FINAL REPORT

As Required by

THE ENDANGERED SPECIES PROGRAM

TEXAS

Grant No. TX E-113-R-1

Endangered and Threatened Species Conservation

Population biology, habitat description and delineation and conservation of Terlingua Creek Cat's-eye (*Cryptantha crassipes*)

Prepared by:

Dr. Bonnie Warnock

TEXAS PARKS & WILDLIFE

Carter Smith
Executive Director

Clayton Wolf
Director, Wildlife

22 March 2012

FINAL REPORT

STATE: Texas **GRANT NUMBER:** TX E-113-R-1

GRANT TITLE: Population biology, habitat description and delineation and conservation of Terlingua Creek Cat's-eye (*Cryptantha crassipes*)

REPORTING PERIOD: 1 Sep 09 to 28 Feb 12

OBJECTIVE(S):

To describe and delineate habitat, collect data on population biology and habitat requirements, and begin conservation efforts of Terlingua Creek Cat's-eye (*Cryptantha crassipes*).

Segment Objectives:

Task 1: September 2009. Create an ecologically friendly access road, with as small a foot print as possible from the 02 ranch proper to the study site. The road will not extend into the study site. All access on the study site will be on foot. This will allow access to the site for data collection.

Task 2: September 2009-May 2010. Find and document all populations on the 02 ranch study site. Boundaries of populations will be geo-referenced and mapped using ESRI ArcMap. This project task addresses USFWS recovery task 4.

Task 3: September 2009- May 2010. Place rain gauges to monitor precipitation at each population location. This project task addresses USFWS recovery task 3.

Task 4: January- March 2010. Observe flowering plants to determine species of pollinator and pollinator activity. This project task addresses USFWS recovery task 3.

Task 5: January –March 2010. Collect seeds to add to the collection at the Desert Botanical Garden for increased seed conservation. This project task addresses USFWS recovery task 2 and 3.

Task 6: January- March 2010. Estimate flower and seed production per plant on a sample of plants and extrapolate to the population. This project task addresses USFWS recovery task 3.

Task 7: March-September 2010. Collect basic population data on all populations located within the study site. Data collected will include number and size of individuals, density, dominance, location within the overall habitat, number of plants infected by "disease," and geo-referenced positions of populations. Information on associated species and the overall habitat characteristics will be collected as well. This project task addresses USFWS recovery task 3.

Task 8: March- September 2010. Collect samples of "diseased" plants and send them off for analysis to determine the mechanism of disease. This project task addresses USFWS recovery task 3.

Task 9: March-September 2010. Survey for seedlings and document number and location. This project task addresses USFWS recovery task 3.

Task 10: July 2010-December 2010. Survey for the conservation fence. This project task addresses USFWS recovery task 1.

Task 11: August 2010-May 2011. Construction of fence along the boundary of the 02 Ranch and Terlingua Ranch to protect the Cat's-eye from traffic threats. This project task addresses USFWS recovery task 1.

Task 12: September 2010-December 2010. Establish permanent monitoring plots for population change over time. This project task addresses USFWS recovery task 3.

Task 13: December 2010- May 2011. Collect soil samples from populations. This project task addresses USFWS recovery task 3.

Task 14: December 2010- July 2011. Analyze soil samples for basic soil properties as well as mineral and nutrient content. This project task addresses USFWS recovery task 3.

Task 15: January- March 2011. Observe flowering plants to determine species of pollinator and pollinator activity. This project task addresses USFWS recovery task 3.

Task 16: January –March 2011. Collect seeds to add to the collection at the Desert Botanical Garden. This project task addresses USFWS recovery task 2 and 3.

Task 17: January- March 2011. Estimate flower and seed production per plant on a sample of plants and extrapolate to the population. This project task addresses USFWS recovery task 3.

Task 18: March-September 2011. Survey for seedlings and document number and location. This project task addresses USFWS recovery task 3.

Task 19: March-August 2011. Survey selected populations to determine if microtopography influences plant distribution. This project task addresses USFWS recovery task 3.

Task 20: May-September 2011. Using ESRI Arcmap, and taxroll information, create a databasae of all landowners that potentially own property with the Cat's-eye. This will give a starting point for conserving populations in addition to those on the 02 ranch. This project task addresses USFWS recovery task 4.

Significant Deviations:

None.

Summary Of Progress:

Please see Attachment A.

Location: 02 Ranch, Brewster County, TX.

Cost: Costs were not available at time of this report, they will be available upon completion of the Final Report and conclusion of the project.

Prepared by: Craig Farquhar **Date:** 22 Mar 2012

Approved by: ____________________________ **Date:** 22 Mar 2012

C. Craig Farquhar

Attachment A

Population biology, habitat description and delineation and conservation of Terlingua Creek Cat's-eye (*Cryptantha crassipes*)

Principal Investigator:

Bonnie J. Warnock, PhD

C-16, Department of Natural Resource Management

Sul Ross State University, Alpine, TX

79832

(432) 847 8706

bwarnock@sulross.edu.

ABSTRACT--This study addressed the life history and habitat requirements for Terlingua Creek Cat's-eye (*Cryptantha crassipes*), an endangered plant species, a forb and member of an edaphic community of gypsum loving plants. Terlingua Creek Cat's-eye is located solely within the drainage of Terlingua Creek and its tributaries in Brewster County, Texas. This narrow endemic is found on what is described as the Fizzle Flat lentil, a geologic rock unit which begins within a half mile up Terlingua Creek from a point near the mouth of Alamo de Cesario Creek. This yellowish lentil formation is comprised of limestone with veins of gypsum and barite. We described Terlingua Creek Cat's-eye populations through map parameters, numbers of Terlingua Creek Cat's-eye individuals and habitat. Soil samples taken indicate a higher level of gypsum present in close proximity to Terlingua Creek Cat's-eye plants. Given that our mapped parameters of Terlingua Creek Cat's-eye are all located on private land, implications regarding conservation status and concerns are highlighted.

INTRODUCTION

Terlingua Creek Cat's-eye (*Cryptantha crassipes*) is a federally endangered plant species found only in what can be described as the harshest part of the Chihuahuan desert ((http://drought.unl.edu/dm). These plants grow on a very specific substrate where vegetation is sparse in the southern part of Brewster County, Texas (Poole, 2007). Texas Parks & Wildlife (TPWD) has been monitoring small populations of both this perennial forb and a rare endemic plant species since 1984 (Poole, 1987). Terlingua Creek Cat's-eye was federally listed as endangered in 1991 (Poole, 1994). When this research began, there were no limits to off road traffic and ATV use in Terlingua Ranch where Terlingua Creek Cat's-eye is located. This research intended to investigate its adaptation to one of the harshest ecological habitats in Texas and call attention to the precarious existence of this endangered plant species. This research is important because it can raise the recovery potential for Terlingua Creek Cat's-eye.

Attempts to locate Terlingua Creek Cat's-eye populations outside of the 10-km radius of the confluence of Terlingua Creek and Alamo de Cesario Creek have been historically unsuccessful (Poole, 1994). Current attempts to locate this species in the approximately 12,140.569 km^2 of adjoining Big Bend State Park and nearby Big Bend National Park have not resulted in locating any new populations. Credible sources from both parks (Poole, 2007) verify that this is the case. Terlingua Creek Cat's-eye has not been found anywhere outside of this area in south Brewster County, Texas. V.L. Cory made the first collection of Terlingua Creek Cat's-eye in 1936 and I.M. Johnston (1939) described the specimen.

