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Understanding the Ecological Role, Population Dynamics, and Geographic Distribution of *Manihot Walkerae*

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UNDERSTANDING THE ECOLOGICAL ROLE, POPULATION DYNAMICS, AND GEOGRAPHIC DISTRIBUTION OF *MANIHOT WALKERAE*

A Thesis

by

GISEL GARZA

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In partial fulfillment of the requirements for the degree of

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UNDERSTANDING THE ECOLOGICAL ROLE, POPULATION DYNAMICS, AND GEOGRAPHIC DISTRIBUTION OF *MANIHOT WALKERAE*

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ABSTRACT

Garza, Gisel., Understanding the ecological role, population dynamics, and geographic distribution of *Manihot walkerae*. Master of Science (MS), May 2021, 91 pp., 11 tables, 21 figures, references, 100 titles.

Walker's Manihot, *Manihot walkerae* Croizat (*Euphorbiaceae),* is an endangered plant that is endemic to the Tamaulipan thornscrub ecoregion. Understanding *M. walkerae's* geographic distribution, populations, and species interactions can provide essential information for the development of sound conservation strategies. To this aim, I asked the following questions: 1) What is the potential geographic distribution for *M. walkerae*? and, will it be affected by climate change? 2) Using global and regional extinction risk assessments, what is the extinction risk category for *M. walkerae* after incorporating species distribution models? 3) What do natural history observations reveal about *M. walkerae's* population composition, and insect interactions? These questions were answered using species distribution modeling, both IUCN and Mexican Risk Assessment methods, and natural history observations. The results of this work could be used to establish national and international strategies to conserve this endangered species.

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tracts of the Lower Rio Grande National Refuge. I am also grateful to Anna Strong and Kimberly Wahl for providing information on *M. walkerae's* distribution and biology. Thank you also to the Texas Natural Diversity Database for sharing distribution data for *M. walkerae,* and to the private landowners who allowed me to conduct field work in their property.

Finally, I wouldn't be where I am today if it wasn't for the support from my family and my boyfriend so I would like to express my thanks to them too.

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CHAPTER I

INTRODUCTION

Background and Threats

The Lower Rio Grande Valley of Texas, U.S.A. (LRGV: Hidalgo, Starr, Cameron, and Willacy counties) and northeastern Tamaulipas, Mexico compose part of the Tamaulipan thornscrub ecoregion, a highly biodiverse area that is home to unique endemic species of plants and animals of which nineteen are federally threatened or endangered and nearly 60 are state-protected species (Cook et al., 2020; Jahrsdoerfer and Leslie, 1988; Leslie, 2016).

With over 95% of the focal region's Tamaulipan thornscrub modified or destroyed, native species of plants and wildlife are faced with the loss of their habitat (Cook et al., 2020; Jahrsdoerfer and Leslie, 1988; Leslie, 2016). Additionally, a part of the Tamaulipan thornscrub ecoregion is found in the southwestern United States, where the greatest temperature increase of any area in the lower 48 states is predicted to occur (Best, Miller, and Cobb, 2009; Pachauri and Reisinger, 2007). Climate change could also cause semi-arid areas like the Tamaulipan thornscrub to experience a decrease in water resources. Collectively, climate change could have adverse effects on native species by restricting their range and increasing the competitive advantage of invasive species (Best, Miller, and Cobb, 2009; Thuiller, Richardson, and Midgley, 2008), such as *Cenchrus ciliaris* buffelgrass (Best, Miller, and Cobb, 2009; Marshall, Lewis, and

Ostendorf, 2012), one of the most detrimental invasive species in the Tamaulipan thornscrub ecoregion and competitor of native plants (Best, Miller, and Cobb, 2009; Clayton, 1993; Leslie, 2016; Vera-Sanchez and Nassar, 2019).

Walker's Manihot, *Manihot walkerae* Croizat *(Euphorbiaceae*), is a federally and state endangered species of Texas that is endemic to the Tamaulipan Thornscrub ecoregion (Best, Miller, and Cobb, 2009; Clayton, 1993; Leslie, 2016; Vera-Sanchez and Nassar, 2019). It is a perennial vine-like subshrub that is characterized by its palmate five-lobed leaves, white flowers, and circular dehiscent fruits. *M. walkerae* is a species with tubers that allow for it to die back during unfavorable conditions such as during droughts or in freezing temperatures. This species flowers during rains starting in spring and it is ongoing into the summer and early fall months, fruiting occurs approximately 4 weeks after flowering (Poole, Carr, & Price, 2007). Like other members of the *Manihot* genus and of the Euphorbiaceae, *M. walkerae* seeds have a seed appendage known as an elaiosome. Elaiosomes are lipid-rich seed appendages that are attractive to ants who then provide the service of seed dispersal (Leal et al, 2014). This type of interaction is known as myrmecochory, and seed dispersal by ants has been recorded in closely related members of *M. walkerae* such as the cassava plant *Manihot esculenta* (Elias et al, 2004). When *M. walkerae* fruits are mature they dehisce and release on average three seeds per fruit. Although ant seed dispersal is believed to occur after *M. walkerae* seeds are released, it has not been previously observed or documented.

Manihot walkerae is found in semiarid, shaded shrublands on xeric slopes and uplands, often on overexposed caliche outcrops (Best, Miller, and Cobb, 2009; Clayton, 1993; Jahrsdoerfer and Leslie, 1988; Leslie, 2016; Vera-Sanchez and Nassar, 2019). Habitat destruction, fragmentation, herbicide application, overgrazing, herbivory by native and introduced wildlife, surface mining of caliche, petroleum and natural gas exploration, urban and residential development and competition by invasive plant species are risk factors that affect *M. walkerae* (Best, Miller, and Cobb, 2009; Clayton, 1993; Vera-Sanchez and Nassar, 2019).

Ecological and Economic Importance

Manihot walkerae serves an ecological role in the Tamaulipan thornscrub ecoregion and shares species interactions with native wildlife (Leslie, 2016). Additionally, *M. walkerae* is a wild relative of the widely utilized agricultural crop Cassava (*Manihot esculenta*). Cassava is a staple worldwide and serves many roles in food, biofuel, and industrial uses (Morante et al., 2010; Saravanan et al., 2016; Zainuddin et al., 2018). A major problem for the Cassava agricultural industry is post-harvest deterioration, a condition which limits the time that Cassava is viable for consumption after its harvest. Studies have found that hybridizing *M. walkerae* with Cassava has resulted in a tuber that is more resistant to post harvest deterioration (Morante et al., 2010; Saravanan et al., 2016; Zainuddin et al., 2018). Furthermore, *M. walkerae* possesses genes that are resistant to prominent diseases of cassava such as Cassava brown streak, and Cassava bacterial blight and it also contains genes for cold resistance (Clayton, 1993). Given the benefits that the genetic constituents of *M. walkerae* provide, it is a crop wild relative (CWR) of great use to improve longevity and disease resistance in Cassava and its extinction could have negative effects on the future of this crop as its genetic diversity would no longer exist (Dempewolf et al., 2014).

Historical Background

Manihot walkerae was first discovered in the late 1800s in Rio Grande City, Texas, but was misidentified as another species (Best, 1996). It was not until 1942 that *M. walkerae* was identified as its own separate species after Mrs. E. J. Walker collected a sample in the city of La

Joya, Texas, and sent it for further identification (Best, Miller, & Cobb, 2009). These historical *M. walkerae* populations are believed to be extinct as a result of extensive land clearing for agriculture use and development; a cutting from the original 1942 individual however, was sent to the University of Texas at Austin and the species was propagated in the San Antonio Botanical Gardens (Best, 1996). Since then, a few sightings of *M. walkerae* were observed in Tamaulipas, Mexico, but after a 30-year period with no sightings it was believed to have gone extinct in the wild (Best, 1996). Consequently, a single *M. walkerae* individual was later discovered in 1990 within private property in La Joya, Texas, and survey efforts led to the discovery of two other small populations in Tamaulipas, Mexico (Best, 1996). This led to the listing of *M. walkerae* as a federally endangered species in 1991, a state endangered species in 1993, a ranking of imperiled (G2) by the Nature Reserve global conservation rank, and most recently it was listed as endangered by the IUCN's Redlist in 2019.

In 1995 local botanists discovered a few other populations in Hidalgo and Starr County, although most do not have enough individuals to be viable (Best, Miller, & Cobb, 2009). The three largest historical *M. walkerae* populations are all found within protected tracts of the Lower Rio Grande Valley National Wildlife Refuge (LRGV NWR) and have 45-90 documented individuals (Best, Miller, & Cobb, 2009). The other few historical populations are inside of private properties and along right of roadways (ROW) in Hidalgo, Starr, and Duval counties in Texas, and Tamaulipas Mexico. It is evident that *M. walkerae* is severely affected by habitat fragmentation as it is found in a few restricted areas that offer no connection to each other. As a result, *M. walkerae* faces added problems such as a lack of gene flow and a small gene pool that would make it difficult for this species to survive when faced with added disturbances (Best, 1996). The successful conservation of *M. walkerae* is therefore dependent on the cooperation between private landowners and conservationists, scientists conducting research to better understand this species and educating the local communities about the significance of *M. walkerae.*

Justification

Manihot walkerae is an example of an endemic endangered species that has been affected by habitat destruction, fragmentation, and invasion by non-native species (Best 1996, Best, 2009). An obstacle for individuals wanting to learn more about *M. walkerae* is the lack of readily available peer-reviewed literature on this species. Current literature consists primarily of reports that are written by individuals in federal and state agencies, and non-governmental organizations that develop plans to conserve endangered species. Additional published literature on *M. walkerae* mention it as a source of genetic material for its widely used agricultural relative cassava, *Manihot esculenta* (Ewa, 2018). There exists an urgent need to conduct studies that will aid in understanding *M. walkerae's* role in local ecosystems as well as its ecological and habitat requirements. The value that *M. walkerae* provides to local ecosystems although unexplored is important, it is a part of the trophic relationships of native communities and its removal could have a cascading effect on other organisms.

In this thesis, I address some questions that could help better understand *M. walkerae*: 1) What is *M. walkerae's* predicted geographic distribution*,* and will it be affected by climate change? 2) Using global and regional extinction risk assessments, what is the extinction risk category for *M. walkerae* after incorporating species distribution models? 3) What do natural history observations reveal about *M. walkerae's* populations, and insect interactions? These questions will be addressed using species distribution modeling, extinction risk assessments, and natural history observations. The ultimate outcome of this thesis is meant to help in ongoing and future conservation efforts for *M. walkerae*.

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CHAPTER II

THE POTENTIAL EFFECTS OF CLIMATE CHANGE ON THE GEOGRAPHIC DISTRIBUTION OF THE ENDANGERED PLANT SPECIES *MANIHOT WALKERAE*

Introduction

Anthropogenic activities have a significant influence on the geographic distribution, rate of extinction and endangerment of many of the world's plant species (Paul et al., 2013). These activities have led to the fragmentation and destruction of plant habitats, as well as the introduction of invasive competitors and pests (Crowl et al., 2008). Climate change is also having resonant impacts on plants and wildlife (Jump and Penuelas, 2005; Thuiller et al., 2005; Walther et al., 2002). It is predicted that there will be a shift in the distribution of plants towards higher elevations and latitudes to attempt to cope with the changing climate. However, for plants that are rare, endemic, have lower dispersion distances, or persist in fragmented areas, this transition will be difficult, and they will tend instead toward extinction (IŞIK, 2011; Jump and Penuelas, 2005). A plant's suitable habitat and distribution is dependent on temperature along with other environmental factors, and with changing temperatures they are expected to expand or restrict (Pulliam, 2000; Soberón and Peterson, 2005). Invasive plant species that find higher temperatures favorable are expanding in range and out-competing native species (Thuiller, Richardson, and Midgley, 2008), while many endemic plants are projected to lose their suitable habitat and are facing extinction. According to the Intergovernmental Panel on Climate Change

(IPCC) global temperatures are projected to increase, with heat waves and heavy precipitation events becoming more frequent (Pachauri and Reisinger, 2007). For endemic plants that are already faced with habitat fragmentation and competition by invasive species, climate change could act as a catalyst for extinction (Brook, Sodhi, and Bradshaw, 2008; El-Keblawy, 2014; IŞIK, 2011; Leimu et al, 2010; Thuiller, Richardson, and Midgley, 2008).

Species distribution models (SDM) are useful tools in conservation planning to project the effects that climate change could have on an endangered species' distribution (Jeschke and Strayer, 2014; Pulliam, 2000). As our global awareness on climate change increases, SDM have progressively been used to project the effect of climate change on the distribution of invasive pests, pathogens, and endangered species (Abolmaali, Tarkesh, and Bashari, 2018; Adhikari, Barik, and Upadhaya, 2012; Ardestani et al., 2015; Jeschke and Strayer, 2014; Khanum, Mumtaz, and Kumar, 2013; Kurpis, Serrato-Cruz, and Arroyo, 2019; Qin et al., 2017; Vieilledent et al., 2013; Yu et al., 2014). Increasingly, studies have also started assessing the effectiveness of protected areas at conserving endangered species at present and in the future by incorporating climate change SDM models (Adhikari, Barik, and Upadhaya, 2012; Ardestani et al., 2015; Hole et al., 2011; Qin et al., 2017; Vieilledent et al., 2013; Yu et al., 2014;). One study conducted by Vieilledent et al, 2013 explored the effects of climate change on three endangered species of Madagascar (*Adansonia grandidieri* Baill., *Adansonia perrieri* Capuron and *Adansonia suarezensis* H. Perrier) and how climate change would modify the effectiveness of protected areas in the future. It was found that in the future, because of climate change, no protected areas were viable for conserving two of these species, which puts them at risk of future extinction (Vieilledent et al., 2013).

The objectives of this chapter are to evaluate the potential effects of climate on the climatic geographic distribution of *M. walkerae* and assess the usefulness of natural protected areas in future conservation. To do this, I constructed species distribution models of the current and future climatic geographic distribution of *M. walkerae* for the years 2050 and 2070 using three different general circulation models (CM3, CMIP5, and HADGEM), and two climate change scenarios (RCP 4.5 and 8.5). I hypothesize climate change could restrict *M. walkerae's* potential geographic distribution and reduce the effectiveness of protected areas at conserving *M. walkerae* in the future.

Methods

Occurrence Data

Occurrence data was obtained from three different sources: 1) Historical populations identified according to Source Features (SF; observations) shapefiles and Element Occurrences (EO) provided by the Texas Natural Diversity Database (TXNDD) (TXNDD 2016). SF and EO are matched with shapes and shapefiles using key identificatory fields (IDs). The EO ID represents populations and contains the complete information that TXNDD has for *Manihot walkerae*, 2) Non-digital data in the form of reports, handwritten notes, pictures and maps obtained from the Texas Parks and Wildlife Department (TPWD), and 3) Shapefiles provided by expert botanists that contain precise latitude and longitude data for parcels within the Lower Rio Grande Valley National Wildlife Refuge.

All gathered occurrences were converted into decimal degrees. Geographic autocorrelation was reduced using the "spatially rarefy occurrence data" tool in the SDM toolbox version 2.2 (Brown, 2014) at a distance of 4-km.

Study area and bioclimatic variables

The climatic potential geographic distribution of *M. walkerae* was generated using the Tamaulipan thornscrub ecoregion as the study area since it encompasses all *M. walkerae* historical occurrences (Figure 2.1). The Tamaulipan thornscrub is characterized by a subtropical, semi-arid vegetation type that occurs on either side of the Rio Grande delta (Cook et al., 2020). Spiny shrubs and trees dominate, but grasses, forbs, and succulents are also prominent. It is located within the physiographical province known as the Coastal Gulf Plain (Cook et al., 2020). The region originates in the eastern part of Coahuila, Mexico at the base of the Sierra Madre Oriental, and then proceeds eastward to encompass the northern half of the state of Tamaulipas, and into the United States through the southwestern side of Texas. Elevation increases northwesterly from sea level at the Gulf Coast to a base of about 300 m (1,000 ft) near the northern boundary of the ecoregion, from which a few hills and small mountains protrude (Cook et al., 2020).

BIOCLIM variables representing current and future conditions were downloaded from WorldClim, a database that provides climatic data derived from monthly temperature and precipitation collected from weather stations around the world, and interpolated onto a surface of approximately 1 km spatial resolution (Hijmans et al., 2005). Nineteen BIOCLIM variables representing current global climate data at a 30 arcseconds spatial resolution were used along with the future bioclimatic variables for three general circulation models (GCM): HadGEM2 (Hadley Centre for Climate Prediction and Research), CMIP5 (Coupled Model Intercomparison Project Phase 5), and CM3 (Geophysical Fluid Dynamic Laboratory) and for the two representative concentration pathways, 4.5 watts/m^2 and 8.5 watts/m^2(Table 2.1; Table 2.2) (Jalota et al, 2018). These scenarios were developed by the International Panel on Climate

Change (IPCC) based on levels of accumulation of greenhouse gas emissions, agriculture area, and air pollution (Pachauri et al., 2014; Van Vuuren et al., 2011). The 4.5 RCP represents an intermediate emissions scenario where temperatures are predicted to increase by approximately 1.5 °C by the end of the 21st century, while the 8.5 RCP represents the most severe scenario with an expected increase of over 2 °C by the end of the 21st century (Pachauri et al., 2014). Highly correlated environmental variables with a correlation value above 0.8 were excluded from modeling using the "remove highly correlated variables" tool in the SDM toolbox (Brown, 2014).

Running MaxEnt and creating consensus models

The spatially rarefied occurrences along with the low correlated BIOCLIM variables were input in MaxEnt (version 3.4.1) using default parameters and the bootstrap function. Twenty replicates were run for the current scenario and for each of the three general circulation models at 4.5 and 8.5 RCP for the years 2050 and 2070 (Kurpis, Serrato-Cruz, and Arroyo, 2019). Consensus models were produced from the twenty replicates following the works of Marmion et al, 2008. Each consensus model was then converted into a binary model using the reclassify tool and a threshold value acquired from the MaxEnt results.

