one mammal species as well, while the native and cotton site shared an amphibian and reptile species. However, the two agro-ecosystems shared animals of all three classes (mammals, reptiles, and amphibians). The cluster dendrograms illustrate this relationship between the \textit{Agave} and cotton sites, although the relationship in amphibians captured is stronger between the native and cotton sites. In considering all animal classes, a larger number of species was
found in the cotton site, though more total captures occurred in the Agave site, indicating higher species richness. The capture rate of the Agave site was much closer to that of the native site than the cotton site. Rank abundance curves reveal an increase in reptiles and mammals captured at all sites over the sampling period, although only the cotton site exhibited an increase in amphibian captures. While amphibians seem to be more sensitive to irrigation in addition to precipitation activity (eight out of the nine total amphibian captures occurred within two days of a rainfall event), the presence of all animal classes increased in response to rainfall events (e.g. accelerated capture rates at the end of July and beginning of August, when rainfall events caused decreased ambient and operative temperatures (Table 10)).

Table 10

*Table 10. A Comparison of Average Rainfall and Average Ambient and Operative Temperatures across Sites.*

<table>
<thead>
<tr>
<th>Date</th>
<th>Avg AZMET Rainfall (mm)</th>
<th>Avg AZMET Temp (°C)</th>
<th>Agave Avg Tₑ (°C)</th>
<th>Native Avg Tₑ (°C)</th>
<th>Cotton Avg Tₑ (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>7/30</td>
<td>0.00</td>
<td>36.1</td>
<td>40.1</td>
<td>49.1</td>
<td>39.4</td>
</tr>
<tr>
<td>7/31</td>
<td>3.05</td>
<td>34.4</td>
<td>40.0</td>
<td>49.9</td>
<td>37.6</td>
</tr>
<tr>
<td>8/1</td>
<td>4.06</td>
<td>28.3</td>
<td>32.2</td>
<td>50.7</td>
<td>31.8</td>
</tr>
</tbody>
</table>

While both the Agave and cotton sites were characterized by monoculture activity, the close proximity of the cotton site to both a large tree plot and the operation’s buildings at the agricultural center could explain the presence of some tree and building-dwelling species captured in the cotton pitfall traps (e.g. *Urosaurus ornatus* (ornate tree lizard)), as well as the heightened Shannon-Weaver Diversity Index associated with the
cotton site when accounting for all classes. It has been found that, in examining patterns of lizard diversity and abundance, lizard diversity is highest in ‘affluent’ or well-developed areas, while abundance is highest in patches of open desert, which the *Agave* field more closely mimics (Ackley et al. 2015).

The *Agave* site’s various agricultural and urban surroundings may have influenced the presence of species captured there as well — for example, *Uta stansburiana*, the common side-blotched lizard, which is one of only two lizards known to occur in areas classified as ‘heavily developed’ in the Phoenix area (Ackley et al. 2015). Additionally, the native site was located on private land, and thus underwent some degree of land management (*e.g.* weeding, clearing of debris, etc.) compared to its surroundings, which might have contributed to the abundance of species found there (Fig. 23).
The diversity index for mammals was greatest at the *Agave* site, as was mammalian abundance. This might be indicative of the fact that small, ground-dwelling mammals (*e.g.* *Xerospermophilus tereticaudus* (round-tailed ground squirrel), *Chaetodipus baileyi* (Bailey’s pocket mouse), *Dipodomys merriami* (Merriam’s kangaroo rat)) prefer crops with broad roots. Observations indicate round-tailed ground squirrels in particular favor *Agave* roots as a food source — this coincides with studies examining the round-tailed ground squirrels’ home range and behavior in the Sonoran Desert region of Arizona, which report that foraging is their primary activity, followed by excavating burrows, both of which are associated with this species actions within the *Agave* field (Drabek 1973). Birds were the second most observed group in the *Agave* field, specifically mourning doves during the sampling period — outside of the sampling period (*i.e.* during the cooler months), *Haemorhous mexicanus* (the house finch) was a
common resident of the *Agave* site — and were seen using the long stems of the *A. americana* as perches. These observations suggest that *Agave* plants serve a functional purpose to surrounding native wildlife, particularly small mammals and birds.

