The Environmental Productivity and Photosynthetic Light Response of *Agave americana*:

A Potential Semi-Arid Biofuel Feedstock

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Nicholas A. Niechayev
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This thesis titled
The Environmental Productivity and Photosynthetic Light Response of *Agave americana*:
A Potential Semi-Arid Biofuel Feedstock

by

NICHOLAS A. NIECHAYEV

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

Sarah C. Davis
Assistant Professor of Environmental Studies

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

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The Environmental Productivity and Photosynthetic Light Response of Agave americana: A Potential Semi-Arid Biofuel Feedstock

Director of Thesis: Sarah C. Davis

The potential for the desert succulent species Agave americana (L.) as an advanced biofuel crop in water limited regions has recently been recognized. However, the potential productivity of A. americana in the United States is not yet fully understood. This study developed an environmental productivity index (EPI) model that can be used to estimate the actual growth of A. americana based on the seasonal patterns of water, temperature, and photosynthetically active radiation (PAR) on a monthly time scale for any given region. Previously published research was used to construct indices that predict growth responses of A. americana to water and temperature. Light responses, however, have not previously been determined for this species, and this study is the first to experimentally resolve the physiological response of A. americana to varying intensities of PAR. The photosynthetic response to light was determined by measuring gas exchange over 24 hours in plants that were acclimated to varied light levels over 10 days. Results were used to derive a predictive index of the growth response to light. Maximum CO₂ fixation rates were observed at a light intensity of 1250 µmol photons m⁻² s⁻¹. A monthly EPI was calculated as the product of the water, temperature, and light indices appropriate for the monthly environmental conditions in Maricopa, AZ, where the first trial of A. americana was recently completed. Growth predicted using the EPI was compared to actual production. The summed EPI values were highly correlated (R² = 0.99) with the
average total biomass of healthy 2 and 3 year old plants. Quantitative relationships derived here between environmental conditions and production of *A. americana* provide a simple tool to estimate and compare potential productivity across regions where this species has not yet been grown, and to determine potential geographic ranges in the future as climate changes.
This work is dedicated to my family, whose routine dinner time conversations have educated me more than any class or book ever has.
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CHAPTER 1: INTRODUCTION

Maximizing yields from agriculture requires an understanding of how environmental conditions affect photosynthetic activity and productivity of the crop species in question. Of the three photosynthetic pathways, crassulacean acid metabolism (CAM) has substantial advantages in semi-arid and xeric regions (Davis et al. 2014a). High-yielding CAM species (*Agave* and *Opuntia*) have been shown to be approximately four times more water use efficient (WUE) than that of agricultural crop species that use *C*₄ photosynthesis, and about six times more WUE than that of agricultural *C*₃ photosynthetic species (Borland et al. 2009; Davis et al. 2014b, Yang et al. 2015). Despite the common perception that CAM species have relatively low annual productivities, *Agave* species have been shown to have annual biomass productivities ranging from <1 to 34 Mg ha⁻¹ yr⁻¹ without irrigation (Davis et al, 2010). The upper end of this range exceeds that of current crops that use *C*₃ or *C*₄ photosynthetic pathways; productivities of *C*₄ photosynthetic species grown for biofuels such as maize, switchgrass, and sugarcane have average productivities ranging between 5-26 Mg ha⁻¹ yr⁻¹, and *C*₃ photosynthetic species grown for biofuels such as oil palm, poplar, and willow have productivities between 2-14 Mg ha⁻¹ yr⁻¹ (Davis et al, 2014b).

*Agave* varieties have been used in the past for making beverages, food, fiber, medicines, shelter, ornamentals, and are now potential advanced biofuel crops (Davis et al, 2010; Borland et. al, 2009; Owen et. al, 2013; Thakur et. al, 2015; Mielenz, 2015; Garcia et. al, 2011). *Agave americana* is one such *Agave* that is an obligate CAM species (Neales et. al, 1968), and has also been indicated as a species that may produce viable yields for cellulosic biofuel production while supporting local wildlife populations in
xeric regions when managed properly (Davis et. al, 2016; Kuzmick, 2015). A defining characteristic of an obligate CAM species is nocturnal assimilation of CO$_2$ by regulating stomatal opening during the night, and closing stomata during the day. This is opposite from the diurnal activity in C$_3$ and C$_4$ plants that are vulnerable to water loss during the hottest part of the day, and is a trait that allows for a greater WUE in CAM species (Ting, 1985).