Statistical Analysis

Receiver Operating Characteristics (ROC) was used to evaluate the models based on the area under the curve (AUC) and partial ROC (pROC) values. The AUC is used to evaluate a model's predictive ability where values range from 0 to 1, with those closer to 1 indicating models with a good predictive ability and a value of 0.5 representing a random predictive ability (Elith et al., 2006). However, the reliability of the AUC to evaluate the models has been brought into question for several reasons summarized by Lobo et al, 2007. Therefore, I also calculated the pROC as an additional statistic to evaluate the model (Peterson, Papeş, and Soberón, 2008) using NicheToolbox, an application that facilitates its calculation (Osorio-Olvera, 2018).

Protected areas maps

Using geographic information systems (ArcGIS) a polygon showing the area lost as a result of climate change was created and overlapped with polygons of natural protected areas in the Tamaulipan thornscrub ecoregion. The chosen protected areas in the U.S. were TPWD lands, and U.S. Fish and Wildlife Service (USFWS) Lower Rio Grande Valley National Wildlife Refuge tracts (LRGV NWR), while for Mexico they are Natural Protected Areas and Priority Terrestrial Regions from the CONABIO data base.

Results

The area under the curve (AUC) values of the consensus models were higher than 0.90, indicating that models had a high predictive ability (Table 2.3). The present geographic distribution consensus model produced from the ten BIOCLIM variables and nineteen spatially rarefied occurrences had an AUC value of 0.925 and overlapped well with known occurrences of *M. walkerae* (Figure 2.2: A) (Table 2.3). Areas of high suitability shown in red are majorly along the US-Mexico boundary and extend towards the southeastern portion of the Tamaulipan thornscrub study area. From the ten bioclimatic variables used to produce the model, the highest contributions were made by BIO 1 (Annual Mean Temperature), BIO 7 (Temperature Annual Range), BIO 19 (Precipitation of Coldest Quarter) and BIO 15 (Precipitation of Seasonality) which collectively contributed 89.4% to the model (Table 1). The bioclimatic variables that contributed least were BIO 2 (Mean Diurnal Range), BIO 3 (Isothermality), BIO 13 (Precipitation of Wettest Month) and BIO 14 (Precipitation of Driest Month) which collectively contributed only 3.4% to the model (Table 2.1).

The present geographic distribution binary consensus model was used to compare the percent change of geographic distribution with the future climatic models (Figure 2.2: B). For the year 2050, the most apparent change in distribution is seen in the northeastern portion of the Tamaulipan thornscrub study area (Figure 2.3). Both the CM3 and CMIP5 GCM at a 4.5 RCP emission scenario projected a slight increase of 7.20% and 7.42% in distribution respectively primarily in the northeastern portion of the study area (Figure 2.3: A & C). However, at a more severe emission scenario RCP 8.5 they both showed restrictions of distribution, most notably in the north and southwestern portion of the study area for the CM3 GCM -9.52% (Figure 2.3: B) and a slight decrease in the southernmost portion of the study area for the CMIP5 GCM -2.08% (Figure 2.3: D). The HadGEM GCM predicted a loss of distribution at both emission scenarios, but notably there is a higher calculated decrease in distribution for the 4.5 RCP -5.60% than the 8.5 RCP -1.19% (Figure 2.3: E & F) (Table 2.4).

All future climatic models for the year 2070 predicted a loss of potential distribution with notable differences in percent lost between the models (Figure 2.4) (Table 2.4). For the CM3 GCM loss of geographic distribution is apparent, especially for the highest emission scenario RCP 8.5 which shows a restriction of -13.63% in all areas especially in the northeastern area, northwestern portion along the border, and in the southernmost portion of the Tamaulipan thornscrub ecoregion (Figure 2.4: B). The largest restriction of distribution is shown by the CMIP5 GCM at the 4.5 RCP scenario -14.37% in all areas especially in the northeastern, northwestern portion along the border, and the southernmost portion of the study area (Figure 2.4:C). The HadGEM GCM at both emission scenarios show slight decreases of distribution in the southernmost portion of the study area (Figure 2.4: D & E) with a greater loss calculated for the most severe emission scenario -4.13% than the intermediate scenario -2.61%.

Discussion

The climate models produced show that the potential geographic distribution for *Manihot walkerae* in the years 2050 and 2070 could be slightly reduced as a result of climate change. As a consensus, the future climate change models show a restriction in future distribution for *Manihot walkerae* with the lowest loss of distribution calculated as -2.08% for the year 2050 with an RCP of 8.5 and the highest, -14.37% for the year 2070 with an RCP of 4.5. While for two of the future climate change scenarios at an RCP of 4.5 for the year 2050 it is predicted that there could be a potential increase of approximately 7% in distribution (Table 2.4). Similarly, another SDM study conducted in the Chihuahan desert found that some endemic plants were shown to be affected by climate change and expanded in distribution (Sosa et al, 2019). The areas that were shown to be most affected by climate change were those in the northeastern and southernmost portions of the Tamaulipan Thornscrub ecoregion (Figure 2.3; Figure 2.4; Figure 2.5). A reason that could explain why *M. walkerae's* geographic distribution wasn't severely restricted and was even shown to increase in some scenarios is because it is a species that is associated with warm temperatures. Temperatures in the Tamaulipan Thornscrub ecoregion are usually warm ranging from 52°F to 97°F and rarely below 39°F or above 102°F, additionally, the MaxEnt results show that *M. walkerae* responds to temperatures that are above 23°C.

Although there are no documented occurrences of *M. walkerae* in these regions, there are some protected areas within the area that was lost and it is predicted that they will not be suitable for *M. walkerae* in the future (Figure 2.5; Figure 2.6). This potential outcome could limit success in the future for conservation efforts such as reintroduction. Successful reintroduction of *M. walkerae* to increase the number of populations of this species would be best in areas that are predicted to have high potential for geographic distribution. Areas that have a high potential for geographic distribution also have the highest potentially suitable habitat for a said species. Species distribution modeling has been used as a tool for reintroduction of endangered species when models show the areas have potentially suitable habitat for a given species (Adhikari, Barik, and Upadhaya, 2012; Ardestani et al, 2015). In Texas, there are several protected lands that have high potential for geographic distribution for *M. walkerae* and that are predicted to be unaffected by climate change (Figure 2.5). These protected lands could be used for future conservation efforts such as the reintroduction of *M. walkerae*. In Mexico, currently there are no protected lands that lie within the areas that are potentially suitable for *M. walkerae*, making the future of this species in Mexico uncertain. In order for successful conservation efforts to be conducted in Mexico, relationships with private landowners that agree to conserve *M. walkerae* on their property would have to be formed.

Some limitations of this study are that only BIOCLIM variables were used and that a small number of occurrences were used for modeling. Using BIOCLIM variables for climate change modeling is common and has been used to model the effects of climate change on the distribution of different species of plants and animals, some of which are endangered and restricted (Hole et al, 2011; Sosa et al, 2019; Borzee et al, 2019). If I had included static topographic variables the reliability of the models could of improved, but in some instances when topographic variables such as elevation and bioclimatic variables are highly correlated, they could hinder the statistic reliability of the model (Stanton et al, 2012). In the case of this study, the AUC and pROC values were higher than random indicating that even though we used a low number of occurrences and bioclimatic variables these models could serve as a good reference for future conservation plans for *Manihot walkerae*. Most importantly, these models show that although there are some protected areas that could conserve this species in southern Texas, in Mexico there are no conservation areas that lay within *M. walkerae* historical occurrences or predicted current and future distribution. A probable reason why there are no protected areas for this species can be attributed to a lack of sufficient data on its biotic inventory, species ecological requirements, and species distribution patterns (Téllez-Valdés and D¡Vila-Aranda, 2003). This study provides valuable information for *M. walkerae's* distribution and can allow for an inference of some of the ecological requirements of this species. The results of the jackknife procedure show that temperature and precipitation are important influencers of *M. walkerae's* distribution.

Although there is a growing collective awareness for the effects of climate change on the world's species, most of the attention is focused on those that are used in agriculture or provide a direct threat or benefit to humans (Rosenweig et al, 2001). There is scarce research done so far that contributes to the conservation of endemic endangered species of the Tamaulipan thornscrub, especially when it applies to rare plant species that are generally unknown. As human populations continue to grow in South Texas and northeastern Mexico, it is probable that there will be a reduction of suitable habitat for *M. walkerae* due to land cover change. Since climate change is not predicted to be an imminent threat to *M. walkerae* populations, but could act synergistically with other harmful factors that threaten this species (e.g., loss of genetic diversity), future studies exploring the effects of land cover change on this species would be of great use for conservation efforts.

While most occurrences for *M. walkerae* in Texas show a close distribution to the U.S.-Mexican border, there is one population further north which is isolated from the others. We constructed models where we omitted this record and found that omitting it did not have an effect on *M. walkerae's* predicted distribution, we decided to include it in our study since it is a historical record for this species. However, this population exists within private property which restricts our access to this population for potential field studies. Additionally, given that there is a lack of connectivity from this population to other historical occurrences that are located near the U.S.-Mexican border, there is some uncertainty of whether this population is native or could have been introduced. Furthermore, an approaching threat for *M. walkerae* and other native species of the Tamaulipan thornscrub ecoregion is the impending construction of additional border wall segments, which are expected to exasperate fragmentation as well as increase anthropogenic disturbance in the known current distributional range (Greenwald et al, 2017). Collectively, the results of this study show that climate change can potentially have an effect on the geographic distribution of this endangered species and although it is not known if the distribution could expand or restrict, protected areas are essential for conserving *M. walkerae* and I recommend that the geographic distribution of this species be taken into account when designating protected areas in Mexico and southern Texas.

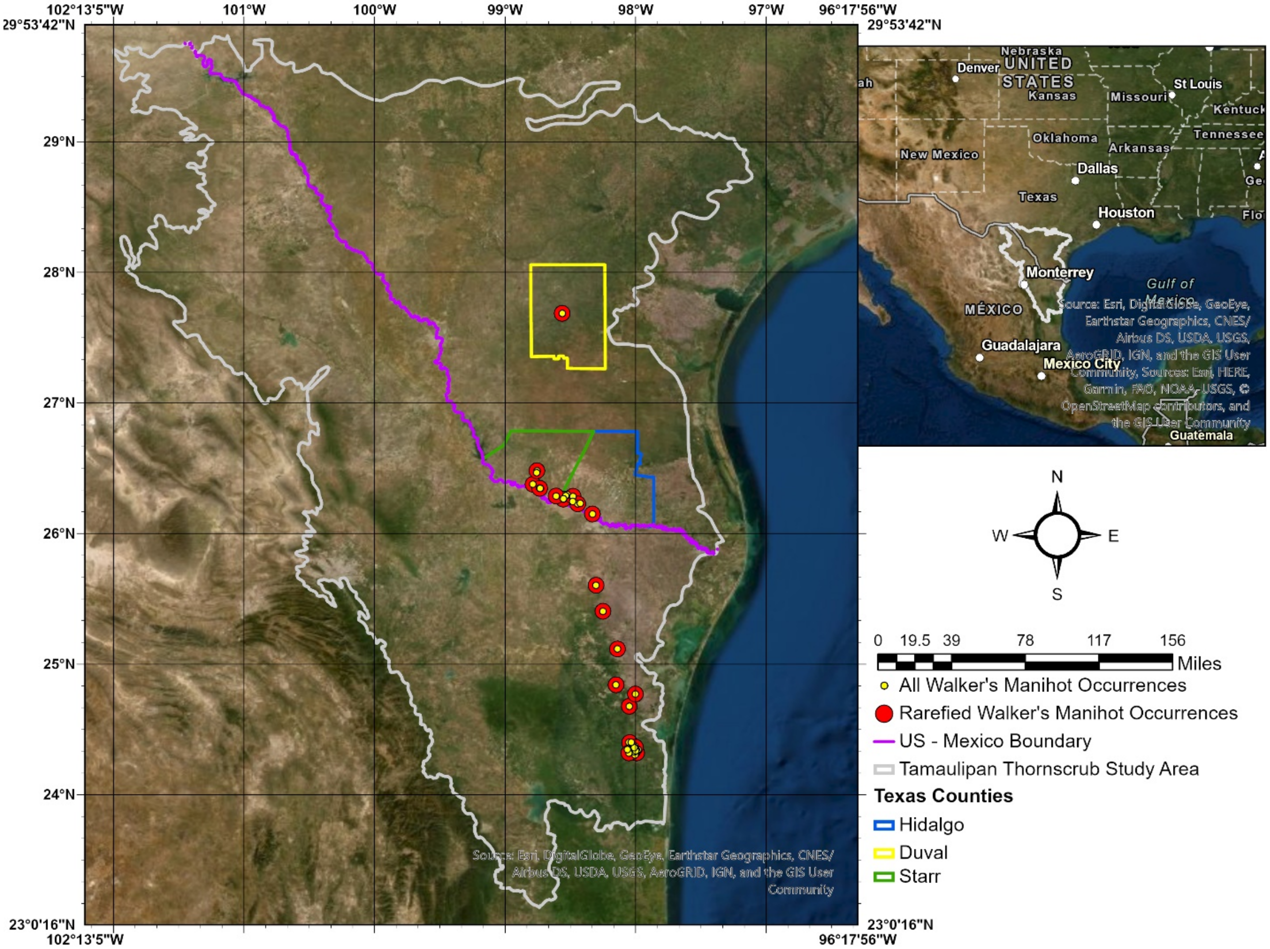

Fig 2.1: Historical occurrences of *Manihot walkerae* in the Tamaulipan thornscrub ecoregion. The yellow dots represent all 399 known occurrences for *M. walkerae*, and the red dots represent the 19 4-km spatially rarefied occurrences used to create the climate change models.

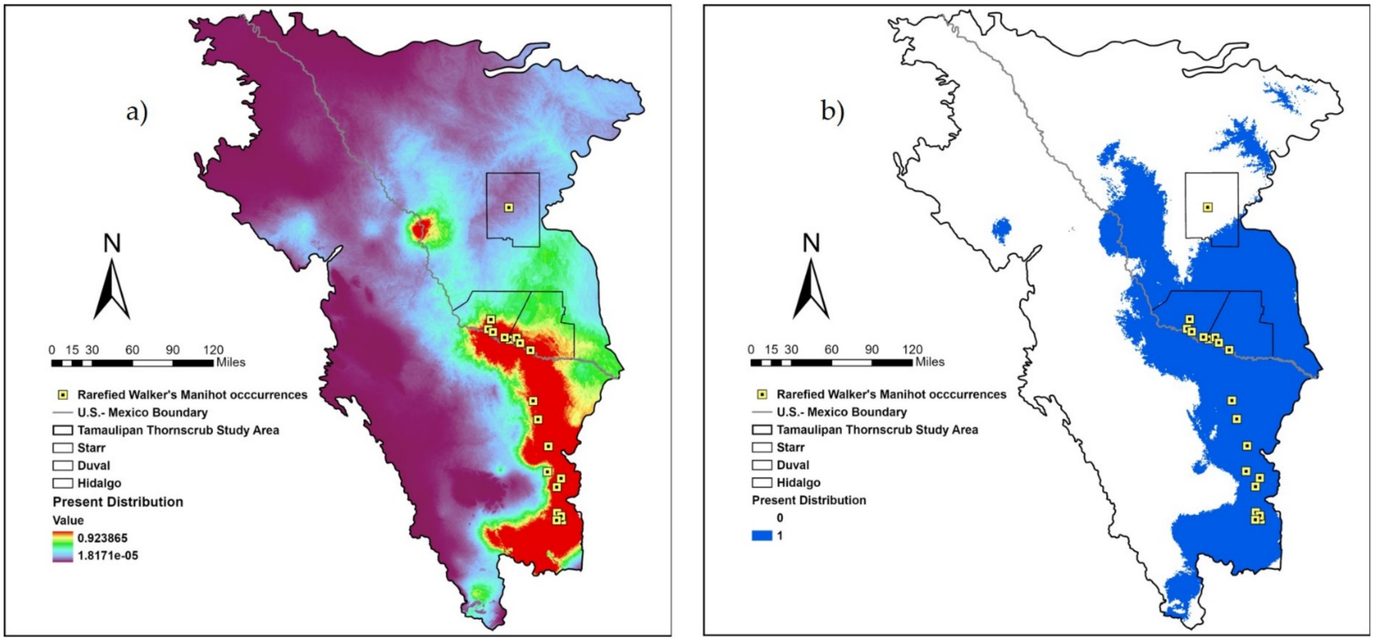

Fig 2.2: Consensus models of present climatic geographic distribution for *Manihot walkerae* based on ten bioclimatic variables and 19 spatially rarefied occurrences. (a) The color scale ranges from blue to red, with blue depicting areas of unsuitable distribution (value: 0), and red areas with highest potential of distribution (value: 1). (b) Binary model, blue areas are potentially suitable. The calculated AUC and pROC values for this model are 0.925 and1.874 which indicate good performance.