The distribution of reptiles and amphibians at the native and cotton sites, respectively, follow what one would predict, as reptiles are commonly found in arid environments with ample sunlight, and amphibians utilize more hydrated settings for their environmental functioning. While the greatest amphibian abundance was observed at the well-irrigated cotton site, all but one amphibian capture at every site occurred within two days of a recent rainfall event. This suggests that amphibian activity is not driven by irrigation as much as it is by precipitation. This trend is supported by the fact that amphibian species richness within a region is largely driven by temperature and precipitation (Buckley and Jetz 2007), as amphibians rely upon moisture for essential reproductive performance (Rodríguez et al. 2005). Likewise, the average rainfall in July 2014 was 54 mm, the second highest amount recorded throughout 2014. Reptiles, contrarily, are known to rely strongly upon solar radiation for environmental requirements (Rodríguez et al. 2005), although thermal trends have not yet been shown to affect their large-scale abundance and species distribution (Ackley et al. 2015). However, this was illustrated at the smaller species-community scale in comparing the shade and sun operative temperatures with observations made between sites throughout the sampling period: the most observed reptile activity at the native site occurred when the “sun” HOBO channel recorded a 3-ºC decrease within an hour, and the second most
observed reptile activity at the native site occurred at the “shade” HOBO channel’s lowest temperature recording (46.8 °C at 10:00 AM).

The ambient temperatures represented through AZMET data proves the highest annual temperatures occurred throughout the study period (June and July of 2014). It is interesting to observe significant differences between the AZMET and operative temperatures recorded at the Agave and cotton sites, and not between AZMET and operative temperatures recorded at the native site, as the native site is further away from the AZMET data-loggers. However, more wildlife activity was observed at the Agave site than at the cotton and native sites when ambient temperatures were slightly higher, suggesting the possible functional use of Agave as a shade agent — round-tailed ground squirrels have been known to rarely leave their burrow during high afternoon temperatures, but utilize shade sources on the rare occasions they have been observed to be active during these times (Drabek 1973). There is also evidence that Merriam’s kangaroo rats prefer operative temperatures between 22 and 25 °C, which might be achieved under sources of direct shade (Yousef and Dill 1971).

The operative temperatures recorded at the native site were higher than those recorded at the agricultural sites, which can be attributed to its more xeric location and lack of irrigation whatsoever. Consequently, the most wildlife activity at the native site was observed at earlier times in the day, when ambient temperatures did not exceed 28 °C. Although the relationship examined between site and operative temperature provides some explanation of class distribution amongst sites, many other factors, such as a species’ life history and behavioral tendencies, as well as the development, surroundings,
and resource availability associated with location, must be taken into account when speculating on reasons for species presence within a site.

**Broader Impacts**

To date, estimated *Agave* yields for bioenergy have been theoretical and based off either the harvests from already established industries (*e.g.* fiber, tequila), or an environmental productivity index (EPI), generated by Park S. Nobel in 1984 (Nobel 1984). This takes into consideration various physiological variables of CAM plants (the original species being *Agave deserti*), including soil moisture content, diurnal and nocturnal temperatures, PAR (*i.e.* light intake), and net CO$_2$ assimilation. This initial field study and application to the EPI resulted in a net productivity of 5.7 Mg ha$^{-1}$ over a five-month period (*i.e.* 13.7 Mg ha$^{-1}$ y$^{-1}$) (Nobel 1984). More recently, this same EPI was used to predict estimated bioethanol potential for four different CAM species — *Agave fourcroydes*, *Agave salmiana*, *Agave tequilana*, and *Opuntia ficus-indica* — based upon maximum dry mass productivities of 30 Mg ha$^{-1}$ y$^{-1}$, 40 Mg ha$^{-1}$ y$^{-1}$, 28 Mg ha$^{-1}$ y$^{-1}$, and 46 Mg ha$^{-1}$ y$^{-1}$, respectively (Garcia-Moya et al. 2011, Nobel et al. 1992), and concluded that bioenergy crops that utilize the CAM pathway may generate significant yields in “environmentally responsible” (ER) sites (quantified by comparing trade-offs in productivity and environmental benefit when cultivated on marginal or degraded lands) (Owen and Griffiths 2014). These theoretical yields have proven to be over-estimates when compared to the realistic biomass potential of *Agave* obtained through this experiment. However, this project’s results can help inform numbers and management practices going forward, as CAM plants are further investigated for their potential in
warming, water-deficient climates. Recently, a roadmap for enhanced research on CAM confronting these issues has been compiled through collaborative efforts, calling for an increased need in CAM field trials, model systems, and data collection (Yang et al. 2015).