Members of this subgenus (*Oreocarya*) occupy zerophytic habitats (Higgins, 1971). Some of the species within the *Oreocarya* such as *Cryptantha crassipes* are sharply limited local, uncommon endemics (Cronquist et al., 1984) that selectively inhabit specific geologic substrates (McLaughlin, 1986; Reveal, 2006). Several *Cryptanthas* within this group possess hepatotoxins (Stermitz et al., 1993).

Terlingua Creek Cat's-eye is located in patches of a soil substrate within an area described as hot desert scrub (United States Department of Agriculture, http://esis.sc.egov.usda.gov).

Terlingua Creek Cat's-eye plants have evolved with periodic drought, in habitat historically utilized for heavy cattle grazing (Keller, 2005). Other than occasional patterns of stem browsing that indicated jackrabbit presence (Elbroch, 2003; Roth et al., 2007) on a few of the Terlingua Creek Cat's-eye plants, herbivory by mammals or insects does not appear to affect Terlingua Creek Cat's-eye. Either what little nibbling occurs is not enough to damage it or nothing eats it.

All known populations are located on private land (Warnock, 2009). Terlingua Creek Cat's-eye and its associates inhabit an ecological habitat that provides a unique set of characteristics. Once these characteristics have been identified and characterized, the plant's continued protection and conservation may be enhanced. Increasing the knowledge of Terlingua Creek Cat's-eye population biology and habitat is a relevant and essential prerequisite to conserve the species, but such knowledge may also help to support its associates with whom it may have evolved (Schemske et al., 1994).

Objective. The objective of the project is to describe and delineate habitat, collect data on population biology and habitat requirements, and begin conservation efforts of Terlingua Creek Cat's-eye (Cryptantha crassipes).

Location. The plants are located in southern Brewster County, Texas on privately owned lands on a geology described as pin clay (Geologic Atlas of Texas, 1979) on a geologic rock unit called the Fizzle Flat Lentil (Moon, 1957). See attached map (Figure 4).

Methods.

Task 1: September 2009. Create an ecologically friendly access road, with as small a foot print as possible from the 02 ranch proper to the study site. The road will not extend into the study site. All access on the study site will be on foot. This will allow access to the site for data collection. Year one fence completed; year two fence completed from the 02 ranch proper to the study site.

Task 2: September 2009-May 2010. Find and document all populations on the 02 ranch study site. Boundaries of populations will be geo-referenced and mapped using ESRI ArcMap. This project task addresses USFWS recovery task 4. All known populations on the 02 ranch study site as well as a new population on Terlingua Ranch have been mapped. We created a digital atlas using the USGS 1:24,000 Long Hills, TX and Packsaddle Mountain topographical maps as the boundaries. We mapped the new Terlingua Creek Cat's-eye populations by creating polygon shape files through the data editor and calculated the polygon area using the table tool from Hawth's tools. We transposed the polygons onto the digital atlas of the USGS maps (see attached map images in results: Figures 1-4).

Task 3: September 2009- May 2010. Place rain gauges to monitor precipitation at each population location. This project task addresses USFWS recovery task 3. Rain gauges were placed at Lower Hill Valley site on the O2 and on the fence of the Section 6 population of the O2. An active rain gauge (8 inch Rain Gauge U.S. Dept. of Commerce NOAA-National Weather Service Contract # Wc 1330-03-CQ-0005: Date: 01 08 serial #3509 Frise engineering company, Baltimore, Maryland.) was already in place at the JW (The Field Lab, Terlingua Ranch population). This was used to calculate precipitation for that population. For the O2 population we used Hobo Rain Gauge, situated at the Longhills site.

Task 4: January- March 2010. Observe flowering plants to determine species of pollinator and pollinator activity. This project task addresses USFWS recovery task 3.

Flowering plants were observed to determine floral visitors. Flowering plants were observed for floral visitors, photographed by hand camera once per week during the 2010 flowering season and with a 24 hour garden camera to determine species of pollinator and pollinator activity in 2011 when blooming occurred only in 1/100 plants.

The floral visitors were identified by family.

Task 5: January –March 2010. Collect seeds to add to the collection at the Desert Botanical Garden for increased seed conservation. This project task addresses USFWS recovery task 2 and 3. Seeds were collected by botanist Patricia Manning (SRSU Environmental Science Technician) according to Millennium seed bank standards. We retained 115 Terlingua Cat's-eye seeds and a bag of flower heads from the three populations for a germination and growth study. The objective of the study was to determine whether Terlingua Cat's-eye seedling growth would be the same if they were grown in different substrates. Seedlings were started in Metro-Mix 510 Sun Gro Horticulture potting medium and transferred to four different substrates and measured weekly from February, 2011 to August 2011.

Task 6: January- March 2010. Estimate flower and seed production per plant on a sample of plants and extrapolate to the population. This project task addresses USFWS recovery task 3. For year 2010 flower and seed production were estimated from the LHV population. Blooming Terlingua Creek Cat's-eye plants (N=398) were sampled out of an approximate 2,300 population of plants.

Task 7: March-September 2010. Collect basic population data on all populations located within the study site. Data collected will include number and size of individuals, density, dominance, location within the overall habitat, number of plants infected by "disease," and geo-referenced positions of populations. Information on associated species and the overall habitat characteristics will be collected as well. This project task addresses USFWS recovery task 3. This area of the O2 Ranch is defined as hot desert scrub and is located within the southern portion of the Trans-Pecos Ecological Region (Keller, 2005, Brown, 2009). Terlingua Creek Cat's-eye plants are accessed by four wheeler and on foot in the O2 populations. The third population (JW), named for landowner John Wells (The Field Lab on Terlingua Ranch, Block 217, Lot 19, and Tract 3090) is located 103 km south and west of Alpine on Texas State Highway 118 on the Terlingua Ranch subdivision. This area consists of populations that are distributed in a patchy section of habitat. Vegetation is sparse (Higgins, 1971; Poole, 2007). Individual plants were counted using a hand-held Trimble 3 GPS unit. Counts of plants were entered and stored with area polygons. Numbers are stored within the attribute tables of each polygon. Vegetative cover was estimated with the assistance of Lynn Loomis, Ph.D (NRCS) and documented during soil assessments. Terlingua Creek Cat's-eye plants with black fungus were counted both with the assistance of Spring Field Ecology class 2010 and in individual stands by this researcher.

Task 8: March- September 2010. Collect samples of "diseased" plants and send them off for analysis to determine the mechanism of disease. This project task addresses USFWS recovery task 3. A specimen of Terlingua Creek Cat's-eye (SRSU herbarium voucher #83) plant with black fungus was sent to Dr. Leslie Gunatilaka, SW Center for Natural Products Research, University of Arizona. See attached report (Appendix A).

Task 9: March-September 2010. Survey for seedlings and document number and location. This project task addresses USFWS recovery task 3. Seedlings were surveyed on foot and through extrapolation. Seedlings were defined as having ten or less leaves. Random stands of Terlingua Creek Cat's-eye plants were chosen and the areas were searched for seedlings on foot. Two Terlingua Creek Cat's-eye seedlings were observed in their natural field habitat (JW population on the Terlingua Ranch site: E636874 N2469377).

Task 9: July 2010-December 2010. Survey for the conservation fence. This project task addresses USFWS recovery task 1.

Task 10: August 2010-May 2011. Construction of fence along the boundary of the 02 Ranch and Terlingua Ranch to protect the Cat's-eye from traffic threats. This project task addresses USFWS recovery task 1. Fence is completed for the O2 populations; for the The Field Lab on Terlingua Ranch, the Terlingua Creek Cat's-eye plants are protected to a degree by the landowner whose construction on the property is the main source of an occasional Terlingua Creek Cat's-eye demise. Adjoining sections with absentee landowners remain vulnerable to off-roading vehicles and other recreational activities such as camping, hunting and four-wheeling.