The fixation of CO$_2$ in an obligate CAM plant is described in four phases that take place over a 24-hour period (Osmond, 1978; Owen & Griffith, 2010; Dittrich et. al 1973). Phase 1 occurs during the night when sunlight is not available. During phase 1 the stomata are open, and CO$_2$ is fixed into a 4-carbon carbohydrate known as pyruvate by phosphoenolpyruvate carboxylase (PEPC). Pyruvate is then converted, and stored in the vacuoles as malic acid. Phase 2 begins at dawn when sunlight is first made available. Phase 2 is characterized as a transitional period at dawn in which not all of the stomata have yet closed, and CO$_2$ is therefore fixed by PEPC as well as directly by ribulose-1,5-bisphosphate carboxylase/oxygenase (RUBISCO). Phase 3 begins when all of the stomata have closed, and malic acid stored in phase 1 is transported out of the vacuoles to be decarboxylated. The decarboxylated CO$_2$ is then fixed by RUBISCO as in the normal C$_3$ photosynthetic pathway. Finally, phase 4 is characterized by stomata beginning to open at dusk, which allows for some CO$_2$ to be fixed directly by RUBISCO while modest amounts of sunlight are still available.

The overnight buildup of malic acid in the vacuoles of CAM plants during phase 1 decreases the tissue pH, and the decarboxylation of malic acid for photosynthesis during phase 3 elevates the tissue pH again until dusk. This change in tissue pH allows
for the quantification of nocturnal CO$_2$ fixation by PEPC via titrations of tissue samples collected at dusk and dawn (Ting, 1985; Osmond, et. al, 1989; Silvera et. al, 2009).

Tissue acidity analysis has been used to determine the percent change in the productivity of CAM plants in response to environmental conditions. These findings can then be used to build a predictive environmental productivity index (EPI) model that is tested, and calibrated with actual field results (Nobel, 1988, 1990, 2009). Plant species that have been studied in this way include *Agave lechuguilla* (Nobel & Quero, 1986), *Agave tequilana* (Nobel & Valenzuela, 1987), *Agave deserti* (Nobel,1976), *Agave salmiana* (Nobel & Meyer, 1985), and *Opuntia ficus-indica* (Nobel & Hartstock, 1984).

The EPI model is typically developed using empirical data from laboratory studies that determine changes in the nocturnal buildup of malic acid in response to changes in the soil water potential, photosynthetically active radiation (PAR), and temperature. The effect of each of these three abiotic factors is tested individually by varying treatment levels of one factor while all other conditions are kept constant. This allows for the determination of the amount of water, PAR, and temperature that is required for optimum productivity, as well as the percent change in productivity that can be expected in response to any deviations from the experimentally resolved optimum conditions. Indices are derived to describe the responses to water, PAR, and temperature, where 1.00 is an optimum condition, and 0.00-0.99 is the proportional index for deviation from the optimum according to experimentally resolved relationships between production and environmental conditions. The product of the light, water, and temperature index values is equal to the environmental productivity index number, or:

\[
\text{Light Index} \times \text{Water Index} \times \text{Temperature Index} = \text{EPI} \quad \text{(Eq. 1)}
\]
Once equations are derived for light, water, and temperature indices, EPI values may be calculated according to environmental conditions at specific geographic locations. Quantitative relationships between EPI and growth can also be developed at field sites where direct measurements of the CAM plant species under study are available. These relationships can then be used to make predictions of production in other regions based on the climatic conditions, even if biomass data are not available (Nobel, 1988).

EPI models are important tools for projecting the potential geographical ranges for successful agriculture of CAM plant species (Garcia, 2009; Nobel & Hartsock, 1986a). Presently, there are no EPI models for *A. americana*, but such a model would be a valuable tool for assessing the economic potential for *A. americana* as a source of bioenergy feedstock, fiber, ornamentals or other bioproducts (Nobel, 1990). The goal of this study is to create an EPI model for *A. americana*, and to test and calibrate the model using results from a field site in Maricopa, AZ.

From 2012 to 2016, an agricultural field trial of *A. americana* was conducted at the University of Arizona Maricopa Agricultural Center (Davis et al. 2016). This was the first field experiment in which the productive yield of *A. americana* was tested under different irrigation treatments in the United States. The results of this field trial provided an understanding of how productivity of *A. americana* is affected by water input, and also provided data to parameterize a water index for the EPI model.