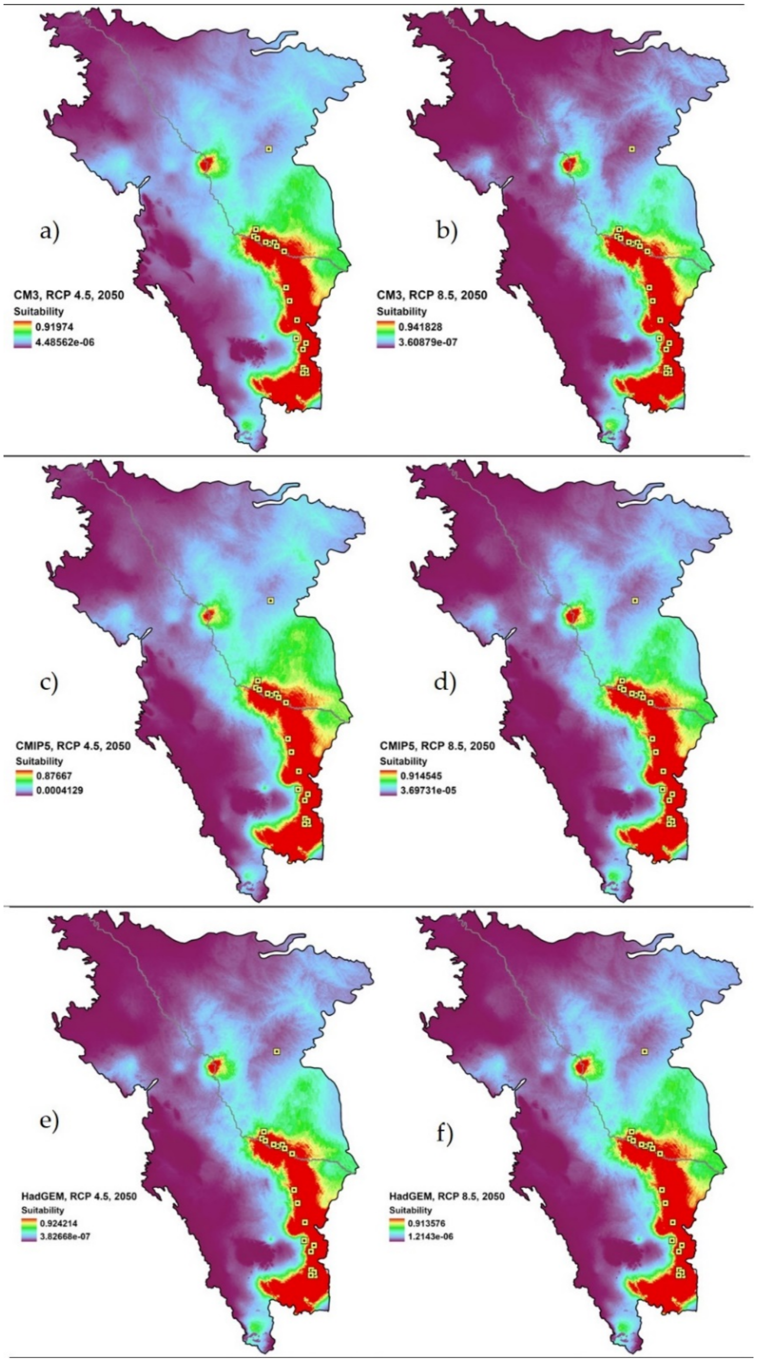

Fig 2.3: Future potential climatic geographic distribution for *M. walkerae* for the year 2050 using ten bioclimatic variables and nineteen spatially rarefied occurrences. (a) and (b) correspond to the CM3 GCM at an RCP of 4.5 and 8.5 respectively. (c) and (d) correspond to the CMIP5 GMC at an RCP of 4.5 and 8.5. (e) and (f) correspond to the HadGEM GMC at an RCP of 4.5 and 8.5.

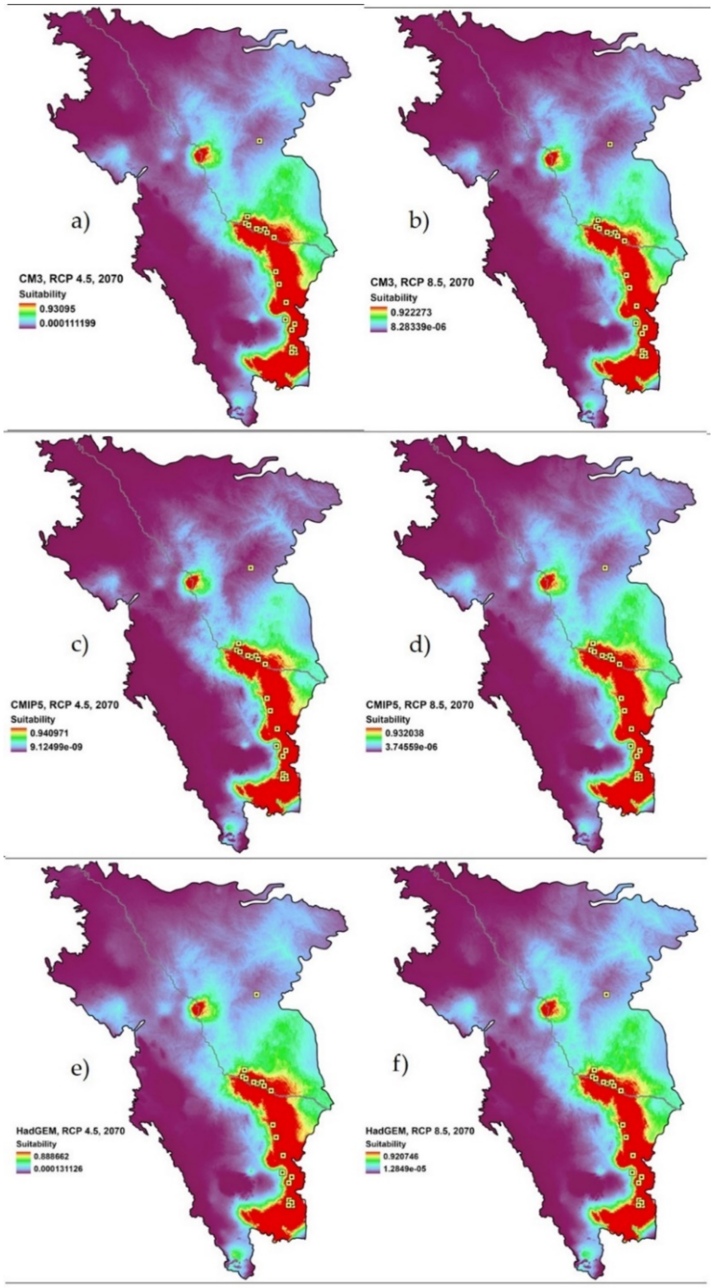

Fig 2.4: Future potential climatic geographic distribution for *M. walkerae* for the year 2070 using ten bioclimatic variables and nineteen spatially rarefied occurrences. (a) and (b) correspond to the CM3 GCM at an RCP of 4.5 and 8.5 respectively. (c) and (d) correspond to the CMIP5 GMC at an RCP of 4.5 and 8.5. (e) and (f) correspond to the HadGEM GMC at an RCP of 4.5 and 8.5.

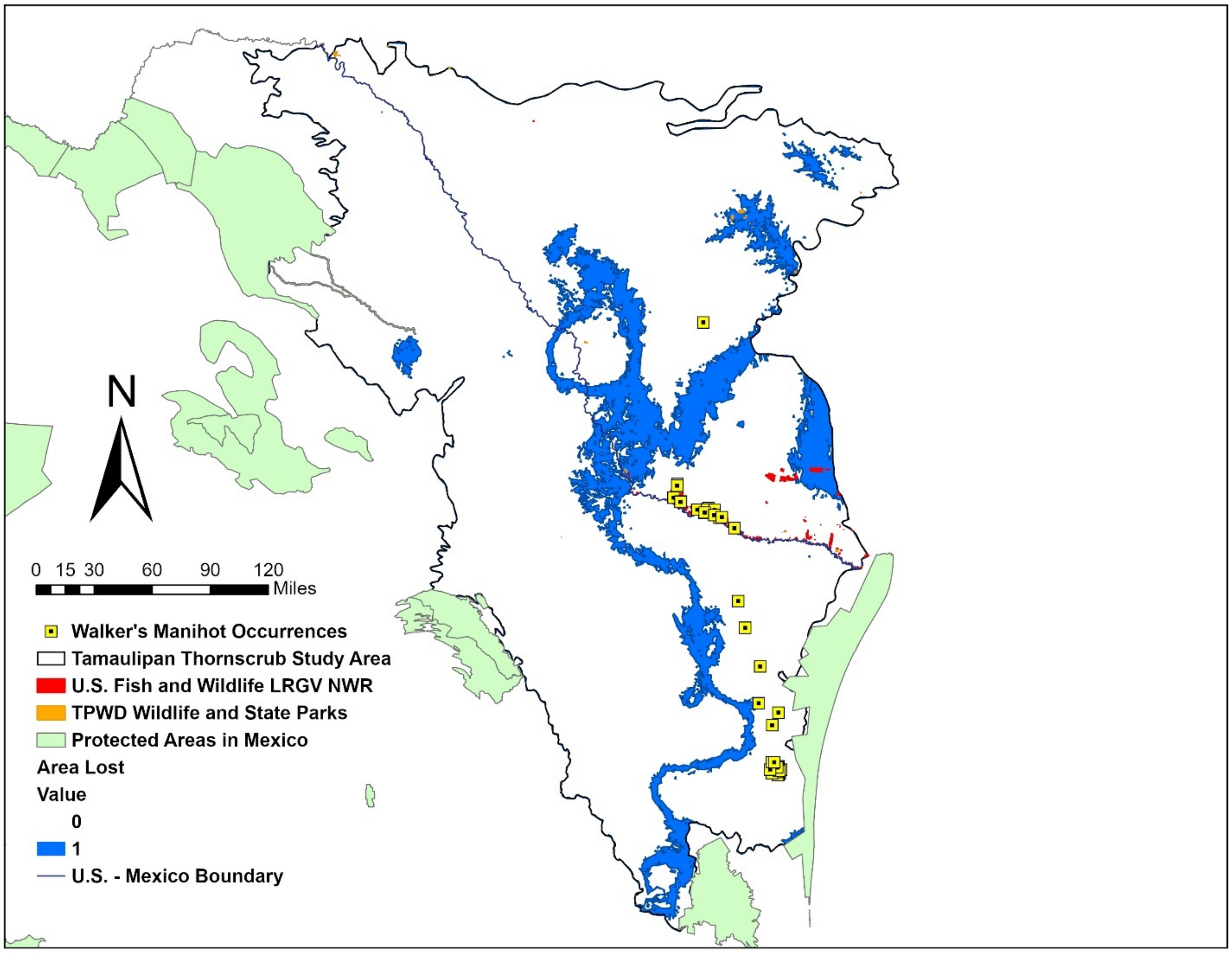

Fig 2.5: The portion of area lost (blue) as a result of climate change for the CMIP5 RCP 4.5 2070 *model* that had the highest predicted loss of distribution -14.37%. The known occurrences of *Manihot walkerae* were shown to not be affected, but some U. S. Fish and Wildlife LRGV NWR protected areas (red) in the northeastern portion of the study area are predicted to no longer be suitable for *M. walkerae* in the future. No protected areas in Mexico are shown to overlap with suitable areas of distribution of *M. walkerae* at present and in the future.

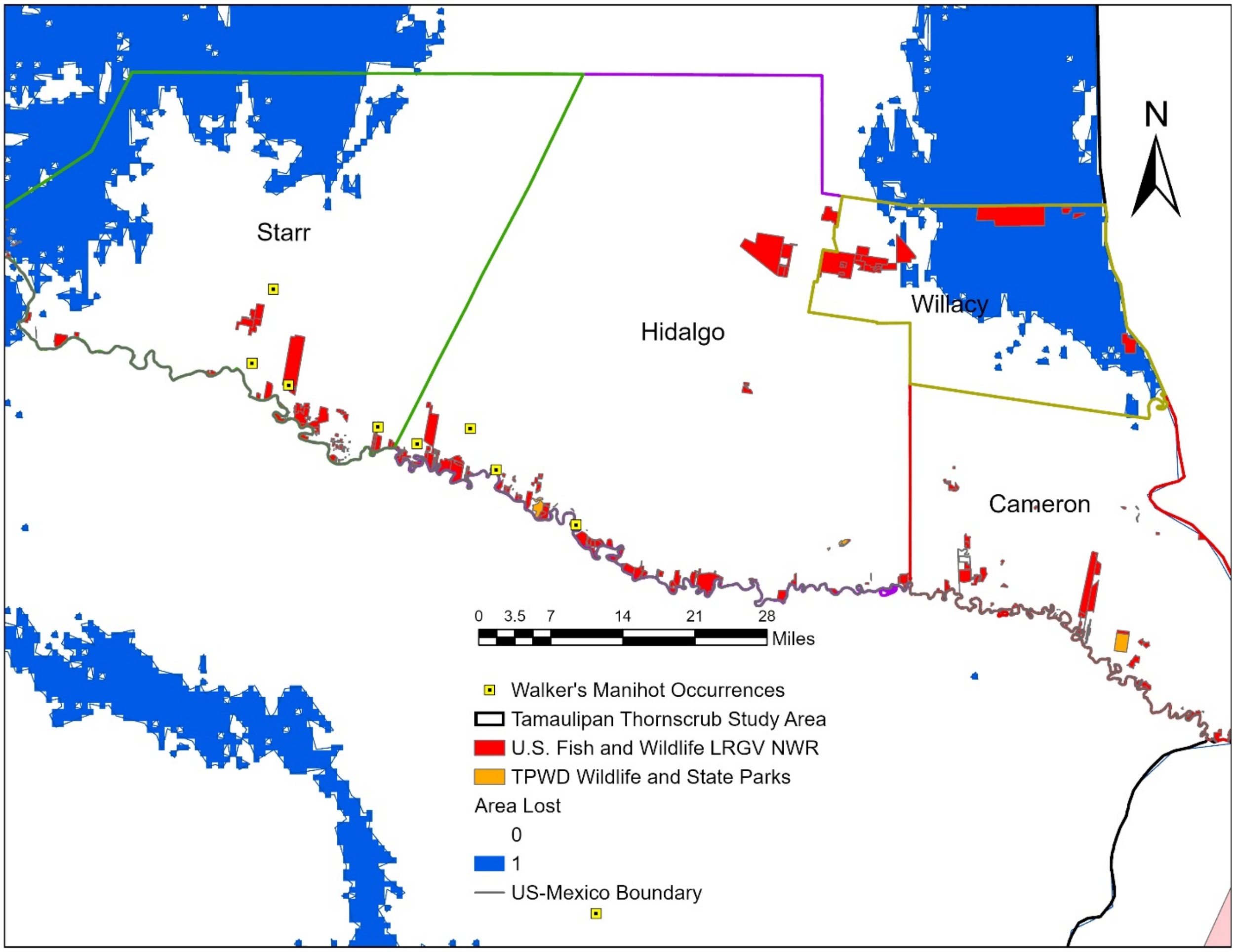

Fig 2.6: Close up of the potential climatic geographic distribution lost (blue) in Lower Rio Grande Valley Texas counties of Starr, Hidalgo, Cameron, and Willacy. The protected areas that are shown to be most affected by climate change are U. S. Fish and Wildlife Service LRGV NWR tracts located in Willacy County.

Table 2.1: All BIOCLIM variables, the ten not highly correlated variables used for modeling of the present and future potential geographic distribution are in bold. The percent contribution of each of the not-highly correlated variables to the present geographic distribution model are also included.

Variable	Explanation	% Contribution
BIO1	**Annual Mean Temperature**	**37.1**
BIO2	**Mean Diurnal Range (Mean of monthly (max temp-min temp))**	**0.3**
BIO3	**Isothermality (BIO2/BIO7)*100**	**1**
BIO4	Temperature Seasonality (standard deviation*100)	
BIO5	Max Temperature of Warmest Month	
BIO6	Min Temperature of Coldest Month	
BIO7	**Temperature Annual Range (BIO5-BIO6)**	**20.3**
BIO8	Mean Temperature of Wettest Quarter	
BIO9	Mean Temperature of Driest Quarter	
BIO10	**Mean Temperature of Warmest Quarter**	0
BIO11	Mean Temperature of Coldest Quarter	
BIO12	**Annual Precipitation**	**7.1**
BIO13	**Precipitation of Wettest Month**	**0**
BIO14	**Precipitation of Driest Month**	**2.1**
BIO15	**Precipitation of Seasonality (Coefficient of Variation)**	**13.7**
BIO16	Precipitation of Wettest Quarter	

Table 2.2: Background information for general circulation models (GCM) (Jalota et al, 2018).

General Circulation Model (GCM)	Name	Explanation
GFDL CM3	Geophysical Fluid Dynamics Laboratory, United States	The atmospheric component of the earth systems model includes physical features such as aerosols (both natural and anthropogenic), cloud physics, and precipitation. The land component includes precipitation, evaporation, streams, lakes, rivers, runoff, and a terrestrial ecology component to simulate dynamic reservoirs of carbon and other tracers. The oceanic component includes features such as free surface to capture wave processes; water fluxes, or flow; currents; sea-ice dynamics; iceberg transport of freshwater; a state-of-the-art representation of ocean mixing; and marine biogeochemistry and ecology.
CMIP5/ GISS-E2-R	Coupled Model Intercomparison Project Phase 5	NASA Goddard Institute for Space Studies (GISS) climate model E 2 with 2 degrees by 2.5 degrees horizontal resolution and 40 vertical layers in atmosphere, with the model top at 0.1 hPa Goddard Institute for Space Studies (NASA), United States.
HadGEM2	Hadley Centre for Climate Prediction and Research	HadGEM2-ES is a coupled Earth system model that was used by the Met Office Hadley Centre for the CMIP5 centennial simulations. The HadGEM2-ES climate model comprises an atmospheric GCM at N96 and L38 horizontal and vertical resolution and an ocean GCM with a 1-degree horizontal resolution (increasing to 1/3 degrees at the equator) and 40 vertical levels. Earth system components included are the terrestrial and ocean carbon cycle and tropospheric chemistry. Terrestrial vegetation and carbon are represented by the dynamic global vegetation model, TRIFFID, which simulates the coverage and carbon balance of five vegetation types (broadleaf tree, needle leaf tree, C3 grass, C4 grass, and shrub). Ocean biology and carbonate chemistry are represented by diat-HadOCC, which includes limitation of plankton growth by macro- and micronutrients and also simulates emissions of DMS to the atmosphere.