Task 11: September 2010-December 2010. Establish permanent monitoring plots for population change over time. This project task addresses USFWS recovery task 3. Three permanent monitoring plots have been established. Three new Terlingua Creek Cat's-eye populations were mapped (Figure 4). Two of the populations are located on 444 km^2 of the southern tip of the O2 Ranch in Brewster County, Texas. The O2 Ranch is located 40 km south of Alpine in Brewster and Presidio counties, Texas, USA. The third population (JW), named for landowner John Wells (The Field Lab on Terlingua Ranch, Block 217, Lot 19, and Tract 3090) is located 103 km south and west of Alpine on Texas State Highway 118 on the Terlingua Ranch subdivision.

Task 12: December 2010- May 2011. Collect soil samples from populations. This project task addresses USFWS recovery task 3. Twenty seven soil samples were collected from three randomly chosen areas within each Terlingua Creek Cat's-eye population. Within these three areas, three distances from a Terlingua Creek Cat's-eye plant were sampled (close proximity to the plant (interior), marginal, and extra-marginal(>100 m).

Task 13: December 2010- July 2011. Analyze soil samples for basic soil properties as well as mineral and nutrient content. This project task addresses USFWS recovery task 3.

We analyzed soil samples at the Sul Ross State University Soils lab and sent samples to Texas A & M System Soil, Water and Forage Testing Laboratory Department of Soil and Crop Sciences 2478 TAMU College Station, TX 77843-2478 for analysis.

Task 14: January- March 2011. Observe flowering plants to determine species of pollinator and pollinator activity. This project task addresses USFWS recovery task 3. Due to unusually harsh drought conditions in 2011, very few (approximately 10/999) Terlingua Creek Cat's-eye plants bloomed in all three populations. See video footage of one that did bloom (JW site) with Lepidoptera floral visitor (Disk #1). This Terlingua Creek Cat's-eye plant was monitored weekly until seeds were produced; one capitate flower head was harvested from the plant. Six nutlets were harvested and planted in the Sul Ross State University greenhouse. One viable seedling germinated but perished after two weeks.

Task 15: January –March 2011. Collect seeds to add to the collection at the Desert Botanical Garden. This project task addresses USFWS recovery task 2 and 3.

Due to the extremely low number of flowering Terlingua Creek Cat's-eye plants during the 2011 spring blooming season, seeds to add to the collection at the Desert Botanical Garden could not be harvested. Terlingua Creek Cat's-eye produced numbers of blooming plants so low that harvesting at the previous year's rate was not advisable. We used the 2010 data to extrapolate nutlet numbers and harvested sparingly in the LHV population of 2,300.

Task 16: January- March 2011. In 2011 we sacrificed approximately 8-10 flower heads for a total harvest of 50 seeds; and 26 seeds from the JW population.   This project task addresses USFWS recovery task 3.

Task 17: March-September 2011. Survey for seedlings and document number and location. This project task addresses USFWS recovery task 3. Seedling searches continued during this study period.

Task 18: March-August 2011. Survey selected populations to determine if microtopography influences plant distribution. This project task addresses USFWS recovery task 3. Terlingua Creek Cat's-eye populations were surveyed on foot with GPS unit in hand, by overlaying elevation layer on the stands of plants (ESRI Arc9 and Arc10), and by use of soil morphology and descriptive resources (Natural Resource Conservation Service: http://esis.sc.egov.usda.gov/esis_report).

Task 19: May-September 2011. Using ESRI Arcmap, and taxroll information, create a database of all landowners that potentially own property with the Cat's-eye. This will give a starting point for conserving populations in addition to those on the 02 ranch. This project task addresses USFWS recovery task 4. We used ESRI Arcmap Texas survey layered over all known Terlingua Creek Cat's-eye sites to identify all landowners that potentially own property that has Terlingua Creek Cat's-eye. See Table 4 in results.

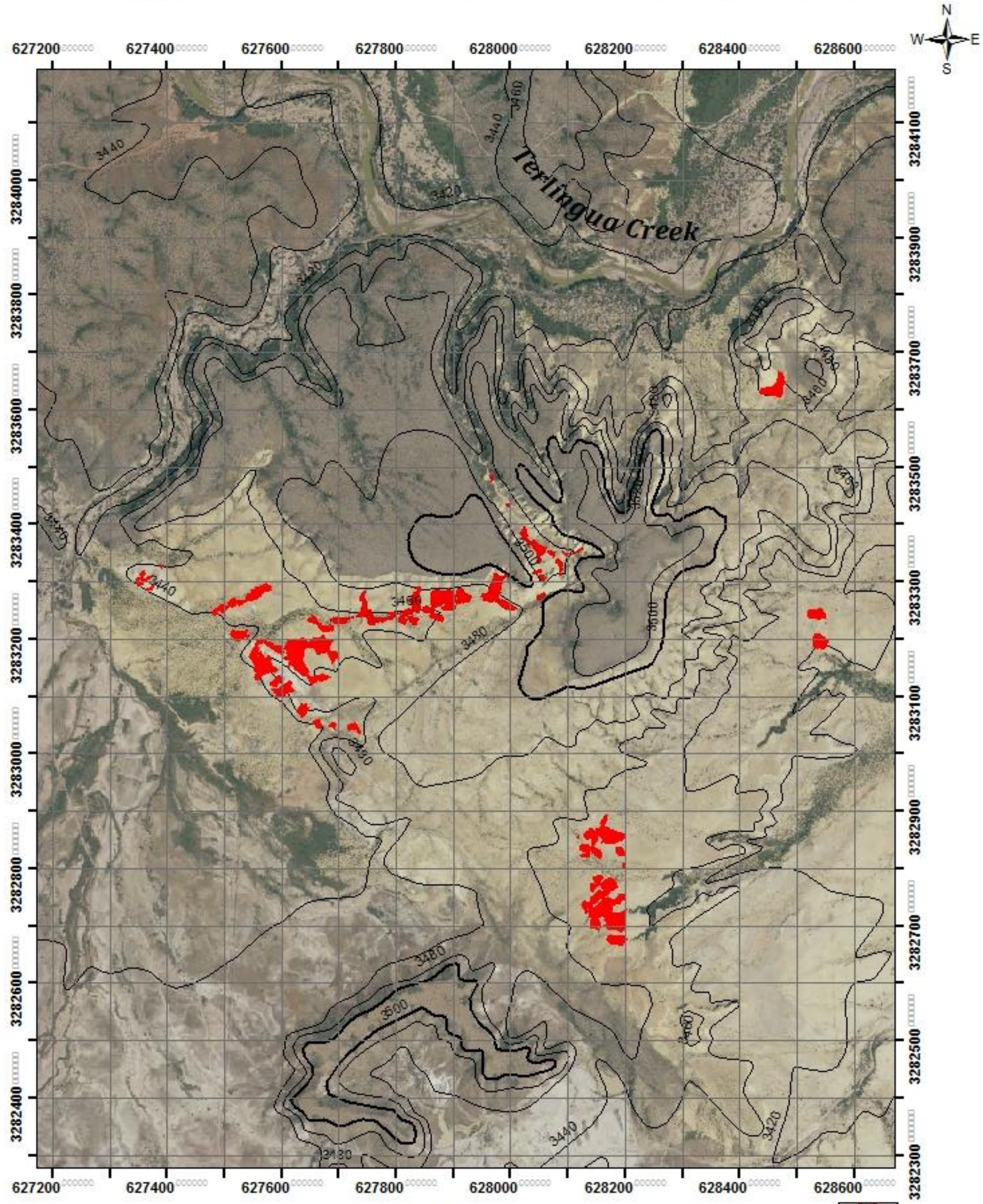
Results.