While literature already exists pertaining to the response of gas exchange in *A. americana* to drought (Ehrler, 1983) and different temperatures (Neales et. al, 1972; Nobel & Smith, 1983), carbon fixation in *A. americana* in response to different levels of
irradiance is still not well understood. As such, a part of this study requires experimental
manipulation of the light environment around *A. americana* plants to develop a light
response curve for this species. Due to the nature of CAM photosynthesis, 24-hour gas
exchange measurements were required to assess the light response of *A. americana*
(Ceusters et al. 2011, 2014; Cui & Nobel, 1994; Haslam et al., 2003; Keller & Luttge,
2005; Martin et al., 1986; Nobel & Hartstock, 1983). Determining the optimum light
conditions and the decrease in photosynthetic activity due to deviations from this
optimum allows for the interpolation of a monthly light index for *A. americana*.

Specific Objectives

1. Quantify, through experimental manipulation, the response of photosynthetic
   activity in *A. americana* to light and develop a light index that is compatible with
   the EPI model.

2. Quantify the response of *A. americana* production to water inputs using
   previously published data from a field site in Maricopa, AZ, and develop a water
   index that is compatible with the EPI model.

3. Develop a temperature index that is compatible with the EPI model using
   previously published datasets that describe the response of *A. americana*
   production to temperature.

4. Calculate monthly index values for water, temperature, and light that correspond
   to environmental conditions measured in Maricopa, AZ, where a field trial of *A.
   americana* was established in 2012.

5. Compare predictions of *A. americana* plant productivity from the new EPI model
to actual above-ground biomass measurements.
CHAPTER 2: METHODS

EPI Construction

Light Response

Beginning in December of 2016, 6 four-year-old *A. americana* individuals grown in a greenhouse at Ohio University were transplanted into cylindrical pots 19cm tall and 70cm in diameter containing approximately 47% Harvest™ organic garden soil, 47% Country Side Accents™ potting soil, and 6% Scotts™ turf builder fertilizer. Plants were then placed in a growth chamber (Conviron growth chamber; Winnipeg, Manitoba, Canada) with 12-hour photoperiods of 25/15°C day/night temperatures. The target photosynthetically active radiation (PAR) for each treatment was achieved by three mixed halogen/fluorescent light banks (Conviron) that remained constant for all treatments and supplemented by three 1200 watt LED grow lights (Roleadro COP) at higher light levels (*Figure 1, D*). The light environment was measured at mid-canopy using a LI-190 Quantum Sensor (Li-Cor, Lincoln, NA, USA) and six targeted light treatments were applied as PAR photon flux densities (PPFDs) of 100, 250, 500, 750, 1000, and 1250 µmol photons m⁻² s⁻¹. All individuals were exposed to each light treatment for 10 days prior to gas-exchange measurements to allow for enzymatic acclimation (Nobel, 1991), and watered to field capacity 48 hours prior to gas-exchange measurements to prevent physiological changes due to water limitations (Erhler, 1982).

At the end of each 10 day acclimation period to each new light treatment, 24-hour gas exchange measurements were collected for all 6 individuals using 3 randomly assigned LI-COR 6400xt portable photosynthesis systems (LiCor Inc.; Lincoln, Nebraska, USA). The exact PAR received at the surface of measured leaves was recorded
and used to calculate the relationship between PAR and 24-hour carbon fixation. For this reason, PAR varied from the target values described above. During the 24 hour measurement period, the leaf-atmosphere exchange of carbon dioxide was measured as micromoles of CO$_2$ fixed per square meter of leaf area per second (µmol CO$_2$ m$^{-2}$ s$^{-1}$) at 5 minute intervals. The integrated net CO$_2$ assimilated over each 24 hour measurement was determined using Simpson's rule (McKeeman, 1962) in the R studio statistical program.

**Light Indices**

The photosynthetic response of *A. americana* to PAR was quantified by the best fit equation that related the PAR treatments to the net moles of CO$_2$ fixed over 24 hours. A light index was generated by converting the dependent light response variable to a proportional value that ranged up to 1.00 at the maximum photosynthetic rate at the horizontal asymptote of the equation for light response.

The monthly average Langley’s recorded at the Arizona Meteorological Network’s (AZMET) automated weather station in Maricopa (https://ag.arizona.edu/azmet/az-data.htm) was converted into the monthly average daily PAR (see appendix 1 for conversion calculation) and used to calculate a monthly light index.