Table 2.3: Mean AUC values for all geographic distribution consensus models.

	Present	*2050*					
		RCP 4.5			**RCP 8.5**		
		CM3	*CMIP5*	*HadGEM*	*CM3*	*CMIP5*	*HadGEM*
Mean AUC	0.925	0.930	0.929	0.926	0.944	0.915	0.919

	Present	*2070*					
		RCP 4.5			**RCP 8.5**		
		CM3	*CMIP5*	*HadGEM*	*CM3*	*CMIP5*	*HadGEM*
Mean AUC	0.925	0.936	0.942	0.898	0.926	0.935	0.920

Table 2.4: Percent change of geographic distribution for *Manihot walkerae* between the current model and the future projected climate change models for the years 2050 and 2070.

	Present	*2050*					
		RCP 4.5			**RCP 8.5**		
		CM3	*CMIP5*	*HadGEM*	*CM3*	*CMIP5*	*HadGEM*
Suitable Area km^2	75901	88186	88571	66345	59657	72352	73870
% Change		+7.20	+7.42	-5.60	-9.52	-2.08	-1.19
	Present	*2070*					
		RCP 4.5			**RCP 8.5**		
		CM3	*CMIP5*	*HadGEM*	*CM3*	*CMIP5*	*HadGEM*
Suitable Area km^2	75901	63995	51398	71449	52653	65156	68845
% Change		-6.98	-14.37	-2.61	-13.63	-6.30	-4.13

CHAPTER III

ASSESSING EXTINCTION RISK FOR *MANIHOT WALKERAE* USING A GLOBAL AND NATIONAL ASSESSMENT METHOD

Introduction

Species do not conform to political boundaries, and endangered species that exist in transboundary regions can frequently be faced with the issue of being protected in one country and unprotected in the other (Gentili et al, 2011). This can be a consequence of using regional/national extinction risk assessments to list species that are distributed across political boundaries (e.g. occur in two countries). An issue that arises when using a regional approach is that it might not consider the species' complete distribution. A means of preventing this problem is using a comprehensive objective method that can be replicated globally on a vast number of different species (De Grammont & Cuarón, 2006).

The International Union for Conservation of Nature (IUCN) Red List provides a comprehensive reliable method for assessing extinction risk in species that can be used at a global level (IUCN, 2012). The IUCN's method consider five criteria that include species' population size, geographical range, and extinction probability analysis that are used to assess a species' extinction risk and to assign them to seven different categories, from species of least concern to those that are extinct (IUCN, 2012). However, a disadvantage of the IUCN's method is that in some cases the data that is needed to assess a species' extinction risk is often not readily available, this makes using some of the five criteria difficult or not possible and the species is then referred to as data deficient by the IUCN (De Grammont & Cuarón, 2006; Feria-Arroyo et al, 2009; Kaky & Gilbert, 2019). The use of species distribution modeling can be used to assess species' extinction risks, specifically to support criteria's B extent of occurrence (EOO) and area of occupancy (AOO) which are both measures of a species' geographic range (Feria-Arroyo et al, 2009; Cassini, 2011; Syfert et al, 2014; Keith et al, 2014; Kaky & Gilbert, 2019). Species distribution modeling is especially helpful when there is not enough data for a species as it has been found to be able to compensate for a lack of distributional data (Kaky & Gilbert, 2019). An added benefit of this method is that it allows for a more rapid assessment of a species extinction risk' by using what data is already available and not having to spend time gathering new species data (Kusumoto et al, 2017). This type of rapid assessment of extinction risk allows for immediate attention to be brought to the species and as a result, the species could potentially have a higher chance of survival since there is more time to address the species' extinction threats (Le Breton et al, 2019).

The designation of a species into an extinction risk category both globally and nationally is of upmost importance for conserving species, and it is often a first step to developing conservation plans (De Grammont & Cuarón, 2006). The benefits of globally listing species, especially in the IUCN's Redlist is that it is seen as reliable comprehensive tool for conservationists when developing strategies, and most importantly, the IUCN's Redlist is readily available to the public thus allowing for global education about species that are at risk of extinction at all levels (Rodrigues et al, 2006; IUCN, 2021). On the other hand, benefits provided by national assessment methods are that when a species is designated as endangered nationally, there is heightened awareness at the community level to protect the species and can result in the conservation of its regional genetic diversity through the creation of protected areas or when it is not possible, in agreements between conservation agencies and private landowners to conserve the species (De Grammont & Cuarón, 2006).

Manihot walkerae is a federally and state endangered species in Texas that is also designated as endangered by the IUCN red list (Vera-Sanchez & Nassar, 2019). However, the species is not nationally listed as endangered in Mexico's Norma Oficial Mexicana SEMARNAT-2010 (NOM-059) also known as Mexico's Redlist of endangered species (SEMARNAT, 2002; Vera-Sanchez & Nassar, 2019; Garza et al, 2020).When species are not designated as endangered regionally, especially nationally like in the case of *M. walkerae*, it is more difficult to advocate for the designation of protected areas for the species in that nation or region, and it is not subject to special protection by the nation itself which makes it vulnerable to exploitation or harm by humans that do not know of its endangered status. The importance of this species besides being an ecological component in its native ecosystem is that it is a crop wild relative of importance to cassava, *Manihot esculenta* (Dempewolf et al., 2014).

Cassava is a widely used and depended on food source worldwide, previous studies have found that *M. walkerae* has genes that can help prevent post-harvest deterioration which is a condition which limits the time that Cassava is viable for consumption after its harvest. Hybrids of these two species have been found to have longer longevity and be more resistant to post harvest deterioration (Morante et al., 2010; Saravanan et al., 2016; Zainuddin et al., 2018). Additionally, *M. walkerae* possesses genes that are resistant to other prominent diseases of cassava such as cassava brown streak, bacterial blight and it also could contain genes for cold resistance (Clayton, 1993). Overall, *M. walkerae* provides many benefits to better cassava longevity and disease resistance, the extinction of this species would not only negatively affect its local ecosystem, but also could be a disadvantage to the future of cassava.

Threats that face *Manihot walkerae*, in its entire range of distribution, are habitat modification and destruction as well as population fragmentation, small population size, herbicide application, overgrazing, herbivory by native and introduced wildlife, surface mining of caliche, petroleum and natural gas exploration, urban and residential development and competition by invasive plant species (Best, Miller, and Cobb, 2009; Clayton, 1993; Vera-Sanchez and Nassar, 2019). With over 95% of the focal region's Tamaulipan thornscrub modified or destroyed, *Manihot walkerae* as well as other native species of plants and wildlife are faced with the loss of their habitat (Cook et al., 2020; Jahrsdoerfer and Leslie, 1988; Leslie, 2016).

Manihot walkerae is of great concern for conservation efforts in Texas since there are few known populations and many occur within private lands and a few in protected areas such as U.S. Fish and Wildlife Lower Rio Grande Valley National Wildlife Refuges (Best, Miller, & Cobb, 2009). Accounts from 1995 state that the largest populations of *M. walkerae* in Texas contained approximately 100 individuals and were in protected reserves (Best, Miller, & Cobb, 2009). However, in field visits conducted in 2019 and 2020 very few *M. walkerae* individuals were found, and to recent knowledge the largest *M. walkerae* population now exists within private property and only contains approximately 34 individuals. Small population sizes bring about their own problems such as low genetic diversity that can make this species more vulnerable to extinction via a disturbance event (Oostermeijer et al, 2003). In Mexico, recent field efforts have revealed one population with over 100 individuals, alarmingly though several populations that were once present in 2005, are no longer present within private properties

(Contreras-Arquieta, 2005). In both countries the effects of anthropogenic land use on this species can be intensified in areas where there are no designated protected areas, but in Mexico since *M. walkerae* is not listed as endangered in the NOM-059 it has no special protection and anthropogenic destruction of habitat may be even worse.

In this chapter, I am using the International Union for Conservation of Nature (IUCN's) risk assessment method and Mexico's risk assessment method (MER) to designate *Manihot walkerae* into a global and national extinction risk category after incorporating species distribution modeling and geographic information systems (Feria et al, 2009). Aside from advocating for the designation of *M. walkerae* as endangered in Mexico, incorporating the predicted geographic distribution of this species with the standard EOO and AOO geographic range values will allow for a comparison of effectiveness of SDMs in extinction risk assessments. The ultimate outcomes of this chapter are a more realistic model of *Manihot walkerae's* geographic distribution that was constructed with environmental variables relevant to this species life history and biology, and a designation of this species into a global and regional risk category after incorporating its predicted geographic distribution.

Methods

Species distribution modeling

The occurrence data used to construct the potential geographic distribution model for *Manihot walkerae* in this chapter, are the same as what I used to construct the climate change models in chapter two. To summarize, the occurrence data used to run the models are 19 4-km spatially rarefied occurrences (please see figure 2.1). The Tamaulipan thornscrub ecoregion was chosen as a base map since it represents a natural boundary that *M. walkerae* is endemic to and contains the full extent of this species' occurrences.

To construct a more realistic model of *M. walkerae's* potential geographic distribution, the environmental variables used to construct the potential geographic distribution model are those that affect its growth and physiology and were recommended by rare plant experts (Chris Best). The variables that were used to generate the distribution models were chosen based on the values of the variance inflation factor (VIF = 1/(1-r2), which allows excluding redundant variation among them (Fois et al., 2015). The correlation coefficient (r) was obtained from multiple regression using the variable with the highest correlation coefficient as the predictor variable and the rest as independent variables. The excluded variables were those that had a VIF greater than 5.0, because their variation was contained in the other independent variables (Fois et al., 2015). The procedure was repeated until no variable had a VIF value greater than 5.0.

Collectively there were 15 not highly correlated environmental variables used to construct the model (Table 3.1). These environmental variables can be separated into two categories, continuous and categorical. Continuous variables which are typically seen in raster format contain cells or pixels with gradually changing data and no distinct boundaries such as in temperature or elevation. Categorical variables on the other hand have clear boundaries that show a change in characteristics such as in soil type or hydrological sub-basins.

The 19 rarefied occurrences for *M. walkerae* were then input into MaxEnt along with the environmental variables (Table 3.1) and run to produce 50 replicates at a random test percentage of 30. This means, that of the 19 species occurrences provided, 70% of them were used to construct the model, and 30% were used to check the model's accuracy. The 50 resulting models were then visualized in ArcGIS and a consensus model was constructed using the raster calculator spatial analyst tool.

I quantitatively evaluated the accuracy of the potential geographic distribution consensus model using the Area Under the ROC curve (AUC) and the partial ROC. As mentioned previously, AUC values range between 0.5 and 1. A value of 0.5 is equivalent to a random prediction, while a value of 1 indicates a perfect prediction (Fielding and Bell, 1997), therefore the maps are accurate, robust, or statistically good. Because there has been some criticism to the use of the AUC (Lobo et al, 2008) I also used an alternative method to evaluate the accuracy of the models called the partial ROC which is a more specific statistic because it only considers regions where data have been observed (Walter, 2005). Values that are significantly higher than 1 and closer to 2 signify a good prediction and a more accurate map (Peterson et al, 2008).

IUCN Assessment

The IUCN's risk assessment method uses 5 different criteria to designate a species into one of seven different categories, least concern (LC), near threatened (NT), vulnerable (VU), endangered (EN), critically endangered (CE), extinct in the wild (EW), and extinct (EX). Criteria A, C, and D, all are associated with population sizes, while criterion B is based on geographic range. Criterion A specifically focuses on population reduction or decline, criterion C focuses on small population size and decline, and criterion D on very small or restricted populations (IUCN, 2012). For a species to be listed as a threatened category under the IUCN Redlist it must meet at least one of the five criteria (IUCN, 2012).

Criterion A can be further divided into four categories (A1-A4) where A1 states that there is an observed, estimated, inferred, or suspected population reduction in the past where the causes of the reduction are reversible, understood, and have stopped, on the other hand, A2 states thatthe causes of the reduction have not stopped, may not be understood, and are not reversible (IUCN, 2012). A3 focuses on a projected reduction of population in the future for a maximum of

100 years, and A4 can be applied only when there is an observed, estimated, inferred or suspected population reduction both in the past and future where the causes of the reduction have not stopped, may not be understood, and are not reversible (IUCN, 2012). These four criterion A categories can be assessed in the following ways: a) direct observation, b) an index of abundance of the appropriate taxon, c) a decline in the AOO, EOO, and/or habitat quality, d) actual or potential levels of exploitation, e) effects of introduced taxa, hybridization, pathogens, pollutants, predators, and parasites (IUCN, 2012). Species or taxa are then designated as either critically endangered, endangered, or vulnerable based on threshold values. *Manihot walkerae* was assessed through category A2 using species distribution modeling to calculate a decline in the AOO, EOO and/or habitat quality by subtracting transformed habitats into cropland and urban and built-up land based on a land use raster from the area predicted as potential geographic distribution (Figure 3.3) (Feria-Arroyo et al, 2009)

Criterion B assesses species' geographic range in the form of the extent of occurrence (EOO) and the area of occupancy (AOO) along with at least two of the following conditions, fragmentation, observed or predicted decline and fluctuation in either the EOO, AOO, quality of habitat, number of locations or subpopulations, or the number of mature individuals (IUCN, 2012). The extent of occurrence is defined as the area within an imaginary boundary that is drawn to include all occurrences for a species, while the AOO is the area within the EOO that is occupied by the species (IUCN, 2012). The distribution area of *M. walkerae* was used to designate it as critically endangered (CE), endangered (EN), or vulnerable (VU) based on criterion B threshold values (Table 3.2). We used the Geospatial Conservation Assessment Tool (GeoCAT) to acquire the EOO and AOO by inputting all 399 historical occurrence records for

M. walkerae (Bachman et al, 2011). The area that the model predicted as potential geographic distribution (PGD) for *M. walkerae* was also compared to criterion B EOO threshold values.

Criterion C is used to assess small population size and decline for a species, where a species with a collective number of 250 individuals in all of its populations is designated as CE, less than 2,500 individuals as EN, and less than 10,000 as VU. Criterion C can be divided into two categories where C1 states that there is an observed, estimated, or projected decline of at least 25% in 3 years or 1 generation (CR), 20% in 5 years or 2 generations (EN), and 10% in 10 years or 3 generations (VU). While for C2, there is an observed, estimated, or projected decline where the (a) number of mature individuals in each subpopulation is also assessed along with the percentage of mature individuals in one subpopulation and (b) extreme fluctuations in the number of mature individuals (IUCN, 2012). The threshold values for C2a are less than 50 mature individuals in a subpopulation (CE), less than 250 (EN), and less than 1,000 mature individuals (VU) with a percentage of 90-100% of mature individuals in one subpopulation designating the species as CE, 95-100% as EN, and 100% as VU (IUCN, 2012). *Manihot walkerae* was assessed using criterion C2 and considering that there is an estimated number of 1,000 mature individuals for this species collectively in all of its populations (Vera-Sanchez & Nassar, 2019).

Under criterion D, which assesses very small or restricted populations, *M. walkerae* could classify as vulnerable under D1 because it does have an estimated number of 1,000 mature individuals. However, it does not meet D2 (extremely restricted AOO) since the AOO for the species is greater than 20 km^2. Regarding criterion E extinction probability analysis, it could not be done, since the generation time for *M. walkerae* is not yet known. It is for this reason that *M. walkerae* was assessed using criteria A2ac, B1 and B2ab, and C2a.

Mexico's Risk Assessment Method (MER)

The MER uses four different criteria to designate species into four risk categories ranging from species of least concern, to those that are believed to be extinct in the wild (Tambutti et al, 2001; Sanchez, 2007). The first is criterion A, the extent of species' occurrence in Mexico which is assessed by comparing the species' geographic distribution in Mexico with the total area of Mexico which is 1,964,375 km^2 (Tambutti et al, 2001). Values that can be used for this assessment are the species' extent of occurrence, area of occupancy, and predicted potential geographic distribution (Feria-Arroyo et al, 2009). Threshold percentages are used to designate species into one of four categories and are as follows: widely distributed species cover >40% of Mexico's area, semi-restricted or vast species cover >15% of Mexico's area but less than 40%, restricted species cover between 5% to 15% of Mexico's area, and very restricted species cover less than 5% of Mexico's area. Each of the categories is assigned a respective value from 1 to 4 with species that are widely distributed having values of 1, and species that are very restricted having values of 4.

The species' extent of occurrence (EOO), area of occupancy (AOO), and the predicted potential geographic distribution (PGD) from the model that was constructed earlier was used to assess *Manihot walkerae* with criterion A. The EOO and AOO values were calculated with only the 33 out of the 399 historical occurrences for *M. walkerae* that are distributed in Mexico, since the MER is a criterion specific to Mexico. I input the 33 occurrences into the Geospatial Conservation Assessment Tool (GeoCAT) to acquire the EOO and AOO (Bachman et al, 2011). I decided that it would be best to crop the potential geographic distribution to exclude Texas instead of running another model with only the occurrences in Mexico's as this could cause a false prediction of suitable habitat (overprediction or underprediction) as the species' complete range is not considered. The percentage of area covered by the species distribution was calculated by dividing the EOO (1,223 km^2), AOO (76 km^2), and PGD (9,986 km^2) by Mexico's total area (1,964,375 km^2) and multiplying by 100.