Task 1: September 2009. An ecologically friendly access road from the 02 ranch proper to the study site was completed. The road does not extend into the study site but can be accessed either through the ranch, on foot or with permission through an adjoining bentonite mine (Cowboy mining Company). This allows access to the site for data collection and projects the O2 Terlingua Creek Cat-s-eye populations. The JW population in Terlingua Ranch is not fenced. This is an ongoing danger to the Terlingua Creek Cat's-eye plants.

Task 2: September 2009-May 2010. All newly identified populations on the 02 ranch study site are mapped (Section 6 and Lower Hill Valley). All plants on the JW population within Terlingua Ranch were mapped (Map 1-3)

Task 3: September 2009- May 2010. Rain gauges to monitor precipitation were placed at each population location. At the JW Terlingua Ranch site, precipitation was: rain total for 2009: 7.5 inches; 2010: 8.78 inches; 2011: 3.82 inches. It did not rain from September 23, 2010 to July 2, 2011. For the O2 populations, we used the Longhills rain gauge already in place by the Rio Grande Research Center, Hobo Rain Gauge. At this site precipitation was: 10/20/09-09/29/10: 5.71 inches; 09/29/10-01/27/11: .05 inches; and, 05/19/11 to 02/09/12: 1.11 inches.

Terlingua Creek Cat's-eye Section 6 Population



0 50 100 200 300 400
Meters

Legend

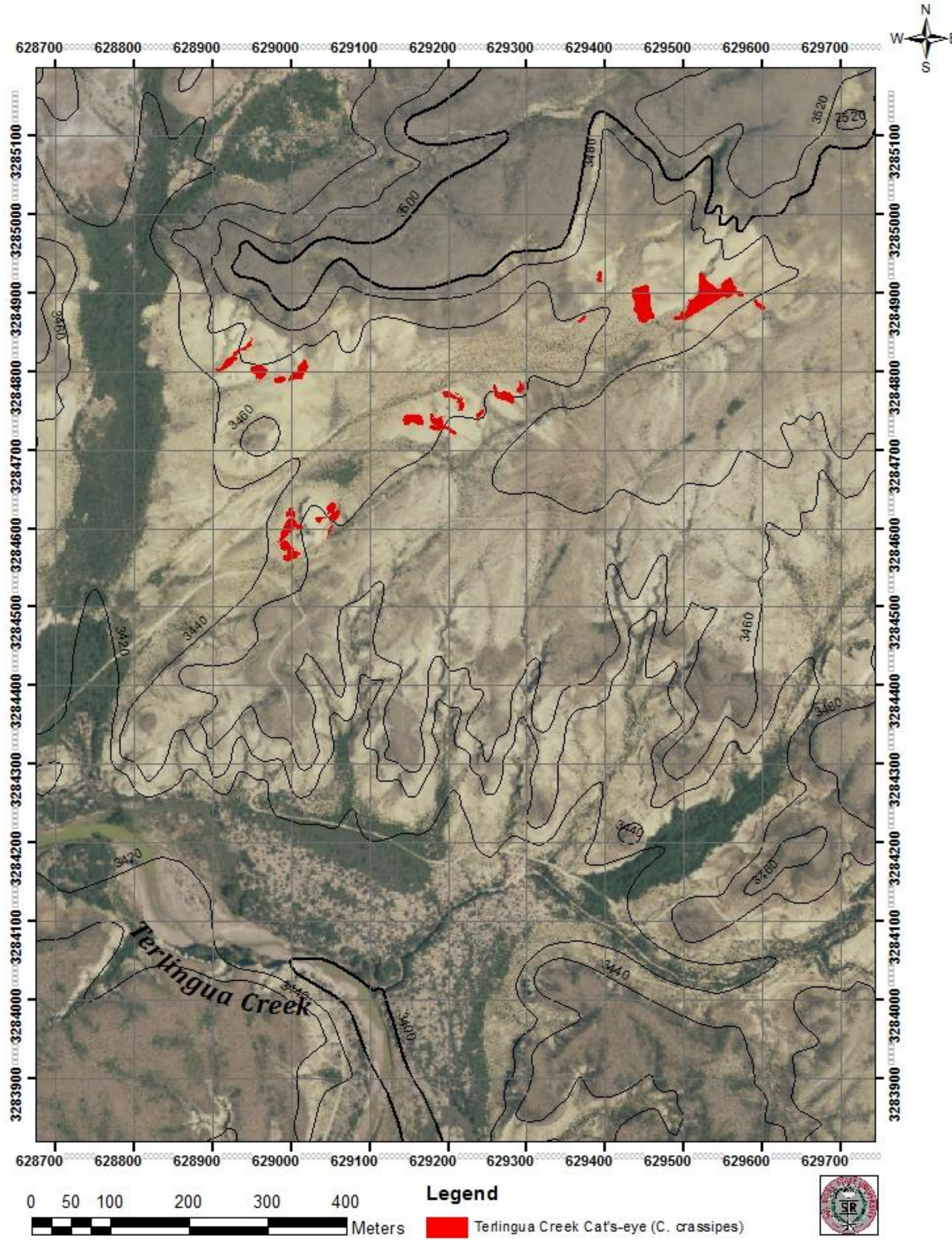
Terlingua Creek Cat's-eye (C. crassipes)