**Water Indices**

Observations of *A. americana* productivity from April 2012 to June 2015 under different irrigation treatments (Davis et. al, 2016) were used to construct an equation describing the water response, and water index of *A. americana*. The average monthly water input for each irrigation treatment was determined by dividing the total annual moisture received in each irrigation treatment by 12 (months). The relationship between
water availability and growth was resolved by regressing the annual average dry biomass against the average monthly water input.

The water index was developed by dividing each of the dry biomass measurements in each water treatment by the highest recorded monthly average dry biomass measurement recorded. This percent of the highest annual dry biomass production was plotted against the monthly total moisture inputs, and the equation of the best fit line through these points was used to calculate the monthly water index values.

The total moisture that each treatment received every month was calculated by combining the total amount of irrigation and total precipitation (mm). The water index value for each month was calculated using the equation of the best fitting line representing the water index and defining the water variable as the total water inputs. Months that received a calculated water index value greater than 1.00 were assigned an index value of 1.00 as actual productivity due to water availability cannot exceed 100%.

**Temperature Indices**

Previous results have shown no reductions in CO\textsubscript{2} assimilation for *A. americana* due to high daytime temperatures until about 45\degree C (maximum heat tolerance in *A. americana* is a staggering 63\degree C) (Nobel & Smith, 1983). The field site used to test the EPI never had an average monthly maximum temperature above 41\degree C. Furthermore, nighttime temperatures are more crucial for the net CO\textsubscript{2} uptake of obligate CAM plant species (Nobel & Hartsock, 1978). As such, the temperature index values were determined using night time temperatures adjusted 2\degree C above the monthly average minimum temperatures recorded at the AZMET meteorological station to correct for
large *Agave* species tissue temperatures being an average of 2°C warmer than nighttime air temperatures (Nobel, 1988).

The change in productivity in *A. Americana* due to shifts in nighttime temperatures was determined by combining results from studies done on the percent buildup of titratable tissue acidity due to experimentally decreased (Nobel & Smith, 1983) and increased (Neales, 1973) nighttime temperatures while daytime temperatures were kept constant. The temperature index was generated by dividing the percent buildup of titratable tissue acidity by 100. The equation used to calculate the monthly temperature indices was derived by plotting the temperature index vs. nighttime temperature (°C) and then fitting the best fit polynomial through the points.

**EPI Calculation**

The monthly EPI values are equal to the product of water, temperature, and light index values. Monthly EPI values were calculated for the field site in Maricopa, AZ according to the monthly average PAR, nighttime temperatures in the experimental field plots, and the total monthly water inputs. EPI values were calculated for three of the four irrigation treatment groups. The EPI of the fourth and highest irrigation treatment tested in the field study was excluded in comparisons with actual productivity measurements because of high mortality due to the presence of the common native pest *Scyphophorus acupunctatus* (Davis et. al, 2016; Waring & Smith, 1986). The sum of the monthly EPI values for each treatment was compared with the average annual dry biomass yields recorded in 2014 and 2015. Finally, the average total dry biomass of 2 and 3 year old *A. Americana* was regressed against the summed monthly EPI predictions to determine how well the new EPI predicts biomass yields.
CHAPTER 3: RESULTS

Light Response

As the mid-level canopy light intensity was increased, both the duration and rate of CO$_2$ assimilation by *Agave americana* plants over the 24-hour measurement periods tended to increase as well (*Figure 1, A-C*). When acclimated and exposed to 100 μmol photons m$^{-2}$ s$^{-1}$, no carbon assimilation in phases 2 or 4 was evident, and assimilation only occurred during the dark periods (phase 1) in *A. americana* individuals. All measurements at 250 μmol photons m$^{-2}$ s$^{-1}$ and above had distinct patterns in carbon assimilation during phase 4 in addition to phase 1. A pronounced period of CO$_2$ assimilation in phase 2 was not observed in any of the light treatments. Occasional negative CO$_2$ assimilation rates were measured during phase 2 and 3 at all light levels (see appendix 2 for all gas exchange measurements).
Figure 1. The 24-hour rate and duration of CO$_2$ assimilation in *A. americana* beginning at 4pm and ending at 4pm the next day at 100 (A), 500 (B), and 750 µmol photons m$^{-2}$ s$^{-1}$ (C). Yellow and black bars along the X-axis represent when lights turned on at 8am and off at 8pm respectively during the 24 hour measurement period. The 6 *A. americana* individuals selected for this study were kept under a 12-hour photo period of 25°C/15°C day/night temperatures (D).