Criterion B is an assessment of the species' natural habitat status with respect to the natural requirements of the species and can be categorized into three categories. The first is a slightly or not limiting habitat which is assigned a value of 1, the second is intermediate or limiting which is assigned a value of 2, and the third is hostile or highly limiting which is assigned a value of 3 (Tambutti et al, 2001). Criterion C is an assessment of the intrinsic biological viability of the species which includes factors such as reproductive strategy, population demography, phenology, genetic variation, and recruitment rate which can be used to designate the species into one of three categories based on the predicted vulnerability of the species to extinction, fromthose that have high vulnerability (value of 3) to species that have low vulnerability (value of 1) (Tambutti et al, 2001).

Finally, criterion D is an assessment of the degree of human impact on the species examples of human impact are habitat destruction, fragmentation, land use change, pollution, use in trade and trafficking, and introduction of invasive species (Tambutti et al, 2001). Species that have a high degree of human impact are assigned a value of 4, species with a medium degree of human impact are assigned a value of 3, and species with a low degree of human impact are assigned a value of 2. Once the species has been assessed with all four criteria, the values that were designated to the species in each criterion are summed and then used to designate the species into one of four categories. Species with a summed value of 9 or less are designated as least concern, species with a value of 11-10 are threatened, and those with a value of 12-14 are endangered, additionally, a species that is believed to be extinct in the wild has a value of 14 along with there being no reasonable doubt that there are no natural populations of the species in the wild (Tabutti et al, 2001).

Criteria B: species' natural habitat status and D: degree of human impact for *Manihot walkerae* in Mexico were assessed using the human footprint raster layer, a data set produced by compiling scores from population density, land transformation, accessibility, and electrical power infrastructure data to yield an estimate of human impact ranging from 0 to 100 (Sanderson et al, 2002). Human footprint ranges were categorized in the following manner: 50 or above (B: hostile or highly limiting value of 3; D: high, value of 4), 25-49 (B: intermediate to limiting value of 2; D: medium, value of 3), 24-0 (B: slightly or not limiting value of 1; D: low, value of 2) (Feria-Arroyo et al, 2009). The human footprint values for *M. walkerae's* range in Mexico was extracted on ArcGIS, to construct a table of values using the 33 occurrences for the species in Mexico. The values were then averaged and equaled to 24 which was then compared to the threshold values for criteria B.

Results

Potential Geographic Distribution

The more realistic potential geographic distribution consensus model for *Manihot walkerae* produced from 50 replicates had a statistically relevant AUC value of 0.93 and a partial ROC value greater than 1.80 (Figure 3.1). This indicates that the map is statistically accurate and can be used for assessing the extinction risk of *M. walkerae* with the IUCN's and MER assessment methods.

The environmental variables that contributed most to the model were water basins, soil type, and elevation (Table 3.1). The variables that contributed least were canopy height, herbaceous percentage, and percent of water per km^2. Most records occur in areas that are predicted as being highly suitable (shown in red), except one historical record at north of their distribution. Highly suitable areas are prominent over the US-Mexico region, and in the southeastern portion of the study area (Figure 3.1).

IUCN Assessment

After assessing *Manihot walkerae* with IUCN criteria A2c, B1, B2ab, and C2a two of these criteria designated the species as endangered, while two categorized it as vulnerable based on threshold values. Criteria A2c which specifically assesses species' population reductions that are observed, estimated, or inferred where the causes of the reduction have not stopped and are not reversible designated *M. walkerae* as a vulnerable species since there is an estimated 48% decline in available potential geographic distribution based on the land use map (Figure 3.3; 3.4). Criterion B1 which was used to assess *M. walkerae's* extent of occurrence designated the species as vulnerable with both the EOO (10,363 km^2) and the predicted geographic distribution (12,274 km^2) since their areas were less than the 20,000 km^2 vulnerable threshold value but greater than 5,000 km^2 endangered threshold value (Table 3.3). However, with criterion B2ab which was used to assess *M. walkerae* restricted area of occupancy it was categorized as endangered since the AOO (132 km^2) is less than the 500 km^2 endangered threshold value and the species also met the conditions of existing in fragmented populations that have declined (Table 3.3). Criterion C2a was used to assess *M. walkerae's* small population size and decline. *M. walkerae* met the endangered threshold of having less 2,500 individuals since the species has an estimated number of 1,000. Additionally, based on the number of mature individuals in each subpopulation which is less than 250 (endangered threshold) and that the percentage of mature individuals in a subpopulation (using the Peñitas population as an example, please see Figure 4.4) is between 95-

100 % the species met the endangered category. Ultimately, *M. walkerae* can be considered an endangered species based on the IUCN's assessment method under criteria B2ac and C2a.

Mexico's Risk Assessment Method (MER)

Manihot walkerae was assessed using all four of the MER's criteria and was designated a threatened species. Criteria A was used to assess the extent of *M. walkerae's* occurrence in Mexico by comparing the species' EOO, AOO, and PSH in Mexico with the total area of Mexico 1,964,375 km^2. All three values the EOO (1,223 km^2), AOO (76 km^2), and predicted geographic distribution in Mexico (9,986 km^2) designate the species as a very restricted species with a value of 4 since it shows that *M. walkerae* covers less than 5% of Mexico's area (0.062 %, 0.0038%, and 0.51%) (Table 3.4). Criteria B: species' natural habitat status and D: degree of human impact for *Manihot walkerae* in Mexico were assessed using the human footprint raster layer and designated the species a value of 24 throughout its distribution in Mexico therefore categorizing it as slightly or not limiting under criterion B (value of 1), and as having a low degree of human impact with criterion D (value of 2) (Table 3.5). Finally, when assessing criteria C, the intrinsic biological viability of the species that makes it vulnerable to extinction we considered the genetic variation and recruitment rate in the populations of *M. walkerae*. Since *M. walkerae* has small populations that are fragmented and disconnected from each other there could be very low genetic variation and genetic diversity for this species that can make it more vulnerable to extinction due to an anthropogenic or environmental disturbance event. Additionally, since *M. walkerae's* population trend is declining, and because recent field visits in Mexico have revealed that populations that were there in the past are no longer present the recruitment rate for this species is low. Collectively, the low genetic variation/diversity along with its low recruitment rate assigned it a value of 3 for a high vulnerability to extinction. The values from all four criteria were then summed in the following manner: criterion A (4) + criterion B (1) + criterion C (3) + criterion D (2) = 10, which designated the species as threatened in Mexico.

Discussion

Extinction risk assessments are crucial tools for the conservation of species both at the global and regional or national level. In this chapter, I used the IUCN's risk assessment method and Mexico's risk assessment method (MER) to assign an extinction risk category to *Manihot walkerae*. By incorporating a model of *M. walkerae's* more realistic predicted potential geographic distribution (PGD) this additional value was used alongside the extent of occurrence (EOO), and area of occupancy (AOO) to assess this species with criteria B of the IUCN and criteria A of the MER (Figure 3.1, 3.2; Table 3.3 & 3.4). The model produced here is being referred to as more realistic because it was made by incorporating different environmental variables that are relevant to this species' growth and physiology as opposed to only climatic variables that were used to construct *M. walkerae's* climatic distribution model. Incorporating this more realistic geographic distribution model is useful since the EOO has previously been regarded as a value that potentially overestimates a species' distribution, while the AOO can underestimate it, and maps that show what areas are suitable and unsuitable for a species can be used in conjunction with these two values to provide a more reliable assessment (Feria-Arroyo et al, 2009). Criteria B of the IUCN revealed that through the EOO and PSH *M. walkerae* can be considered a vulnerable species as these areas cover more than 5,000 km^2 endangered threshold but less than the threshold for vulnerable of 20,000 km^2 (Figure 3.2). However, since the AOO was found to be 132 km^2 which is less than the 500 km^2 endangered threshold the species can be designated as endangered under criteria B2.

Additionally, in this study the PGD model was used to estimate *M. walkerae's* population decline as a result of land use transformation to areas of urban and agricultural development for criterion A of the IUCN (Feria-Arroyo et al, 2009). This revealed that approximately 48% of *M walkerae's* predicted PSH could be disrupted as a result of land use change and is a potential cause of past, present, or future population loss (Figure 3.4). If the PGD area that is left after subtracting transformed habitats due to land use change would be assessed against EOO threshold values (5,938 km^2) it would be very close to meeting the endangered threshold value under criteria B1 (Figure 3.4). Although this value did not meet criterion A2's endangered threshold of 50% loss, it is important to note that 48% is closer to the endangered threshold value of 50% than it is to 30% vulnerable threshold value for criteria A2 and if another measure of criteria A2 such as direct observations of population reduction was also included then the species could be designated as endangered as well under A2. Observations at the population level that could allow for an assessment of *M. walkerae's* population reduction in the future are in progress. Already, field visits have revealed that compared to previous surveys conducted in the late 1990s and early 2000s there are less *M. walkerae* individuals being found in the present than in the past. These population studies could also potentially allow for an extinction risk probability analysis to be performed for *M. walkerae* therefore assessing the species by criterion D. Additionally, since *M. walkerae* has an estimated number of 1,000 individuals and has less than 250 mature individuals per population it met the endangered criterion of criteria C2a. Using the data that has been collected thus far for the *M. walkerae* population in Peñitas Texas, it was determined that the composition of individuals in this subpopulation is between 95-100% which again allows the species to meet the endangered category for criteria C2a (please see Figure 4.4).

Some of the criteria of the IUCN were found to be similar to those in Mexico's Risk Assessment method (MER), such as criterion B of the IUCN and criterion A of the MER which both assess geographic distributions. Like with criterion B, when assessing *M. walkerae* with criterion A of the MER, the EOO, AOO, and PSH were compared against the total area of Mexico to determine if the species is restricted in this country. It was found that nationally, *M. walkerae* is a very restricted species in Mexico as it covers less than 5% of Mexico's area (0.062 %, 0.0038%, and 0.51%) (Table 3.4). Upon a closer look of the land use maps one can see that along historical occurrences the land use type that dominates in these areas in Mexico are mostly croplands (Figure 3.3). This raises a concern as far as if designating protected areas for this species in Mexico is a viable option as many of these croplands are private ranches and could put this species at a greater risk to anthropogenic disturbance if this species is not listed in the NOM-059. In terms of criteria B and D which assess species natural habitat status and degree of human impact I used a method like Feria-Arroyo et al 2009 where a layer of human footprint was used to determine these values. The human footprint data set was produced by compiling scores from population density, land transformation, accessibility, and electrical power infrastructure data, to yield an estimate of human impact ranging from 0 to 100 (Sanderson et al, 2002). The human footprint layer assigned *M. walkerae* a value of 24 along its occurrences in Mexico which ultimately designated the species as one that has a slightly or not limiting habitat and a low degree of human impact.

Ultimately, through the IUCN assessment method *M. walkerae* was designated as endangered and through the MER it was designated as a threatened species. The IUCN assessment performed here supports the previous IUCN assessment done by Vera-Sanchez and Nassar in 2019. While in Mexico, no previous assessment for this species has been attempted in the past. The hope is that the work done here can help advocate for the listing of *M. walkerae* as a threatened species under Mexico's NOM-059.

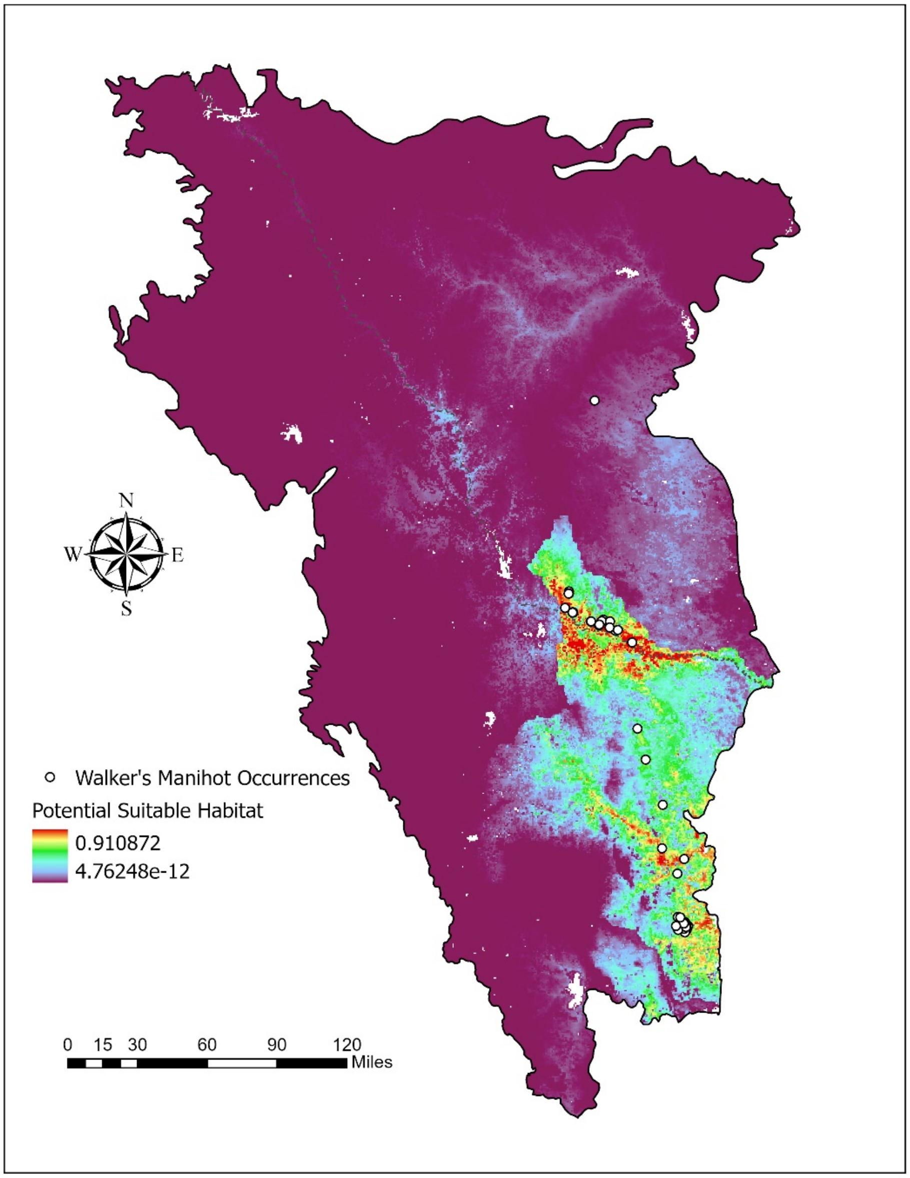

Fig. 3.1: Map of more realistic potential geographic distribution for *Manihot walkerae,* the color scale ranges from blue to red, with blue depicting areas of unsuitable habitat, green neutrally suitable habitat, and red the area of highest suitable habitat. The final AUC value for the consensus map was calculated as: 0.93098; and the partial ROC value was higher than 1.80. This indicates that the map is accurate and can be used for assessing the extinction risk of *M. walkerae* using the IUCN's and MER assessment methods.

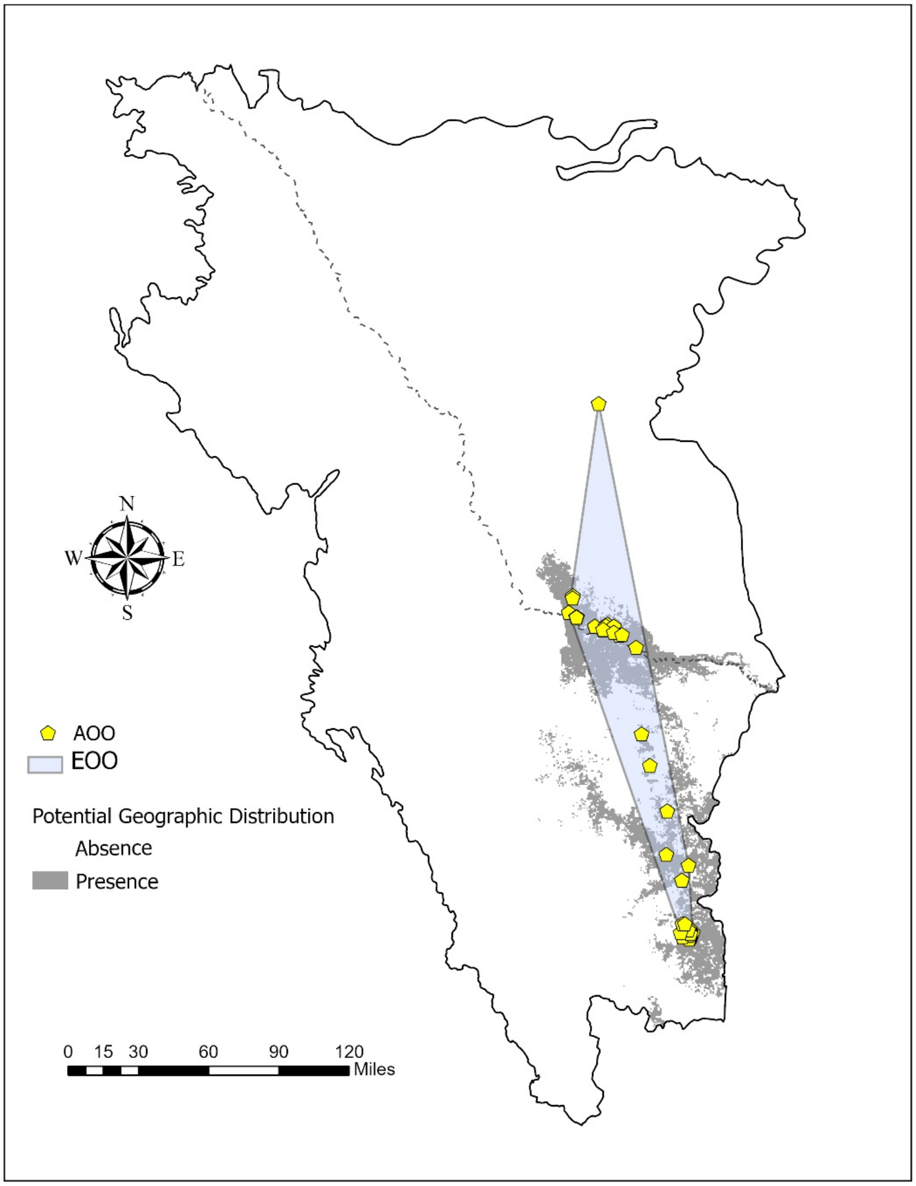

Fig. 3.2: Map showing *Manihot walkerae's* extent of occurrence (EOO), area of occupancy (AOO) and predicted potential geographic distribution (PGD). The area covered by each of these values is: EOO 10,363 km^2, AOO 132 km^2, and PGD 12,274 km^2. Both the EOO and PSH designate this species as vulnerable (less than 20,000 km^2), while the AOO designate it as endangered (less than 500 km^2).