Datum: NAD 83

Map produced by Maria Williams, SRSUGIS

22 Febuary 2012

Terlingua Creek Cat's-eye Lower Hill Valley Population

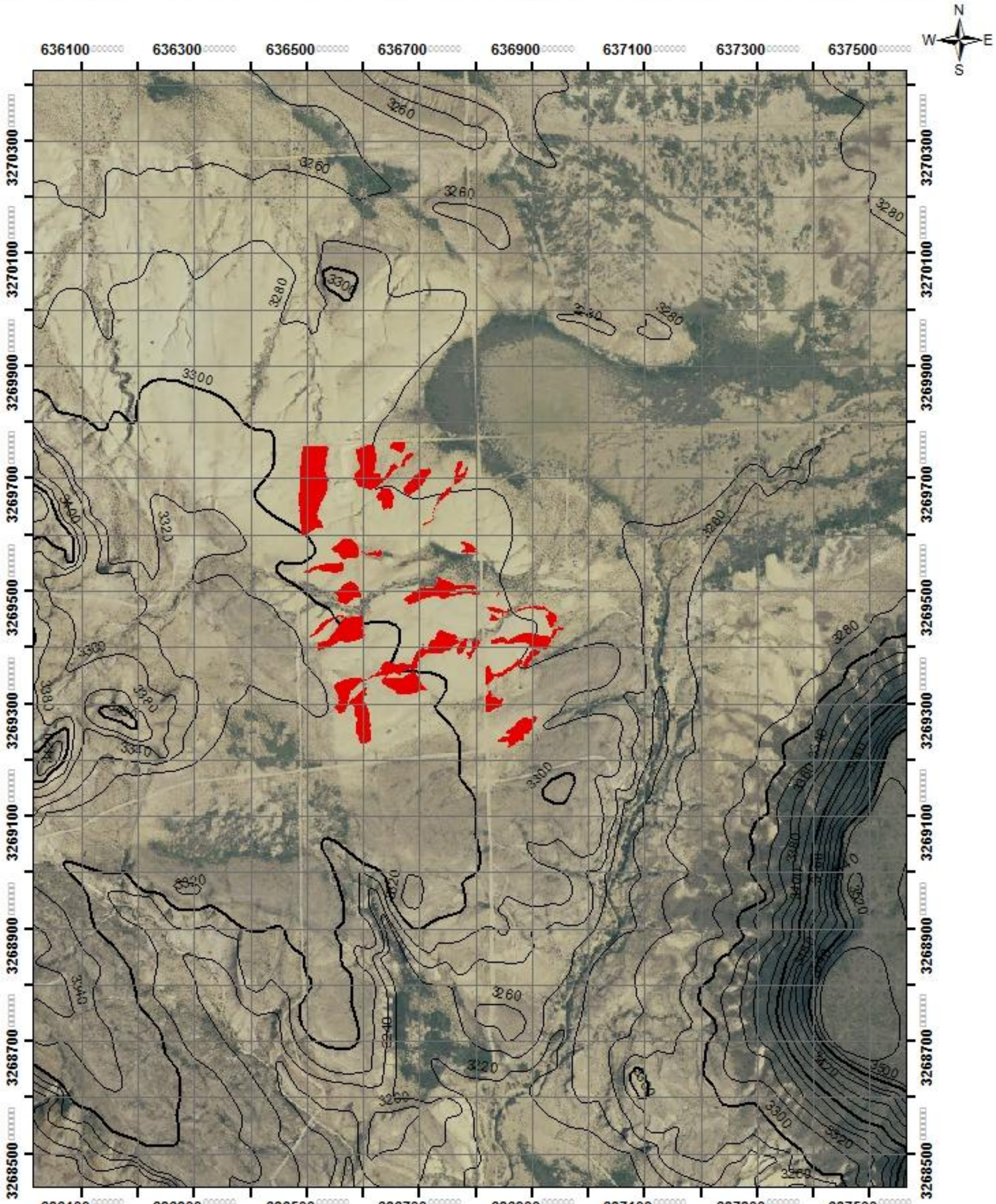


Datum: NAD 83

Map produced by Maria Williams, SRSUGIS

22 Febuary 2012

Terlingua Creek Cat's-eye Terlingua Ranch JW Population



0 50 100 200 300 400
Meters

Legend

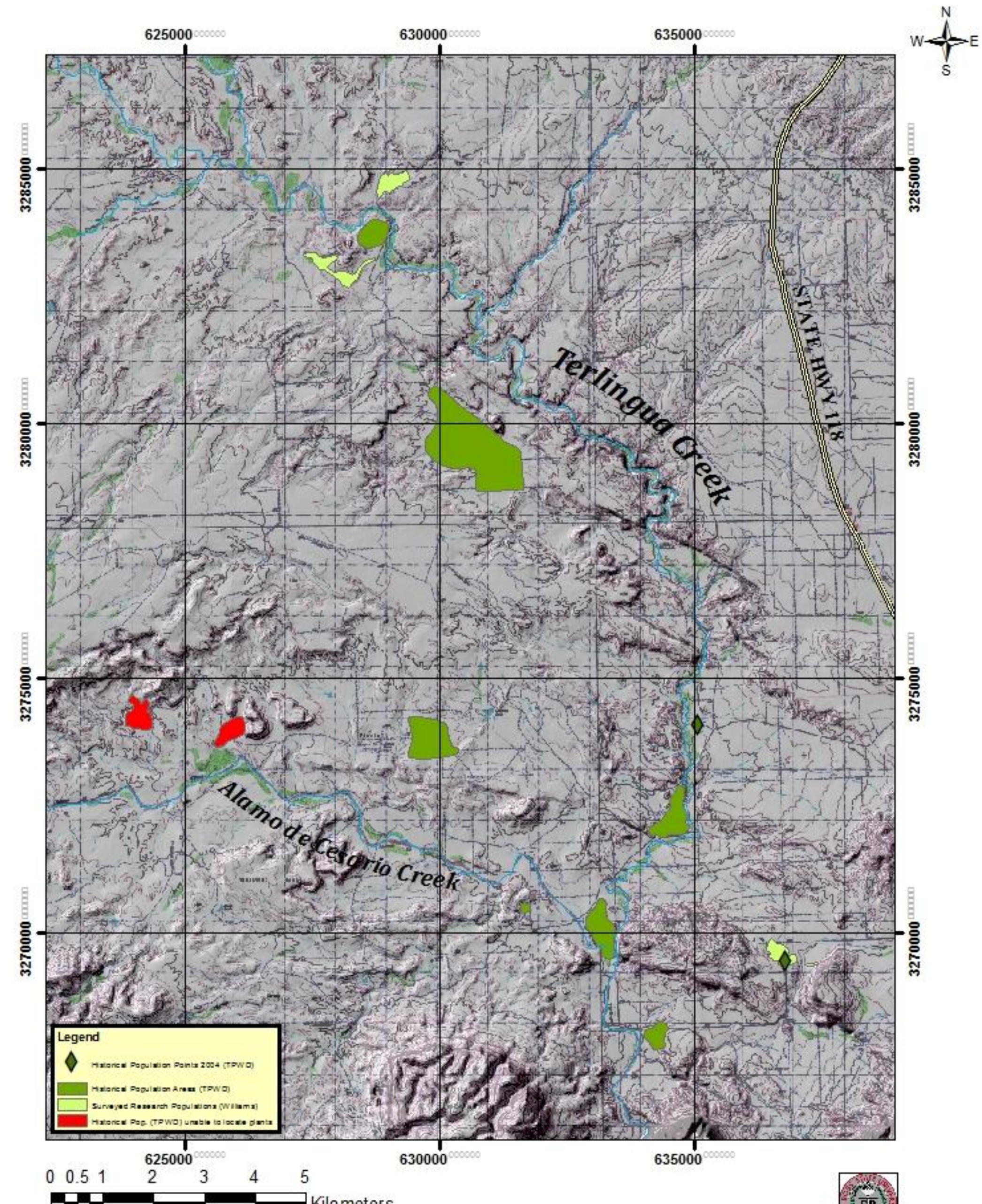
Terlingua Creek Cat's-eye (C. crassipes)

Datum: NAD 83

Map produced by Maria Williams, SRSUGIS

22 Febuary 2012

Terlingua Creek Cat's-eye (Cryptantha crassipes) Populations



Datum: NAD 83

Map produced by Maria Williams, SRSUGIS

23 Febuary 2012

Task 4: January- March 2010. The floral visitors were identified by family as Lepidoptera (Photo 1) and Hymenoptera (Photo 2 and 3).

Photo 1. Funeral Sootywing floral visitor, March 2010.

Photo 2. Hymenoptera floral visitor, March 2010.

Photo 3. Hymenoptera floral visitor, March 2010.

Task 5: January –March 2010. We collected seeds to add to the collection at the Desert Botanical Garden for increased seed conservation (2010). This project task addresses USFWS recovery task

2 and 3. Approximately 1,797 seeds were shipped to the Desert Botanical Garden in Phoenix, AZ to the conservation manager, Brandi Eide and two bags of flower heads were hand-carried to them for germination studies and storage. Ten nutlets were sent (SRSU herbarium voucher number 83) for a *Cryptantha* molecular study at San Diego State University, San Diego. Through our Terlingua Creek Cat's-eye and germination study, we found that seedlings grown in different substrates demonstrated a significant difference in leaf number and height ($P<0.05$) (Kruskal-Wallis, SPSS 19). Terlingua Creek Cat's-eye seedlings grown in substrate N (Native soil) in the greenhouse exhibited the slowest rate of growth and the lowest survival rate while those grown in substrate P (Potting medium) had the highest survival rate. Leaf number was highest in substrate NS(½ native soil, ½ sand) while seedling height was highest in substrate NP (½ native soil and ½ potting medium). At the time of this writing, 27 of these seedlings are approaching their second year. The only known documentation of Terlingua Creek Cat's-eye seedlings grown to adulthood (Hughes, 1992) states that they flowered at two years of age. Patricia Manning (Environmental Science Technician-SRSU greenhouse) continues to maintain this population. We are hopeful that these plants will flower.