The best fit 2$^{nd}$ order polynomial describing the relationship between PAR intensity (µmol photons m$^{-2}$ s$^{-1}$) and carbon assimilation (24-hour net moles) was equal to 

$$y = -4E-07x^2 + 0.001x - 0.0006 (Figure 2, A),$$

and peaks at a net 24 hour CO$_2$ assimilation of 0.6244 moles at a light intensity of 1250 µmol photons m$^{-2}$ s$^{-1}$ ($R^2 = 0.7356$). Two of the measurements made at 100 µmol photons m$^{-2}$ s$^{-1}$ had a net negative 24-hour carbon assimilation rate (Figure 2, A).
Figure 2. The relationship between the net moles of CO$_2$ assimilated over 24 hours vs. the acclimated 12-hour photoperiod PAR exposure (A) and the derived PAR index relationship (B) in *A. americana* with best fit 2$^{nd}$ order polynomials (equations and R$^2$ values shown on figure). The PAR intensity is equal to the actual measured PAR experienced by the leaf being measured.

**Water Response**

The maximum mean annual biomass recorded in the experimental field trial was 9.27 Mg ha$^{-1}$ y$^{-1}$ when the mean monthly annual input was 45 mm (*Figure 3, A*). The relationship between the mean annual dry biomass gain and water inputs was represented by the linear equation: $y = 0.2591x - 2.6443$ with an R$^2$ value of 0.9675 where x is equal to the monthly mean water input (mm) and y is equivalent to the annual dry biomass gain (Mg ha$^{-1}$ y$^{-1}$).
Figure 3. The annual gain in dry biomass in response to the mean monthly water input as described by Davis et al, 2016 (A), as well as the calculated water index relationship (B). Error bars represent standard error. Linear functions and $R^2$ values are shown on their respective plots.

Temperature Response

The relationship between the percent of total titratable tissue acidity and nighttime temperature ($^\circ$C) was best fit by the 5th order polynomial $y = -2E-05x^5 + 0.0013x^4 - 0.0169x^3 - 0.3875x^2 + 10.527x + 35.194$ with an $R^2$ value of 1 where $x$ is equal to the nighttime temperature and $y$ is the percentage of titratable acidity (Figure 4, A). The reported consensus on optimum nighttime temperature is 15$^\circ$C, and productivity ceases when nighttime temperatures are at or below -3$^\circ$C and at or above 38$^\circ$C (Nobel & Smith 1983; Neales, 1972).
**Figure 4.** Change in nocturnal tissue acidity in *A. americana* tissues in response to variation in nighttime temperatures as shown by the percent of maximum recorded titratable acidity (A) and the calculated temperature index relationship (B). In plot A, blue circles represent data collected by Nobel & Smith 1983 (graphically extrapolated), and red X’s represent data from Neales, 1972. The equations of the best fit 5th order polynomial and $R^2$ values are displayed on the plots.

**Productivity Indices**

The PAR, Water, and Temperature indices can be calculated as

\[
(PAR \text{ Index}) \quad y = -7E^{-7}x^2 + 0.0016x - 0.001 \quad \text{(Eq. 2)}
\]

where $x$ is equal to each months average daily PAR in $\mu$mol photons m$^{-2}$ s$^{-1}$ (*Figure 2, B*),

\[
(Water \text{ Index}) \quad y = 0.0279x - 0.2851 \quad \text{(Eq. 3)}
\]

where $x$ is equal to the monthly total moisture in mm (*Figure 3, B*), and

\[
(Temperature \text{ Index}) \quad y = -0.2E^{-7}x^5 + 0.13E^{-4}x^4 - 1.66E^{-4}x^3 - 3.878 \times 10^{-3}x^2 + 0.10524x + 0.35195 \quad \text{(Eq. 4)}
\]

where $x$ is equal to the average monthly minimum nighttime temperature in ºC (*Figure 4, B*).

The monthly PAR, water, and temperature indices were determined for the field site in Maricopa, AZ using the average monthly PAR, monthly total water inputs (including irrigation and precipitation), and monthly average minimum nighttime temperatures over a three year period from spring of 2012 through spring of 2015 (*Figure*...
Water was most limiting to productivity, falling to an index value of 0.00 for several months for all irrigation treatments after plants were established in AZ (Figure 5, B2). PAR availability was the second most limiting (Figure 5, B3), and overall temperature was the least limiting of the three abiotic variables, with an index value of 1.00 during the summer months (May-August) (Figure 5, B1).