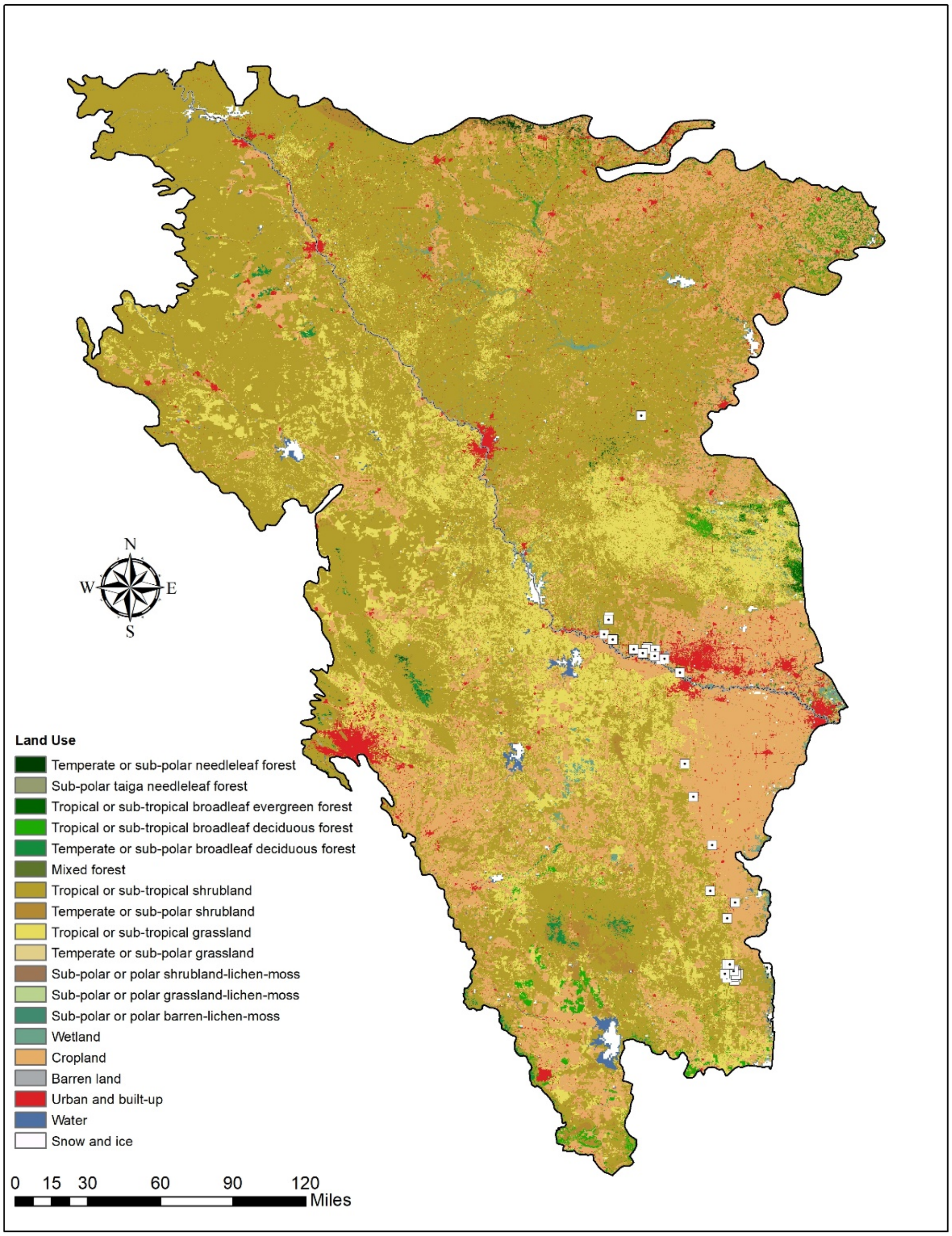

Fig. 3.3: Land use in the Tamaulipan thornscrub study area where the native vegetation types are tropical of subtropical shrubland (mustard brown) and temperate or subtropical grassland (yellow). Many of the areas where *Manihot walkerae* is historically distributed have been converted to cropland (peach orange) and urban and built-up land (red).

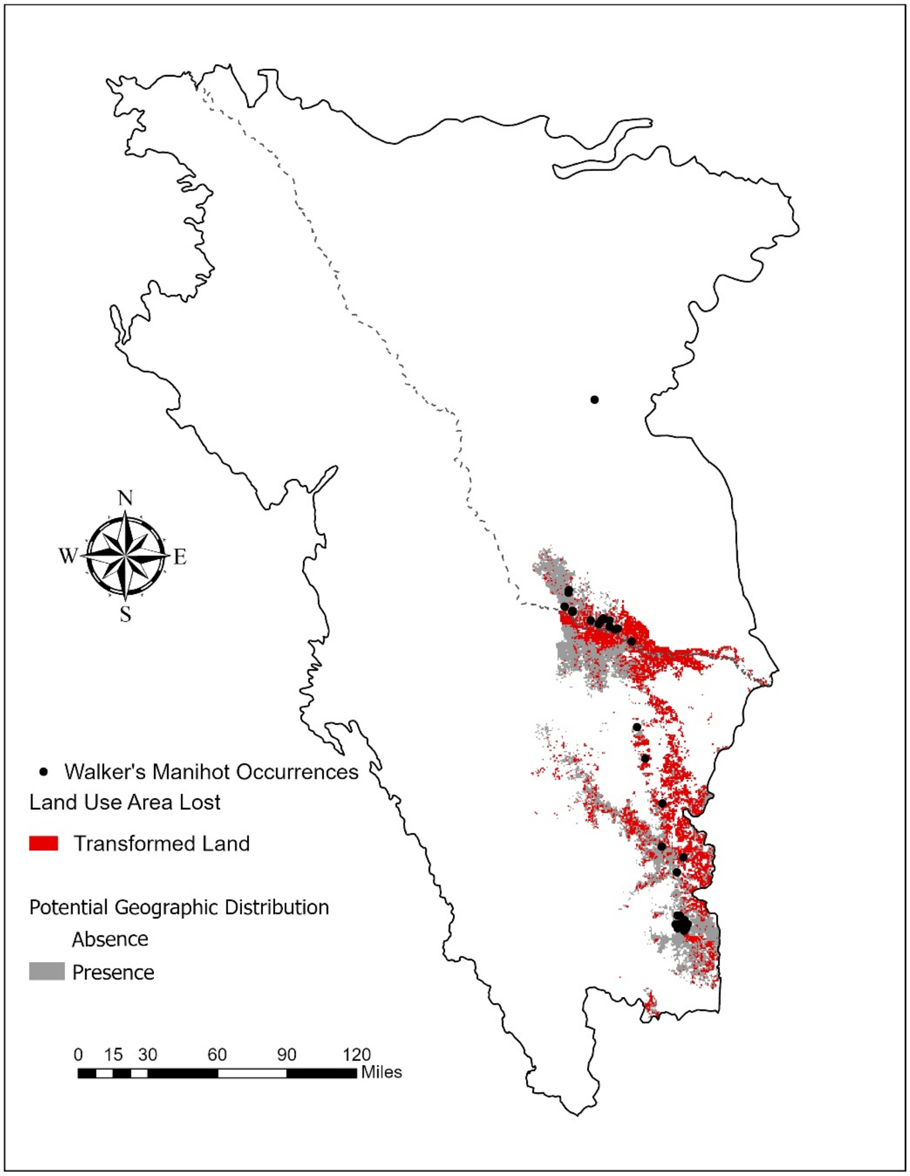

Fig. 3.4: Map showing *Manihot walkerae's* potential geographic distribution habitat lost due to the transformation of habitat to cropland and urban and built-up land (red). The area lost (5,938 km^2) is approximately 48% of the predicted geographic distribution (12,274 km^2). This data was used to assess *M. walkerae* under criterion A2 of the IUCN where there is an estimated population size reduction based on a decline in the AOO, EOO and/or habitat quality and categorizes it as vulnerable since the predicted population reduction is less than 50%.

Table 3.1: The environmental variables used to construct *Manihot walkerae's* potential distribution model and their percent contribution to the model.

Environmental Variable Name	**Type**	**% Contribution**
Biomes	Categorical	6.7
Water basins	Categorical	59.7
Soil Type	Categorical	9.4
Percent of water per km^2	Continuous	0.1
Canopy height	Continuous	0
Percentage of bushes per km^2	Continuous	3.2
Percentage of deciduous broadleaf trees per km^2	Continuous	1.3
Ecoregion	Continuous	0.5
Elevation	Continuous	11
Herbaceous percentage per km^2	Continuous	0.2
Humidity index	Continuous	1.3
Maximum temperature in the coldest four-month period (° C)	Continuous	4.1
Total annual precipitation	Continuous	0.1
Solar radiation for the month of June	Continuous	0.3

Table 3.2*:* IUCN criterion B threshold values.

	Critically Endangered	Endangered	Vulnerable
Extent of Occurrence (EOO)	<100 km^2	<5,000 km^2	<20,000 km^2
Area of Occupancy (AOO)	<10 km^2	<500 km^2	<2,000 km^2

Table 3.3: Assessment of IUCN criterion B: restricted geographic range for *Manihot walkerae* through its extent of occurrence, area of occupancy, and predicted geographic distribution habitat values in its complete range of distribution (South Texas and Tamaulipas). Both the EOO and predicted geographic distribution categorize this species as vulnerable (less than 20,000 km^2), while the AOO designates it as endangered since it is less than 500 km^2.

Criterion B	**Values (km^2)**	**Criterion B Category**
EOO	10,363 km^2	Vulnerable
AOO	132.00 km^2	Endangered
Potential geographic distribution	12,274 km^2	Vulnerable

Table 3.4: Assessment of MER criteria A: extent of species distribution in Mexico for *Manihot walkerae* through its extent of occurrence, area of occupancy, and predicted potential geographic distribution. All three values categorize *M. walkerae* as a very restricted species in Mexico with an assigned value of 4 since it covers less than 5% of Mexico's area (1,964,375 km^2).

Criterion A	**Values (km^2)**	**% of Mexico's area (1,964,375 km^2)**	**Criterion A Category**	**Criterion A Value**
EOO Mexico	1,223 km^2	0.062%	Very restricted species	4
AOO Mexico	76.00 km^2	0.0038%	Very restricted species	4
Potential Geographic Distribution in Mexico	9,986 km^2	0.51%	Very restricted species	4

Table 3.5: Assessment of MER criteria B: species' natural habitat status and D: degree of human impact for *Manihot walkerae* in Mexico using the human footprint raster layer, a data set produced by compiling scores from population density, land transformation, accessibility, and electrical power infrastructure data, to yield an estimate of human impact ranging from 0 to 100. Human footprint ranges were categorized in the following manner: 50 or above (B: hostile or highly limiting value of 3; D: high, value of 4), 25-49 (B: intermediate to limiting value of 2; D: medium, value of 3), 24-0 (B: slightly or not limiting value of 1; D: low, value of 2). The human footprint raster layer assigned *M. walkerae* a value of 24 throughout its distribution in Mexico therefore categorizing it as slightly or not limiting under criterion B, and as having a low degree of human impact with criterion D.

Criterion	**Human footprint value**	**Category designation**	**Category value**
B	24	Slightly or not limiting	1
D	24	Low degree of human impact	2

CHAPTER IV

NATURAL HISTORY OBSERVATIONS OF *MANIHOT WALKERAE*

Introduction

Natural history studies have long been considered as critical for plant conservation as they provide a foundation for the understanding of a plant's life history, species interactions, and ecosystem role which are used for the development of conservation strategies (Bury, 2006; Heywood and Iriondo, 2003; Garcia, 2003; Oostermeijer et al, 1996). For some endangered species, it might not be ideal to conduct manipulative studies in a lab, but through an observational approach one can gather this important information without causing disturbance to the plant and its ecosystem (Farnsworth, and Rosovsky, 1993) These observations whether they be done at species or population level can then be incorporated into a risk assessment method such as that of the International Union for Conservation of Nature (IUCN) when conducting an extinction probability analysis (Bury, 2006; IUCN, 2012).

Field observations allow for one to better understand how endangered plants interact with their ecosystem (Del-Claro et al, 2013). Some significant interactions are shared between plants and insects as they can serve as mutualists, predators, and parasites. The mutualistic relationship of pollination has led to the diversification of flowering plants as both insect and plant species become specialized to each other. Herbivorous insects have caused plants to evolve multiple direct and indirect defenses to better protect themselves against herbivory. The benefits that insects provide plants is vast, they pollinate flowers, cycle nutrients, disperse seeds, and control pest populations (Boada, 2005). The insect-plant relationship has been shown to be a limiting factor for the survival of other endemic endangered plant species, but no studies have been performed that identify these relationships for *M. walkerae* (Tscharntke & Brandl, 2004).

Documentation of what insects are seen interacting with *M. walkerae* would provide information that is currently missing from this species' conservation efforts. In the past, local botanists have learned some general information about *M. walkerae's* insect interactions, such as that the flowers of the plant possess nectaries that are visited by bees and flies (Fisher, 1998). *M. walkerae* is a perennial herbaceous plant of the spurge family with tuberous roots (Mild, 2003). It is characterized by its five-lobed palmate leaves and white flowers that typically appear from May to June and September to November following rains (Mild, 2003). *M. walkerae* is monoecious with female and male flowers on the same plant, but with unisexual flowers. The fruits are 3-lobed capsules that dehisce revealing an average number of 3 seeds per fruit (Mild, 2003). This species can lose its above ground vegetation down to its tubers during unfavorable conditions such as during drought or freezes which makes *M. walkerae* individuals difficult to account for at certain times (Mild, 2003). Like other members of the *Manihot* genus and of the Euphorbiaceae, *M. walkerae* seeds have a seed appendage known as an elaiosome. Elaiosomes are lipid-rich seed appendages that are attractive to ants who then provide the service of seed dispersal (Leal et al, 2014). This type of interaction is known as myrmecochory, and seed dispersal by ants has been recorded in closely related members of *M. walkerae* such as the cassava plant *Manihot esculenta* (Elias et al, 2004). When *M. walkerae* fruits are mature they dehisce and release on average three seeds per fruit. Althoughant seed dispersal is believed to occur after *M. walkerae* seeds are released, it has not been previously observed or documented.

The objectives of this chapter are to observe the natural history of a *M. walkerae* population in order to 1) evaluate the different roles of age classes in the population, 2) to see what age classes contribute to reproduction (flower + fruit production together are reproductive effort), and 3) to determine when are the time periods when flower and fruit production are highest. Additionally, another objective is to observe the insects that interact with *M. walkerae* and share a relationship potentially as pollinators, herbivores, and seed dispersers.

Methods

Selecting population to study

Starting in summer of 2019 and ongoing to fall of 2020, field visits to *Manihot walkerae* historical populations were made in Hidalgo and Starr county to determine which population could potentially be used for natural history studies and insect observations. Of the historical populations visited, three were in Lower Rio Grande Valley National Wildlife Refuges, one was in private property in Peñitas TX, and another was in along a road in a right of way. Of these, only three populations had any visible *M. walkerae* present, but the maximum number of *M. walkerae* individuals found in a population was 34, with the other two having only 13 and 6 individuals. It is important to note that although *M. walkerae* is known to be difficult to identify during times of drought and winter because it loses its above ground vegetation and is left solely as a tuber, the majority of the field visits were done during the rainy season (May-September) when this species is known to be prominent and easy to identify which made the discovery of so few individuals very concerning (Poole, Carr, & Price, 2007). Field visits that were planned in the area that is between the LRGV counties of Hidalgo and Starr and the northernmost historical occurrence of Duval county were not possible due to COVID-19 restrictions and privacy agreements made with the private property owners of the land where the *M. walkerae* population in Duval is located that did not allow for disclosure of the location or for the property to be visited. It was for this reason that it was decided the population used for population dynamic studies would be the one in Peñitas Texas since it has the largest number of individuals out of the three.

Description of study area

The study population is in Peñitas Texas (26.23° N, -98.44° W), and it contains 34 *Manihot walkerae* individuals in an area that measures approximately 100 m x 15 m (1,500 m^2), other plants in this area are *Celtis pallida, Acacia rigidula, Prosopis glandulosa,* and *Opuntia spp.* (Figure 4.1). The average temperature in Peñitas Texas is 31.36 °C while average precipitation is 11.81 mm.

Collecting field population data

First, X and Y distances were documented for each *M. walkerae* individual in the study area and then they were marked with an orange stake flag, this data was used for georeferencing (Figure 4.2). The number of individuals in the population, along with the plant height and the basal diameter of individual stems were documented in August of 2019, and plant height, basal diameter, and number of individuals were gathered again for all individuals in the population in July of 2020. The basal diameter in this study is being used to convey the age of the individuals, with those having thicker diameters being considered as older than individuals with thinner basal diameters which are assumed to be younger (Gatsuk et al 1980; O'Brien et al, 1995). Additional information that was also gathered monthly was the number of flowers and fruits seen on *M.*

walkerae individuals as this data was used to determine what age classes are reproductive and which months had the highest flower and fruit production.