Task 6: January- March 2010. Estimated flower and seed production per plant on a sample of plants and extrapolated to the populations is as follows: Each plant averaged 4.8 capitate flower heads per individual. Each (per) capitate head averaged 13 flowers. Each flower had an average of .75 nutlets.

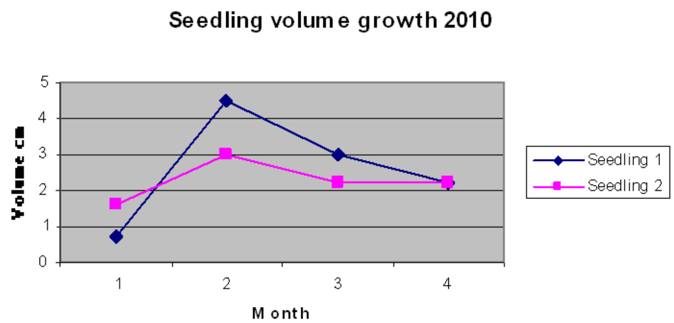
Task 7: March-September 2010. Collect basic population data on all populations located within the study site. Data collected will include number and size of individuals, density, dominance, location within the overall habitat, number of plants infected by "disease," and geo-referenced positions of populations. Within the two O2 populations we mapped an approximate total of 8,700 individual C. crassipes plants. The JW population has 999 plants on his section with plant populations expanding to adjacent sections (see map JW image). Vegetative cover was estimated at 10% with Terlingua Creek Cat's-eye the dominant species. Vegetative associates collected from the Terlingua Creek Cat's-eye habitat include: Castilleja ridida; Polygala lindheimeri var. parvifolia; Eriogonum havardii; Anulocaulis leiosolenus var. lasianthus; Mentzelia mexicana; Isocoma pluriflora; Aristida pansa; Xylorhiza wrightii. Terlingua Creek Cat's-eye plants with black fungus occur in all populations at an approximate 10% of plants. Only Terlingua Creek Cat's-eye plants that were alive and had the black fungus were counted. Groups of plants tended to be infected and on downward slopes

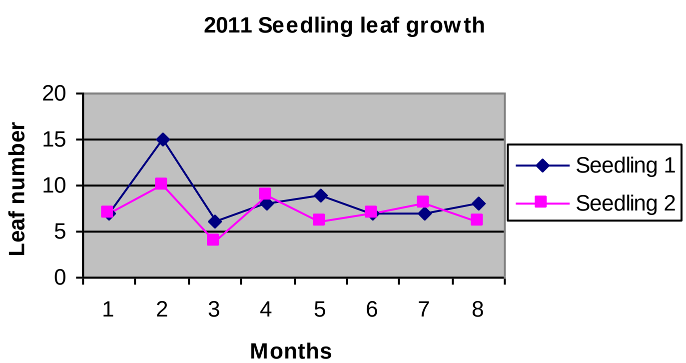
Task 8: March- September 2010. Collect samples of "diseased" plants and send them off for analysis to determine the mechanism of disease. This project task addresses USFWS recovery task 3. Per the report of Dr. Leslie Gunatilaka, SW Center for Natural Products Research, University of Arizona, nine fungal strains were isolated (Appendix A). Extra funding was required to identify each strain to family so this identification did not occur. It is hypothesized that some plants divert their energy to fighting off microorganisms and pathogens in their native soil by producing toxic pyrrolizidine alkaloids as a defense (Hol and Van Veen, 2005). The cost for this activity is a slower growth rate. The same pyrrolizidine producing plants grown in sterilized soil grew faster (Joosten et al., 2009) than those in their own soil. Since Terlingua Creek Cat's-eye plants possess 10 to 50 times more pyrrolizidine alkaloids (Williams et al., 2011) than their closest relatives (Stermitz et al., 1993), this chemical mechanism may be a trade-off that allows this species to exist in a harsh desert habitat. Faster growing desert scrub associates that live on the marginal habitats do not have the same tolerance.

Task 9: March-September 2010. Survey for seedlings and document number and location. This project task addresses USFWS recovery task 3. Seedling counts range from 2 to 7 seedlings per randomly chosen stand of Terlingua Creek Cat's-eye plants in the O2 populations (Polygon # 11 LHV N3284766 E629206 and Section 6 Polygon #1 N3283249 E626820). For the JW population, seedlings ranged from 2 to 16 per randomly chosen stand (Polygon #2 N3269718 E636610). The two seedlings (JW population) remained nearly the same size after one year of observation. At the beginning of the observation, the heights of field seedlings were measured and found to be 1.4 cm and 1.6 cm. By August 2010 each was 1.5 cm and 1.5 cm. By September 2011, the seedlings appeared brittle and were back to their original size.

Measurement changed from volume to counting leaf number it was difficult to determine whether the seedlings are alive as they were desiccated and brittle. Leaf number declined then remained nearly the same for several months (Figure 1). The study period took place during the worst Texas drought since record-keeping began 116 years ago (http://drought.unl.edu/dm).

Figure 1. Seedling characteristics for seedlings monitored at the JW population site.





Task 10: August 2010-May 2011. Construction of fence along the boundary of the 02 Ranch and Terlingua Ranch to protect the Cat's-eye from traffic threats. This project task addresses USFWS recovery task 1. Fence is completed for the O2 populations; for the The Field Lab on Terlingua Ranch, the Terlingua Creek Cat's-eye plants are protected to a degree by the landowner whose construction on the property is the main source of an occasional Terlingua Creek Cat's-eye demise. Adjoining sections with absentee landowners remain vulnerable to off-roading vehicles and other recreational activities such as camping, hunting and four-wheeling.

Task 11: September 2010-December 2010. Establish permanent monitoring plots for population change over time. This project task addresses USFWS recovery task 3. Three permanent monitoring plots have been established. Three new Terlingua Creek Cat's-eye populations were mapped (Figure 4). Two of the populations are located on 444 km^2 of the southern tip of the O2 Ranch in Brewster County, Texas. The O2 Ranch is located 40 km south of Alpine in Brewster and Presidio counties, Texas, USA. The third population (JW), named for landowner John Wells (The Field Lab on Terlingua Ranch, Block 217, Lot 19, and Tract 3090) is located 103 km south and west of Alpine on Texas State Highway 118 on the Terlingua Ranch subdivision.

Task 12: December 2010- May 2011. Collect soil samples from populations. This project task addresses USFWS recovery task 3. Twenty seven soil samples were collected from three randomly chosen areas within each Terlingua Creek Cat's-eye population. Within these three areas, three distances from a Terlingua Creek Cat's-eye plant were sampled (close proximity to the plant (interior), marginal, and extra-marginal(>100 m).