Figure 5. Monthly average PAR (A1), total moisture (A2), and max/min temperatures (A3) at a field site in Maricopa, AZ were used to estimate production relative to the theoretical optimum based on predicted responses to light (B1), water (B2), and temperature (B3) from April of 2012 to June of 2015. There were 4 different irrigation treatments at the AZ field site, generating 4 different monthly EPI values (shown in A2, and B2 as gray scale lines where 780mm=black, 530mm=dark grey, 460mm= grey, 300=light grey).
EPI Estimates

The total summed monthly environmental productivity index values for the 300, 460, 530 and 780mm irrigation treatments from April 2012 to June 2015 in AZ field site were calculated as 14.47, 17.17, 20.58, and 23.59 respectively. The biomass measurements from the 780mm treatment were not compared with EPI estimates due to mortality of the plants caused by the local native pest *Scyphophorus acupunctatus* (Davis et. al, 2016; Waring & Smith, 1986). The monthly EPI values were equal to the product of PAR, water, and temperature index values for each month (*Figure 6*).

![Figure 6](image)

*Figure 6.* Calculated monthly EPI values for the 300, 460, 530, and 780mm annual irrigation treatments in Maricopa, AZ from April 2012 to June 2015.

For all irrigation treatments, the average gain in total dry biomass increased significantly (*p* > 0.05) from 2014 to 2015 (*Figure 7*). In all treatments, the summed EPI also increased from 2014 to 2015. In 2014 and 2015, the average annual dry biomass gain and the summed EPI values both increased with increasing irrigation treatments as well.
Figure 7. Average annual recorded dry biomass gain from 2014 and 2015 (bars) shown with the corresponding summed EPI values (slashes) by irrigation treatment. Error bars are used to represent standard error. The increase in total biomass was significant (p>0.05) from 2014 to 2015 for all irrigation treatments.

The summed monthly EPI values were strongly correlated ($R^2=0.9988$) with the average total dry biomass of healthy 2 and 3 year old *A. americana* individuals in AZ, with a linear relationship $y=0.7607x-14.774$, where $x$ was equivalent to the summed monthly index values and $y$ is equal to the average total dry biomass (*Figure 8, A*). When, however, the summed monthly EPI values and the average total biomass of all plants, including those infested with *S. acupunctatus*, are compared, the correlation coefficient is reduced ($R^2=0.7451$) (*Figure 8, B*). This is due to *S. acupunctatus* causing a
reduction in the total average biomass that is not accounted for in the EPI model for *A. americana*.

*Figure 8.* The total gain in biomass vs. the summed monthly EPI estimates for healthy *A. americana* individuals in Maricopa, AZ (A) as well as the total gain in biomass vs. the summed monthly EPI estimates for all *A. americana* individuals in which measurements including infested individuals are shown in red (B). The best fit linear equations and $R^2$ values are shown on their respective plots. Error bars are representative of corresponding standard error values.
CHAPTER 4: DISCUSSION

This study resolved for the first time the photosynthetic response of *Agave americana* to light and developed a simple EPI model that can be used to provide a convenient index for predicting and comparing the potential productivity of this valuable crop species around the globe. The optimum light conditions were found to be at a higher light saturation point than that of other large agricultural CAM species. Other highly productive CAM species typically have a light saturation point of 700 µmol photons m\(^{-2}\) s\(^{-1}\) (Nobel, 2003, 1988), and the experimentally determined light saturation point of *A. americana* was found here to be 1250 µmol photons m\(^{-2}\) s\(^{-1}\). This finding suggests that *A. americana* is adapted to areas that receive relatively longer time periods of high light intensity throughout the year. The actual total biomass accumulated by *A. americana* individuals grown in Maricopa, AZ were highly correlated with EPI estimates for 2 and 3 year old plants in all irrigation treatments. The mathematical relationship derived from this regression may serve as a tool for projecting potential productivity of *A. americana* in areas where no yield data are available.