Comparing wildlife activity to temperature reveals that local wildlife communities are sensitive to increased daytime temperatures, particularly during the hottest months of June and July. It would be interesting to observe wildlife activity amongst native and agricultural sites in Arizona during the cooler months of the year, which might be more compatible to more animals and different species. However, as desert wildlife continues to thrive throughout the summer, it is important to note that the Agave site supported the highest species richness, and that the high abundance of small, desert mammals observed at the Agave site also indicates that this crop may serve as an important habitat component to native species. There is further evidence of the beneficial ecosystem services native plants provide in desert ecosystems, which include supporting local biodiversity and providing resources for animal foraging (Bidak et al. 2015). In addition to benefits seen by implementing a native terrestrial crop into an agro-ecosystem, wildlife richness and diversity has increased with the replacement of annual crops with perennials (Meehan et al. 2010). This offers benefits similar to those associated with strip harvesting, as it allows for vegetation to remain and support an ecosystem for more than one year at a time. Understanding the inherent nature behind an agricultural system facilitates the success of that system, as underlying ecological mechanisms support ecosystem services and functioning. This has been observed in comparing agro-
ecosystems in Mexico as well, where greater tradeoffs exist between yield and soil quality, and between pest control and biodiversity, in conventionally managed systems (González-Esquivel et al. 2015).

Conclusions

In addition to possibly supporting local wildlife communities, the results depicting potential biofuel productivity indicate that, while issues such as excessive drought and pest infestation decrease productivity, these crops are resilient in their WUE and succulence, regardless of the degree of irrigation treatment. This allows for possible cultivation and implementation as a bioenergy crop even in less than ideal environmental conditions. Therefore, water resources might be conserved with the adoption of *Agave* as a cropping system to replace conventional crops in the southwestern U.S., offer an energy source to drought-stricken, degraded, and resource-limited areas of the globe, and potentially provide functional uses to wildlife, specifically where *Agave* is native to the region.

Advanced energy cropping systems in particular have the ability to mitigate, and perhaps reduce, atmospheric carbon dioxide levels, but only with environmentally conscious decisions that fit well with the land and surrounding ecosystems. Unfortunately, there is no single solution. Renewable energies will have to be employed where it makes the most ecological sense, and this will require more scientific data, enforced regulations, and perfected techniques, while considering location, resource, societal, and climatic factors. Oftentimes, renewable energy locations are chosen based upon monetary viability and land availability, without considering environmental
compatibility. Implementing wildlife protection regulation, such as Environmental Impact Assessments, would more successfully preserve ecosystems. It is important that renewable energy development look past the economic feasibility of execution and consider both direct and indirect environmental consequences as well — disregarding ecological evaluation of renewable energy practices defeats the initial purpose for displacing environmentally damaging fossil fuels and confronting rising carbon emissions, while still striving to meet our global energy needs.
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APPENDIX A: CITI CLEARANCE AND INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE APPROVAL

COLLABORATIVE INSTITUTIONAL TRAINING INITIATIVE (CITI) WORKING WITH THE IACUC CURRICULUM COMPLETION REPORT

Emily Kuzmick (ID: 4181819) Environmental Studies 3305230068 ek160508@ohio.edu

Ohio University 05/29/2017

Basic Course/1 05/30/2014 13089458

LEARNER DEPARTMENT PHONE EMAIL INSTITUTION EXPIRATION DATE

INVESTIGATORS, STAFF AND STUDENTS

COURSE/STAGE: PASSED ON: REFERENCE ID:

ELECTIVE MODULES

Introduction to Working with the IACUC Working with the IACUC Federal Mandates The Veterinary Consultation


DATE COMPLETED

05/29/14 05/29/14 05/29/14 05/29/14 05/29/14 05/30/14 05/30/14 05/30/14 05/30/14 05/30/14 05/30/14 05/30/14 05/30/14 05/30/14 05/30/14 05/30/14 05/30/14 05/30/14

For this Completion Report to be valid, the learner listed above must be affiliated with a CITI Program participating institution or be a paid Independent Learner. Falsified information and unauthorized use of the CITI Program course site is unethical, and may be considered research misconduct by your institution.

Paul Braunschweiger Ph.D. Professor, University of Miami Director Office of Research Education CITI Program Course Coordinator

IACUC Protocol/Addendum: 13-L-021
APPENDIX B: SCIENTIFIC COLLECTING PERMIT STIPULATIONS

Calendar Year 2014
Ohio University- Biology Dept.
DONALD B. MILES

The following are agents under this permit for the activities below: **Emily Kuzmick**

The permittee OR the agent(s) MUST be present at all activities conducted under authority of this permit and must have a copy of the permit and stipulations present at all times while conducting activities.

**Signature:**

**Email:** ek160508@ohio.edu