Task 13: December 2010- July 2011. Analyze soil samples for basic soil properties as well as mineral and nutrient content. This project task addresses USFWS recovery task 3. Average pH of the three Terlingua Creek populations: 8.24; averages for soil in close proximity to Terlingua Creek Cat's-eye: pH: 8.27; marginal: 8.23; ex-marginal: 8.22. Average gypsum percent: interior: 7.36% (range from 1.25% to 26.34%); margin: 1.58% (range from 0.81% to 2.17%); extra-marginal: 1.30% (range from 0.80% to 1.88%). Gypsum percent and pH are higher closer to Terlingua Creek Cat's-eye plants. Percent saturation of soil was higher for the interior soil samples as was clay content (Tables 1-4)

Table 1. Soil texture and saturation percentages of soil samples taken from three populations of Terlingua Creek Cat'seye. Samples were taken near plants, at the edge of a stand of plants and in soil adjacent to stands of plants.

Sample	Saturation Percent	Texture	SD
1 Interior Section 6	48%	Clayey fine	8.08
25 Interior Section 6	48%	Clayey fine	8.08
22 Interior Section 6	34%	Sandy clay	8.08
4 Interior LHV	35%	Sandy clay loam	4.51
10 Interior LHV	39%	Loam	4.51
13 Interior LHV	44%	Sandy clay	4.51
7 Interior JW	39%	Clayey fine	3.06
19 Interior JW	33%	Clayey fine	3.06
16 Interior JW	35%	Sandy clay loam	3.06
2 Margin Section 6	38%	Clayey fine	4.58
23 Margin Section 6	32%	Sandy clay loam	4.58

26 Margin Section 6	29%	Sandy clay loam	4.58
5 Margin LHV	27%	Clayey fine	9.54
11 Margin LHV	43%	Sandy clay loam	9.54
14 Margin LHV	44%	Silty clay	9.54
8 Margin JW	36%	Sandy clay loam	2.51
17 Margin JW	39%	Clayey fine	2.51
20 Margin JW	41%	Clay loam	2.51
3 Ex-mar Section 6	24%	Sandy clay loam	6.02
24 Ex-mar Section 6	36%	Loam	6.02
27 Ex-mar Section 6	31%	Clay loam	6.02
6 Ex-mar LHV	39%	Clayey fine	4.04
12 Ex-mar LHV	26%	Sandy clay loam	4.04
15 Ex-mar LHV	36%	Sandy clay loam	4.04
9 Ex-mar JW	31%	Sandy clay loam	2.08
18 Ex-mar JW	35%	Sandy clay loam	2.08
21 Ex-mar JW	32%	Sandy clay loam	2.08

Table 2. Soil gypsum percent and mineral content within Terlingua Creek Cat's-eye stands.

Interior Location	Gypsum Percent	pH	Nitrate ppm	P ppm	K ppm	Ca ppm	Mg ppm	Na ppm	Sulfur ppm
Sec 6-1	6.6%	8.2	49	1	84	43, 168	474	109	117
Sec 6-22	4.24%	8.5	3	1	86	36,819	256	75	24
Sec6-25	14.7%	7.8	3	0	81	42,663	187	91	4,069
LHV-4	1.25%	8.3	4	3	95	38,099	226	25	85
LHV-10	2.23%	8.4	11	0	110	41,467	437	46	88
LHV-13	7.02%	8.3	6	0	83	36,018	337	84	68
JW-7	26.34%	8.2	120	1	143	41,733	468	457	342
JW-16	2.31%	8.4	3	1	73	34,837	360	86	22
JW-19	1.52%	8.3	5	1	83	34,141	325	86	25

Table 3. Soil gypsum percent and mineral content at the margin of Terlingua Creek Cat's-eye stands.

Marginal Location	Gypsum Percent	pH	Nitrate ppm	P ppm	K ppm	Ca ppm	Mg ppm	Na ppm	Sulfur ppm
Sec 6-2	0.9%	8.4	3	2	85	43,453	355	91	24
Sec6-23	0.98%	8.4	4	1	59	35,882	232	79	24
Sec6-26	0.81%	8.2	4	0	68	36,569	206	87	100
LHV-5	1.57%	8.1	4	3	101	39,971	169	97	50
LHV-11	2.17%	8.4	4	1	74	41,312	258	84	23
LHV-14	1.88%	8.1	5	5	208	20,705	191	85	25
JW-8	1.86%	8.2	5	7	214	32,716	198	108	51
JW-17	2.11%	8.1	20	7	173	29,115	204	94	38
JW-20	1.94%	8.2	2	12	120	28,577	134	92	29

Table 4. Soil gypsum percent and mineral content on soils adjacent to Terlingua Creek Cat's-eye stands.

Extra marginal location	Gypsum Percent	pH	Nitrate ppm	P ppm	K ppm	Ca ppm	Mg ppm	Na ppm	Sulfur ppm
Sec6-3	1.06%	8.2	6	30	208	13,140	127	90	23
Sec6-24	1.57%	8	12	0	83	37,246	167	94	114
Sec6-27	1.33%	8.3	3	3	70	32,113	239	88	51
LHV-6	1.40%	8.1	7	1	81	42,383	208	96	26
LHV-12	1.88%	8.2	7	22	180	23,061	175	75	28
LHV-15	1.44%	8.3	4	3	129	31,546	415	121	43
JW-9	0.8%	8.2	5	23	174	19,911	161	77	24
JW-18	1.15%	8.3	3	1	67	37,356	143	83	35
JW-21	1.05%	8.4	4	0	77	36,968	195	87	27

Task 14: January- March 2011. Observe flowering plants to determine species of pollinator and pollinator activity. This project task addresses USFWS recovery task 3. One blooming Terlingua Creek Cat's-eye plant (JW population; see video footage of floral visitor attachment) was monitored weekly until seeds were produced; one capitate flower head was harvested from the plant. Six nutlets were harvested and planted in the Sul Ross State University greenhouse. One viable seedling germinated but perished after two weeks.

Task 15: January –March 2011. Collect seeds to add to the collection at the Desert Botanical Garden. This project task addresses USFWS recovery task 2 and 3. We were unable to harvest at the previous year's rate due to a lack of blooming plants. We did harvest approximately 76 seeds (LHV population and MPO2 from the JW population).

Task 16: January- March 2011. In 2011 we sacrificed approximately 8-10 flower heads for a total harvest of 50 seeds from LHV population; and 26 seeds from the JW population. Estimated flower and seed production per plant on a sample of plants was 51% lower than the previous year's yield. Seven seedlings germinated and perished after two-three weeks. At the time of this writing, one 2011 Terlingua Creek Cat's-eye seedling remains alive (MPO2 JW population). We have 26 two year old Terlingua Creek Cat's-eye plants in the SRSU greenhouse from our 2010 seed collection. They are monitored by Patricia Manning, Environmental Science Technician.

Task 17: March-September 2011. Survey for seedlings and document number and location. This project task addresses USFWS recovery task 3. Seedling number did not change within the populations from 2010-2011. The two Terlingua Ranch (JW population: N 3269374 E 636849) field seedlings were monitored for growth rate, first by volume then by leaf number from May, 2010-September, 2011. By the last field visit, September, 2011, it was difficult to determine whether the seedlings were still alive. They are encircled on the JW population site with local rocks.

Task 18: March-August 2011. Survey selected populations to determine if microtopography influences plant distribution. This project task addresses USFWS recovery task 3. Terlingua Creek Cat's-eye plants are located on gentle slopes (1-7% grade) on and between the alluvial fans or fan piedmont land forms (Peterson, 1981) of small hills. The soil color is a light yellow for Terlingua Creek Cat's-eye substrate (2.5 y/r-NRCS Field Book for Describing and Sampling Soils, 2002). This is considerably lighter than the dominant surrounding soil (Mariscal and Cesario loams). Aspect appears to be random as evidence by the ESRI identification tool used on aspect layer.