The EPI developed for *Agave americana* grown in Maricopa, AZ revealed, not surprisingly, that water was the most limiting of the three abiotic factors in question. While results showed that the yearly EPI predictions are strongly correlated with the average total gain in biomass in healthy *A. americana* individuals, EPI predictions are less correlated with the average total biomass when *A. americana* individuals infested with the native pest *Scyphophorus acupunctatus* (agave snout weevil) are included in comparisons. Higher irrigation (530 and 780mm) treatments had a higher percent of infestation by the snout weevil than that of the lower irrigation treatments (Davis et al.)
The EPI model can only be used to predict the productivity of healthy individuals as infestation and disease reduce productivity in ways not accounted for in the current EPI. The establishment and presence of *S. acupunctatus* is often difficult to diagnose in *A. americana*, as individuals remain in a vegetative state before showing symptoms (Davis et al., 2016; Kelly & Olsen, 2011, Warring & Smith, 1986). Future efforts to produce biofuels from *A. americana* will depend upon developing means to control *S. acupunctatus* infestation (Kelly & Olsen, 2011), or growing *A. americana* in areas where *S. acupunctatus* does not currently exist such as Australia (Holtum et al., 2011).

For the prediction of plant productivity in individuals older than 3 years of age, a high correlation between the summed EPI and average total biomass may also depend upon the knowledge of how PAR responses in *A. americana* may change as the plants grow (Nobel, 1986, 2003). That is to say that the photosynthetic response to sunlight in *A. americana* changes as the leaf area, angles (de Cortázar & Nobel, 1986; Nobel, 2003; Woodhouse et al., 1980) and spectral reflectance (Christensen & Goudriaan, 1993) change with plant growth. The *A. americana* used to determine the photosynthetic light response in this experiment were 4 years of age, and grown in pots. Future studies to understand how the photosynthetic response changes due to the changing geometry of *A. americana* rosettes, leaf size, spectral reflectance, and angle with age could be used to calibrate the light index equation in order to ensure the accuracy of EPI estimates for all ages (Davis et al. 2015).

The variation in the net moles of CO₂ fixed over 24 hours when individuals were acclimated and exposed to the same light level was likely an effect of the differences between genotypes (Sultan, 2000), as the *A. americana* individuals used for gauging light
response were not genetically identical. It is appropriate then to use the experimentally resolved light response relationship to represent the photosynthetic light response for plants grown in the Arizona field site since those individuals are not genetically identical either. The relationship defined here should be a reasonable approximation of the average photosynthetic response experienced by *A. americana* in the field.

The equation describing the water index for *A. americana* was derived by comparing the average annual biomass gain of plants in the Maricopa, AZ field site with the mean monthly water input per year. Since comparisons between the EPI estimates and actual growth in this study were also compared by using results pertaining to the same field site, the water index is still in need of validation in other field sites and/or through comparing EPI predictions with other *A. americana* growing operations. While research exists on how volumetric soil moisture content affects the transpiration rate in *A. americana* (Erhler, 1983), it would be highly valuable to have an understanding of how nocturnal carbon uptake responds to a decrease in the soil water potential, from field capacity to permanent wilting point. Combining results from such a study with already existing models that predict how desert soil water potentials are altered by precipitation and drought (Reynolds et al, 2000, 2004; Young & Nobel, 1986) would allow for more precise predictions of productivity for *A. americana* in desert habitats of all soil types.

This same method has been used for constructing the water index for several *Agave* and *Opuntia* species (Nobel, 2003, Nobel & Quero, 1986; Nobel & Valenzuela, 1987; Nobel, 1976; Nobel & Meyer, 1985; and Nobel & Hartstock, 1984).

Analysis of past studies aimed at understanding how net CO$_2$ uptake in *A. americana* is affected by temperature have resolved that low and high nighttime
temperatures limit gas exchange more so than do low and high daytime temperatures in xeric and semi-arid regions (Neales, 1973; Nobel & Smith, 1983; Nobel & Hartsock, 1978). *A. americana* had negligible mortality rates in the field site due to low nighttime temperatures (Davis et al, 2016). Cold tolerance in *A. americana* has been experimentally determined to acclimate by 1.8°C per 10°C decrease in day/night temperatures, and the minimum cold tolerance (point of 50% cell death) is -7.4°C (Nobel & Smith 1983). The average monthly minimum temperatures in Maricopa never dropped below 0°C (Figure 5, C3).