Since this species is known to flower after rains a graph of average temperature and precipitation was constructed to see how *M. walkerae* responded to monthly climate fluctuations. A regression analysis was performed on JMP statistical software to see if there was a relationship between flower and fruit production and temperature and precipitation.

Insect observations

Observations of the insects interacting with *M. walkerae* were done during the 1-2-hour periods that monthly population flower and fruit data were gathered. The insects seen interacting with the plant were pictured and later identified by insect order and when possible by species. An approximate count of each insect order was made per field visit, and the area of the plant that the insect was interacting with was also documented. Evidence of insect feeding damage was also pictured and documented. Additionally, since it is suspected that harvester ants are seed dispersers for *M. walkerae*, a survey was done in the study area to account for any harvester ant nests and I measured the distance from the nest to the nearest *M. walkerae* plant. I found two red harvester ant nests, the first was an active ant nest that was located in the southern portion of my study area at a distance of 23 ft or approximately 7 m away from the closest *Manihot walkerae* plant (individual 33). The other was also a red harvester ant's nest but unlike the first it appeared to be inactive, and it was located in the northern portion of my study area approximately 26 ft or 8 m away from the closest *M. walkerae* plant (individual 2). To support my hypothesis that red harvester ants *Pogonomyrmex barbatus* are attracted to *M. walkerae* seeds I found a mature fruit with developed seeds and placed them near the active ant's nest to see how they would react. Since I only found one fruit at the time (November, 2020) and because *M. walkerae* fruits contain three seeds I documented three observations. I placed each seed one at a time one foot away from the active ant's nest and started documenting the length of time (sec) that it took for the ants to first start interacting with the *M. walkerae* seed, the length of time that it took for them to start moving the seed, as well as the distance than the seed was moved, and where the seed was placed.

Statistical Analyses

A mosaic plot and a Chi-square analysis were performed on JMP statistical software to assess if there is an observed statistical significance between the two categorical variables age class and year of study (2019, and 2020) (SAS, 2021). I hypothesized that the composition of the individuals in the Peñitas population would be the same between the years of 2019 and 2020.

Results

The population density for the *Manihot walkerae* individuals was calculated by dividing 34 (number of individuals in the population in 2019) by 1500 m^2 (study area covered) and was found to be 0.02 ind/m^2 which is low. The specific distribution of *M. walkerae* individuals can be seen in the scatterplot made from the X and Y georeferenced locations of each individual in the study area, and they appear to have a clustered distribution (Figure 4.3).

The 34 individuals were separated into five different age classes based on their basal diameter and ability to reproduce (Table 4.1). The age classes are distributed in the following manner, Juvenile (J) 0.01-0.30 cm, Young Reproductive (YR) 0.31-0.60 cm, Mature Reproductive 1 (MR1) 0.61-0.90 cm, Mature Reproductive 2 (MR2) and Old Reproductive (OR) >1.21 cm (Gatsuk et al, 1980; Caswell, 2001) (Table 4.1). The chi-square test revealed that there is no statistically significant difference in the distribution of age classes between the years of 2019 and 2020 (*P*: 0.7941, X^2: 1.68, DF: 4) (Figure 4.4) (SAS, 2021). However, if we look at the distribution of the age classes themselves, one can see that the population is dominated by the MR1, YR, and MR2 groups who respectively contain 38%, 26%, an 23% of the individuals in the population, while the J and OR groups collectively make up only about 12% (*P*: <0.0001) (Figure 4.4).

In terms of flower and fruit production (reproductive structures), the highest production of reproductive structures was made by the MR1 and MR2 age classes followed by the YR and OR, while the J group did not contribute at all (Figure 4.5). During the yearlong study period of August 2019 to August 2020 *M. walkerae* was documented flowering from March until October with the highest production of flowers taking place in May of 2020 (Figure 4.6). Fruits were produced from May to October with the month of October producing the greatest number of fruits (Figure 4.7). *Manihot walkerae* did not flower or produce fruits during the months of low precipitation which are during the late fall and winter months (Figures 4.6 and 4.7). The average yearly temperature in Peñitas was 31.36 C° with the hottest average temperature occurring in the summer month of August 2019, and the lowest average temperature of 25 C° taking place in February of 2020. Average yearly precipitation was 11.81 mm with a noticeable increase in precipitation occurring in July 2020 which coincides with the arrival of hurricane Hanna (70.36 mm), while the lowest average precipitation was also recorded in the month of February 2020 (1.01 mm). Regression analysis revealed that there is no statistically significant relationship between temperature and precipitation and flower and fruit production (Flowers and Precipitation R^2= 0.05, P= 0.4651; Fruits and Precipitation R^2= 0.09 P= 0.3182; Flowers and Temperature R^2= 0.08 P= 0.3645; Fruits and Temperature R^2= 0.15 P= 0.1922).

When looking at the composition of the insect community the Hymenoptera and Lepidoptera orders were seen interacting most with *M. walkerae* (bees, ants, wasps, butterflies, caterpillars) while the Hemiptera, Orthoptera, and Diptera were seen interacting least (Figure 4.8). In terms of what part of the plant was interacted with, Hymenoptera interacted with the flowers (bees & wasps) (Figure 4.9), and the stems and seeds (ants) (Figure 4.11), while Lepidoptera interacted with flowers (butterflies) (Figure 4.9), and leaves and stems (caterpillars) (4.10), the Hemiptera and Orthoptera order were seen interaction with the leaves and stems, while Dipterans were seen only interacting with flowers.

For my first ant observation I saw that the harvester ants started interacting with the seeds approximately 50 sec after it was placed on the ground and began to move it shortly after at 85 sec. As expected, the harvester ants were most attracted to the elaiosome and when they moved the seed their mandibles were grasping this appendage (Fig 4.11). I observed the ants moving the seed for 20 minutes (1,200 sec) after the interaction started to see if they took it to their nest, which is what I expected (Table 4.2). However, I saw that after 20 minutes they had moved the seed a few feet away (~4 ft) and left it under a shrub which could be a potentially suitable microhabitat for *M. walkerae* seeds which typically grow under other plants and are believed to participate in nurse plant associations. Similarly, when I conducted by second and third observations, the ants started interacting with the seed 40 sec and 60 sec after it was placed and moved it soon after (60 sec and 77 sec respectively). Like in my first observation, I observed the ant's interaction with the seed for 20 minutes for the second and third trial and saw that for the second trial the seed was moved a few feet away (~5 ft) and was left out in the open, and the third seed was moved a similar distance and was left under another plant (Table 4.2). I did not see any *M. walkerae* seeds taken to the nest within the full length of time that I was observing these interactions (1 hour).

Discussion

A problem that faces many endangered plant species is that because they are rare, natural history data such as data of their populations, floral phenology, and species interactions, is not readily available for use in development of species-specific conservation plans (Essi, 2020). The preliminary results provided in this chapter, although only being for one population, could be used as a starting point for understanding *Manihot walkerae* at a population level. Although extinction risk of *Manihot walkerae* has previously been assessed it is mainly through the use of geographic data, and specific population level data could improve extinction risk assessments for this species in the future. Of importance is that although the data presented here was for only for a year-long study period, it is imperative that longer observational studies be conducted for *M. walkerae* in order to know more about this species, such as it's generation length which is currently unknown and could be used to conduct an extinction probability analysis.

Results for the *Manihot walkerae* population in Peñitas showed that most individuals fall into the reproductive age classes (Figure 4.4) while few individuals belong to the oldest and juvenile age class. This could mean that at least for the near future, the *M. walkerae* population in Peñitas will continue to have individuals present if no anthropogenic disturbance event destroys the population (e.g. complete removal of plants). Monthly data presented for flower and fruit production of the *M. walkerae* individuals in Peñitas revealed that for the study period of August 2019 to 2020 flower production is ongoing from late spring until early fall with flower production showing to start after precipitation and diminish in the months when precipitation is the least (August & September 2019) (Figure 4.7). However, regression analysis showed that there is no statistical relationship between precipitation and temperature and flower and fruit production.

Regarding the insect interactions observations, the orders that were seen interacting with *M. walkerae* the most were Lepidoptera and Hymenoptera. I expected insects like bees and ants to interact most with *M. walkerae* as the flower appears to have a bee pollination syndrome because of its white color and yellow anthers, and because of the elaiosomes that are found on the seeds. Although the data that I documented could help support that red harvester ants are attracted to *M. walkerae* seed elaisomes, it is not enough to say that ants are *M. walkerae* seed dispersers and more studies on this interaction is needed. Concerning to me, was that of the insects seen acting as herbivores on *M. walkerae* one of them was the cassava hornworm *Erinnyis ello*, a significant pest of cassava and a specialized feeder of the *Manihot* genus (Barrigossi et al, 2002). The cassava hornworm's natural range includes Texas and Mexico, so it overlaps with *M. walkerae's* historic distribution and could potentially be a risk factor to this species. I recommend that studies look more closely at the interaction between *M. walkerae* and *E. ello* are conducted to assess the risk of the cassava hornworm on this species.

Fig. 4.1: Picture of study area in Peñitas Texas. The study area is in private property that is right across from the San Antonio Cemetery in Peñitas Texas, and it measures 100 m x 15 m (1500 m^2) (Source Google Earth).

Fig. 4.2: A *Manihot walkerae* individual from the population in Peñitas TX. Picture taken May 26th, 2020 by Gisel Garza.

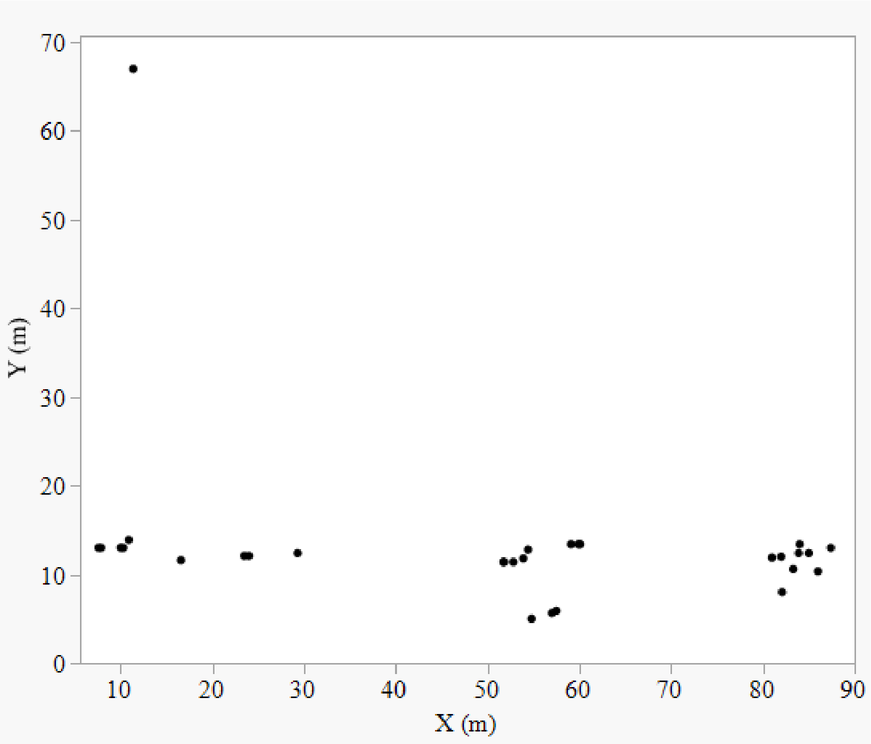

Fig. 4.3: Spatial distribution of *Manihot walkerae* individuals in the study area using X and Y georeferenced points. Most individuals are shown to be distributed fairly close to the fence that separates the private property from the San Antonio Cemetery with the exception being one individual that is distributed on the other side of the study area (please see Fig 4.1 as well).

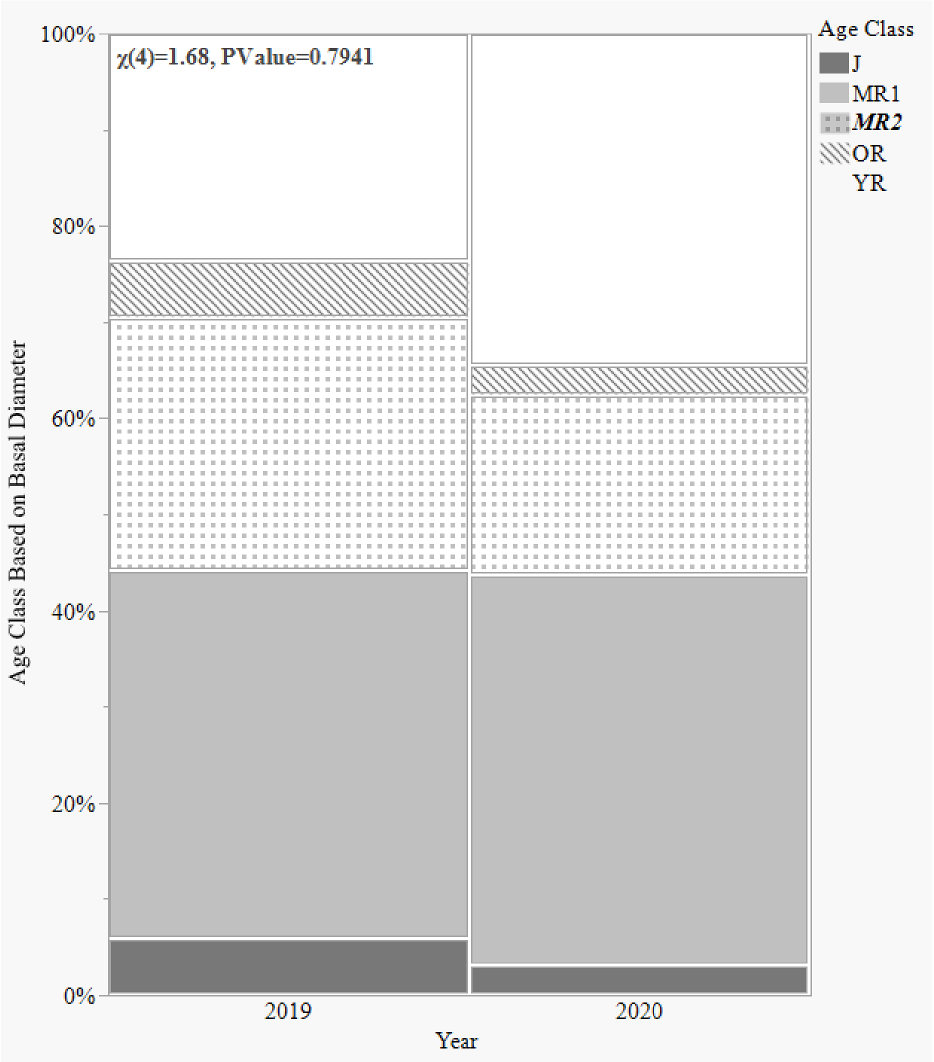

Fig. 4.4: Mosaic plot showing the distribution of the five age classes of *Manihot walkerae* in the Peñitas Texas population for the years of 2019 and 2020. The age classes are based on the plant's basal diameter and are as follows: Juvenile (J) 0.01-0.30 cm (dark grey), Young Reproductive (YR) 0.31-0.60 cm (white), Mature Reproductive 1 (MR1) 0.61-0.90 cm (light grey), Mature Reproductive 2 (MR2) (dots) and Old Reproductive (OR) >1.21 cm (diagonal lines). Chi-square analysis showed that there is no statistically significant difference in age classes between the years of 2019 and 2020 (P-value: 0.7941, X^2: 1.68).

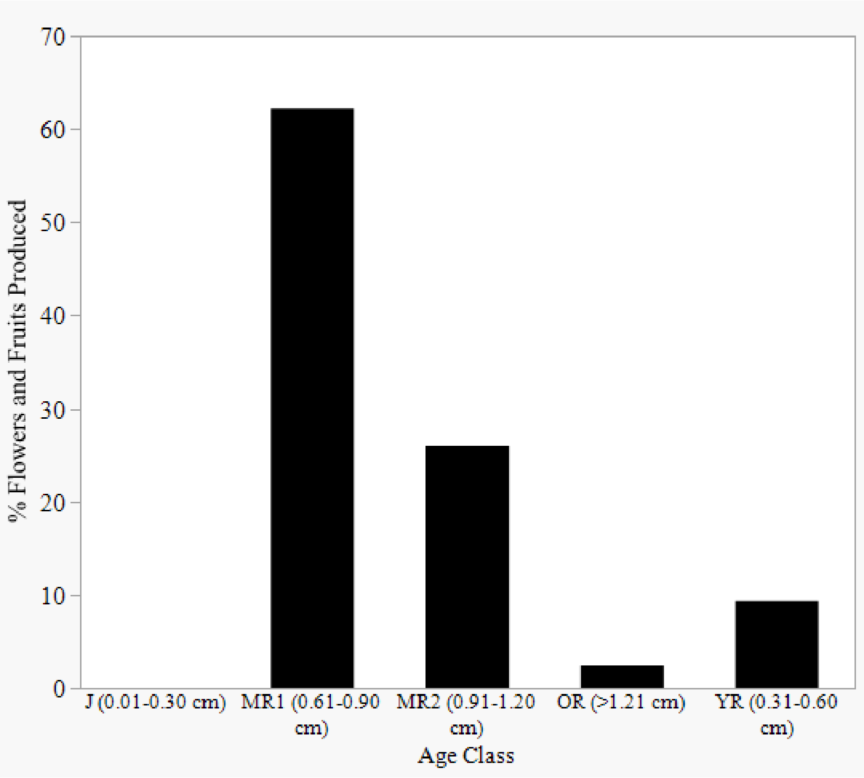

Fig. 4.5: A comparison of the percentage contributed by each of the five age classes to flower and fruit production which together are referred to as reproductive effort. Collectively, the young reproductive (YR), mature reproductive 1 (MR1) and mature reproductive 2 (MR2) age classes contributed the most to *M. walkerae's* reproductive effort by producing 97.58% of the flowers and fruits in the population, while the old reproductive age class (OR) only produced 2.42% and the juvenile class (J) produced no reproductive structures.