Alternatively, high-temperature hardening in *A. americana* acclimates 3.3°C for every 10°C increase in day/night temperatures, and has a maximum high temperature tolerance of 63.8°C (Nobel & Smith, 1983) which never occurred in the field site evaluated here (Figure 5, A3). When considering alternative sites for *A. americana* agriculture, high and low daytime temperatures of some regions may be extreme enough to have a noticeable effect on nocturnal gas exchange, and therefore the temperature index may need calibration for such cases.

Although the goal of this study was to use photosynthetic responses of light, water, and temperature to parameterize a productivity model, adding a nutrient index parameter may also improve estimates of productivity for certain regions in that it would assimilate edaphic influences in calculated estimates (Nobel, 1988, 1989). Adding a nutrient index into the EPI model for *A. americana* would likely have a negligible change on the EPI values calculated for the Maricopa field site, as all irrigation treatment groups were fertilized on an annual basis (Davis et. al, 2016). However, a well-designed study to determine the photosynthetic response of *A. americana* to variation in soil nutrient
content may prove useful in defining the minimum amount of nitrogen fertilizer necessary for optimum nocturnal carbon fixation. It has been suggested that CAM photosynthesis may have evolved not only as a way to use water more efficiently, but also increase nitrogen use efficiency (Baattrup-Pedersen & Madsen, 1999; Lüttge et. al, 1991; Santos & Salem, 1991; Nobel & Hartsock, 1986b; and Winter et. al, 1982), and therefore understanding the photosynthetic response to nitrogen availability in *A. americana* may improve the efficiency of fertilizer inputs for future production.
CHAPTER 5: CONCLUSION

A simple environmental productivity model developed for *Agave americana* is a useful starting point for predicting potential yields in semi-arid and xeric regions around the world. The EPI model will be valuable for understanding geographical ranges that will become favorable for *A. americana* as climate changes in the near future.

The accuracy of the current EPI for predicting growth is limited to conditions where physiological limitations are dominated by light, temperature, and water conditions. However, the simplicity of this model allows for calibration of the already defined index equations and the addition of other abiotic or biotic indices to improve prediction of productivity in areas where other limitations on growth are already well-described.
REFERENCES


APPENDIX 1: LANGLEY’S TO PAR CONVERSION

The AZMET weather station in Maricopa, AZ recorded daily solar radiation in Langley’s which is a unit of energy distribution over an area (www.nist.gov). 1 Langley is equivalent to 11.622 watt-hours of light in the visible spectrum (390-700nm) per square meter. In order to compute the light index, Langley’s were converted into units of photosynthetically available radiation (PAR). PAR is equal to the number of micro-moles of photons hitting one square meter per second within the spectrum typically used by plants for photosynthesis (400-700nm) (Enoch & Kimball, 1986).

In order to convert daily solar radiation (Langley’s) to the average daily PAR received that day, Langley’s was first converted to watt-hours per square meter. Secondly, the number of watt-hours per square meter was divided by the number of daylight hours to give watts per square meter. Then, watts per square meter was multiplied by the average global annual luminous efficacy value of 110 lumens per watt (Littlefair, 1985) in order to convert to lux (lumens per square meter). Finally, lux was converted into the average daily PAR using the relationship 1 klux = 18 µmol photons m⁻² s⁻¹ of daylight (LI-COR, 1982). A sample calculation of the average daily PAR for a 12 hour long day that received a total solar radiation of 600 Langley’s is shown below as:

\[
600 \text{ Langley's} \times \frac{11.622 \text{ watt hours}}{m^2} \times \frac{1 \text{ Day}}{12 \text{ Hours daylight}} \times \frac{110 \text{ lumens}}{m^2} \times \frac{1 \text{ lux}}{1000 \text{ klux}} \times \frac{18 \mu \text{mol photons m}^{-2} \text{ s}^{-1}}{klux} = 1150 \mu \text{mol photons m}^{-2} \text{s}^{-1}
\]
APPENDIX 2: LIGHT RESPONSE RESULTS

Below are the 24-hour gas exchange measurements used for determining the photosynthetic light response in *A. americana*. The main title has the individual *Agave* identification number followed by the PAR the sampled leaf was acclimated to. y-axis is the photo rate in µmol of CO$_2$ fixed m$^{-2}$ s$^{-1}$, and the x-axis is seconds into the 24-hour measurement. Yellow and black bars along the x-axis represent when lights were on and off respectively. Some measurements are not shown due to sampling, and mechanical errors (blown fuses, bad chemicals, dud CO$_2$ cartridges, and etc.).