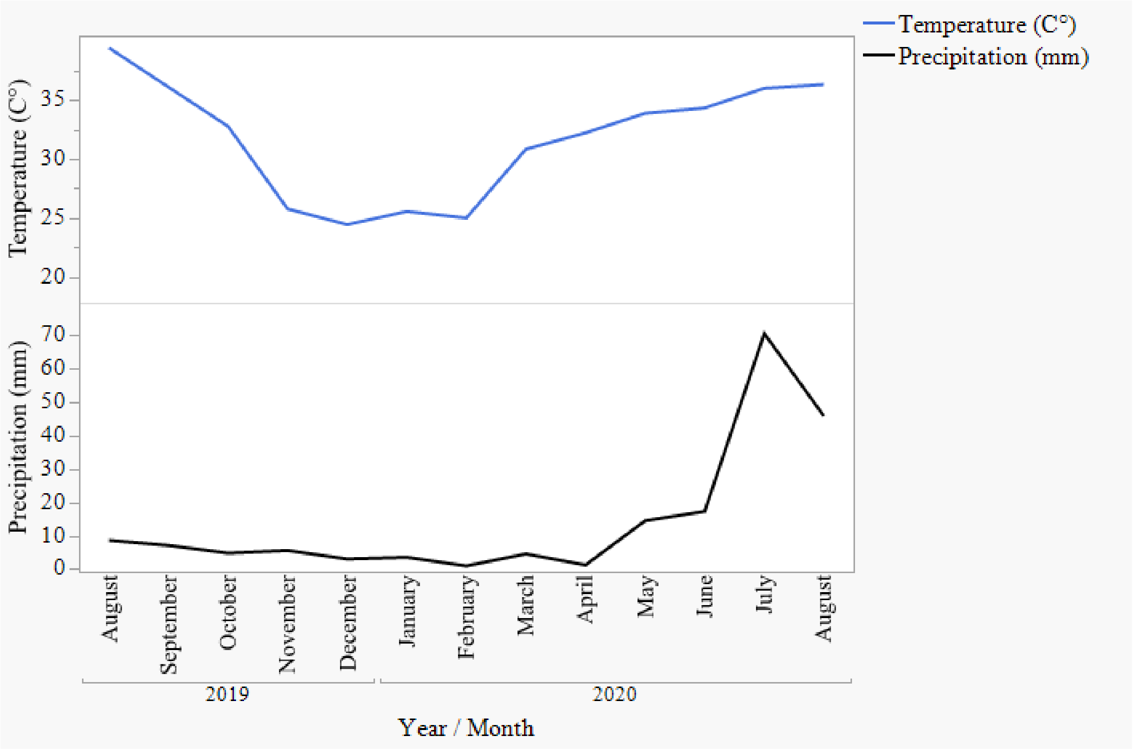

Fig. 4.6: Average monthly temperatures (C°) and precipitation (mm) in the *Manihot walkerae* population of Peñitas Texas during the time span of August 2019 to August 2020. The average yearly temperature in Peñitas was 31.36 C° with the hottest average temperature occurring in the summer month of August 2019, and the lowest average temperature of 25 C° taking place in February of 2020. Average yearly precipitation was 11.81 mm with a noticeable increase in precipitation occurring in July 2020 which coincides with the arrival of hurricane Hanna (70.36 mm), while the lowest average precipitation was also recorded in the month of February 2020 (1.01 mm) (Source AccuWeather).

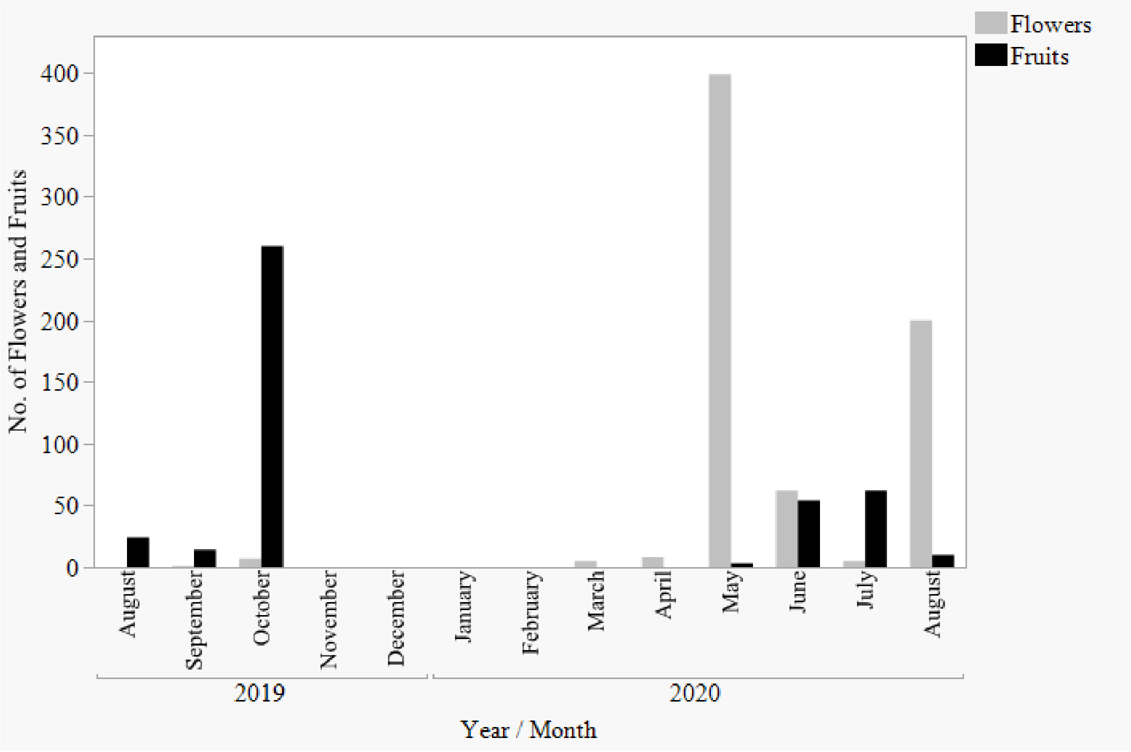

Fig. 4.7: Distribution of flowers and fruits produced by *Manihot walkerae* in the months of August 2019 through August 2020 in the population of Peñitas Texas. Flowering is shown to start in March and continue until the month of October, with the month of May producing the highest number of flowers in the year. *M. walkerae* fruits start to be produced from the months of May to October, with the month of October having the greatest number of fruits counted.

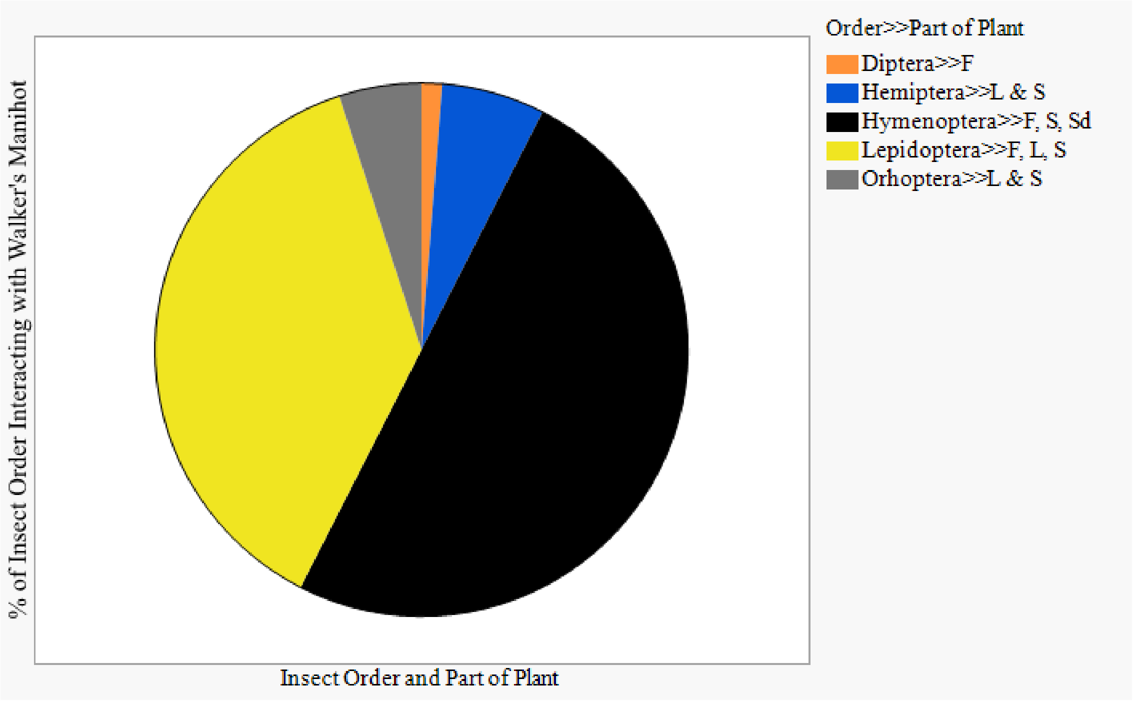

Fig. 4.8: Composition of insect community by order that were seen interacting with *Manihot walkerae*. The Hymenoptera (black) and Lepidoptera (yellow) orders were seen interacting most with *M. walkerae* (bees, ants, wasps, butterflies, caterpillars) while the Hemiptera (blue), Orthoptera (grey), and Diptera (orange) were seen interacting least. In terms of what part of the plant was interacted with, Hymenoptera interacted with the flowers (bees & wasps), and the stems and seeds (ants), while Lepidoptera interacted with flowers (butterflies), and leaves and stems (caterpillars), the Hemiptera and Orthoptera order were seen interaction with the leaves and stems, while Dipterans were seen only interacting with flowers.

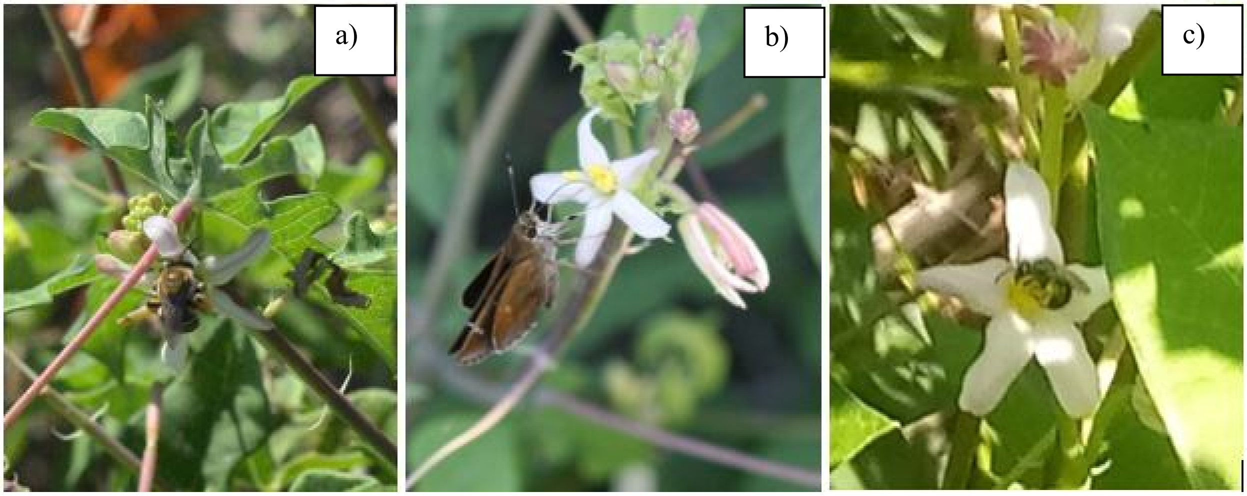

Fig. 4.9: Observed floral visitors of *Manihot walkerae*, a) shows a bee (Hymenoptera) interacting with the flower, while b) shows a butterfly (Lepidoptera) with an inserted proboscis inside the flower possibly acquiring nectar, c) shows a wasp (Hymenoptera) interacting with the flower, all interactions lasted longer than 2 minutes.

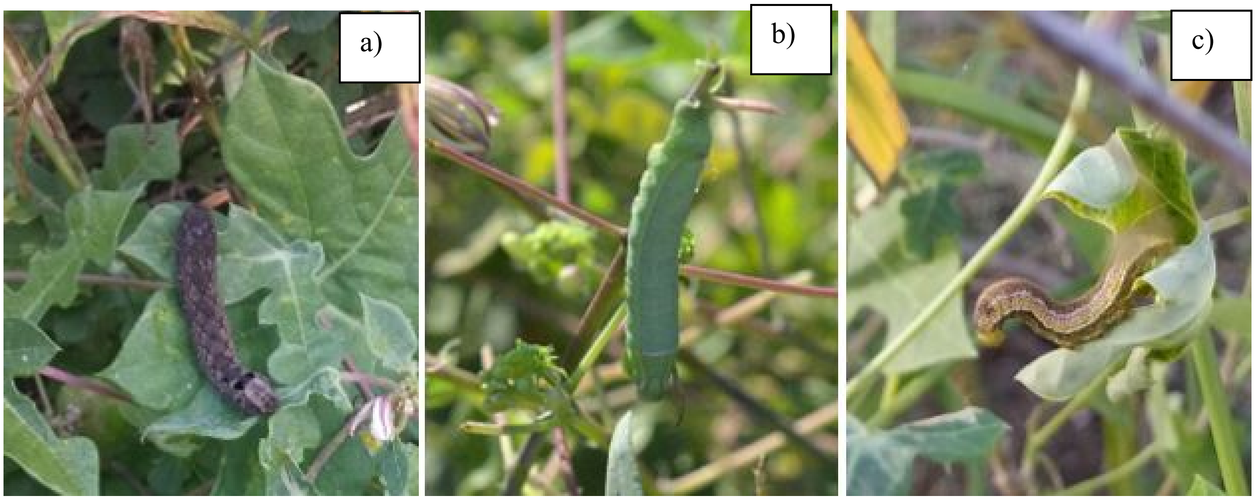

Fig. 4.10: Lepidopteran leaf and stem visitors of *Manihot walkerae*. a) Unidentified Lepidoptera caterpillar b) Cassava hawkmoth *Erinnyis ello* (Sphingidae) caterpillar on *M. walkerae* stem picture taken August 21st, 2020, c) Fall armyworm *Spodoptera frugiperda* (Noctuidae) seen on a *M. walkerae* leaf picture taken October 15th, 2019.

Fig. 4.11: Red harvester ants interacting with a *Manihot walkerae* seed. The ants appear to be most attracted to the seed's elaiosome (yellow arrow).

Table 4.1. Distribution of age classes for the 34 *Manihot walkerae* individuals in the Peñitas Texas population based on basal diameter. The age classes are distributed in the following manner, Juvenile (J) 0.01-0.30 cm, Young Reproductive (YR) 0.31-0.60 cm, Mature Reproductive 1 (MR1) 0.61-0.90 cm, Mature Reproductive 2 (MR2) and Old Reproductive (OR) >1.21 cm.

Age class	Basal diameter	No. of individuals 2019	No. of individuals 2020
Juvenile	0.01-0.30 cm	2	1
Young Reproductive	0.31-0.60 cm	9	11
Mature Reproductive 1	0.61-0.90 cm	13	13
Mature Reproductive 2	0.91-1.20 cm	8	6
Old Reproductive	>1.21 cm	2	1
Total		**34**	**32**

Table 4.2: Length of time to start of ant-seed interaction, time to ant movement of seed (sec), distance of seed dispersal, and location of *Manihot walkerae* seed after 20 min of observation.

Observation	**Time to start of ant-seed interaction (sec)**	**Time to ant seed movement (sec)**	**Distance moved (ft)**	**Location of seed after 20 min**
1	50 sec	85 sec	4 ft	Under shrub
2	40 sec	60 sec	5 ft	Open area
3	60 sec	77 sec	4.5 ft	Under shrub

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BIOGRAPHICAL SKETCH

Gisel Garza graduated from the University of Texas Rio Grande Valley with a Bachelor of Science degree in biology with a minor in chemistry in May 2019 and received the honor of Magna Cum Laude. She then earned a Master of Science in Biology at The University of Texas Rio Grande Valley in May 2021and received the Presidential Graduate Research Assistantship and the Ruth and Katherine Dugger scholarship. As an undergraduate student, Gisel was part of the program "Training the next generation of agricultural scientists: coping with food security and climatic change challenges" (TACFSA) and conducted research in the field of plant pathology focusing on citrus greening disease. As part of her master's thesis she focused on contributing to the conservation of the endangered endemic species Walker's Manihot, *Manihot walkerae* and has published her research about the potential effects of climate change on *M. walkerae's* geographic distribution in a high-quality journal.

As a student at UTRGV she has gained many opportunities to present her research in local, regional, and national conferences where she has been awarded for her presentations. Focusing on native endangered plants, Gisel is experienced in creating species distribution models with MaxEnt and in using geographic information systems (GIS). Gisel is passionate about plant conservation, was president and founder of the Native Plant Conservation Club and hopes to continue addressing global change issues in the future through research and her future career.

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