Task 19: May-September 2011. Using ESRI Arcmap, and taxroll information, create a database of all landowners that potentially own property with the Cat's-eye (Table 5). Actual landowner names can be obtained from tax records for the identified locations.

Table 5. Texas land survey information identifying potential owners of Terlingua Creek Cat's-eye.

Poly #	Survey Name	Block	Section	Name	160 acre quarters
6	T&STL RR CO	214	16	Dorr, CC	NW, NE, SW
6	T&STL RR CO	214	17		NE, SE
6	T&STL RR CO	214	21		NE
1	T&STL RR CO	214	6	Benson, GM	NE, SE
1	T&STL RR CO	214	5		NW, SW
0	T&STL RR CO	213	60	Benson, GM	NE, SW
4	T&STL RR CO	210	35		SW, SE
4	T&STL RR CO	204	36	Worthington, GW	NW
5	T&STL RR CO	SL	2	Robinson, TP	NW
3	T&STL RR CO	215	4	Minchen, M	NW
3	T&STL RR CO	215	5		NE
0	T&STL RR CO	215	1		NW
8	T&STL RR CO	215	11		NE, SE
2	T&STL RR CO	215	16	Parker, ZE	NE
0	T&STL RR CO	215	15		NE, SE
2	T&STL RR CO	215	13		SW, SE
2	T&STL RR CO	215	24	Dorr, CC	NE
3	T&STL RR CO	217	19		NW
1	T&STL RR CO	215	26	Adam, S & Kendrick, KB	NW, NE

Discussion.

Through this research, we found information that may contribute to the recovery of this federally endangered species. In the seedling growth experiment with Terlingua Creek Cat’s-eye seedlings were able to grow in different substrate combinations but they did not grow at the same rate, with growth being higher in all artificial media as compared with native soil. While Terlingua Creek Cat’s-eye will grow in a variety of substrates in ideal greenhouse conditions, it appears to be an obligate gypsophile in the field, based on Meyer’s (1986) definition. Other plant species defined as obligate gypsophiles that do not grow naturally outside of their native gypseous substrate grew to maturity successfully when germinated and grown in the greenhouse in other studies (Powell and Turner, 1977).

As with halophytes who can tolerate low water potentials, competitive exclusion may explain why Terlingua Creek Cat’s-eye is not found outside of its gypseous substrate (Ungar, 1974). Terlingua Creek Cat’s-eye’s restricted distribution may be related to the gypsum present within its habitat (Moon, 1957) but for more reasons than gypsum provides chemically. Meyer, (1986) documents that gypseous soils had higher moisture contents than the adjacent soils. Gypseous soils can also form a crust that interfered with seedling establishment (Meyer and Garcia-Moya, 1986). Another aspect important to plant adaptation to a gypsum substrate relates to plant physiology and the production of osmoprotective compounds (Roberts, 2005).

One hypothesis relating to plant adaptation in a harsh, dry desert environment concerns the production of pyrrolizidine alkaloids as a consequence of water stress (Briske and Camp, 1982). Some pyrrolizidine alkaloids have a zwitterionic charge. Molecules with zwitterionic charges are small, electrically neutral molecules that are non-toxic at molar concentrations. Zwitterions stabilize proteins and membranes against the denaturing effect of high concentrations of salts and other harmful solutes (Yancey, 1994). This confers cellular protection from the pressure of lowered osmotic potential in soil lacking water or high in soluble salts (Hanson et al., 1994; Roberts, 2005). Terlingua Creek Cat’s-eye’s pyrrolizidine alkaloids (Williams et al., 2011), toxic to most generalist herbivores (Fu et al., 2004), may have allowed for adaptation to a harsh desert environment.

Pyrrolizidine alkaloids also may function as a defense against microorganisms and pathogens (Hol and Van Veen, 2005). The physiological cost for this activity is a slower growth rate. In other studies the same pyrrolizidine producing plants grown in sterilized soil grew faster (Joosten et al., 2009) than those in their own soil. This strategy works for Terlingua Creek Cat’s-eye who cannot compete with faster growing desert scrub associates who live on the margins. This mirrors the growth pattern of two Terlingua Creek Cat’s-eye seedlings observed in their natural habitat that remained nearly the same size after more than a year of observations.

Terlingua Creek Cat’s-eye were found to have nine distinct fungal growth patterns when we sent a specimen for analysis of microorganisms to the Natural Products Laboratory, University of Arizona (L. Gunatilaka et al., in litt.). We do not know the nature of the relationship between Terlingua Creek Cat’s-eye and its fungal associates. It could be a symbiotic (Barrow et al., 2008) one or may be responsible for the blackening noted on Terlingua Creek Cat’s-eye plants that, once infected with it, die (Poole, 1987, 1994).

Soil characteristics play an essential part in what species can grow in a specific area. The distribution of vegetation and nutrients is a function of edaphic soil factors that control the distribution of native desert plant species as well as post-forest pastures (Hallmark and Allen, 1975; Numata et. al., 2007).

We suggest that Terlingua Creek Cat's-eye's adaptation to what has been described as one of the harshest environments in Texas may involve a set of several adaptive strategies. Terlingua Creek Cat's-eye has not been found outside of a narrow range of geology and soils. Soil morphology of Terlingua Creek Cat's-eye habitat consists of a combination of soils that include Cesario and Fizzleflat loams, Cheosa and Loyplace soils, and Mariscal-rock outcrop complex. An interesting characteristic of all these soil types is the capacity to transmit water within their most limiting layer (Natural Resource Conservation Service: http://esis.sc.egov.usda.gov/esis_report). Terlingua Creek Cat's-eye does not fit into the soil descriptions of the soils in its vicinity.

There are three characteristics in which the substrate on which Terlingua Creek Cat's-eye grows differs from the official Mariscal soil description. Mariscal has a B horizon where Terlingua Creek Cat's-eye has only an A horizon to bedrock. The soil color is considerably lighter for Terlingua Creek Cat's-eye substrate 2.5 y/r compared to Mariscal with 10 y/r. Mariscal has a wetter climate with a mean annual precipitation of 28 cm whereas the annual precipitation for Terlingua Creek Cat's-eye substrate for 21 cm. There are some similarities between Mariscal and Terlingua Creek Cat's-eye soil substrate as well. Both soil types have violent effervescence reactions in the A horizon. This fact strongly infers that the parent material for Terlingua Creek Cat's-eye soils is platy limestone (Moon, 1957). The soil in closest proximity to the Terlingua Creek Cat's-eye plants is higher in gypsum percent and in its percentage of clay. Clay and colloidal materials increase the amount of water available to plants (Milford, 1997). Documented populations of Terlingua Creek Cat's-eye occur on site elevations from 955-1,045 m (Poole, 1994). Terlingua Creek Cat's-eye could be benefiting from its position on the soft hills and slopes where the plants are found. Guerrero-Campo et al. (1999) documented that the high infiltration capacity of gypsum soils leads to the existence of a reduced runoff of rainwater but a more intense ion washing.

Land use at the Terlingua Creek Cat's-eye population sites was historically used to graze cattle (Keller, 2005). Landowners are presently exploring sustainable harvest of desert plants and other ecologically based conservation projects (Brown, 2009) although cattle still frequent the areas where Terlingua Creek Cat's-eye are located. For Terlingua Creek Cat's-eye, its limited distribution is one of the factors that keep it endangered. All known populations are located on private land (Poole, 1994; Warnock 2009). As part of a conservation strategy for wild populations, strategies should include the owners of the resource. Owners can become interested in preventing the extinction of a rare endangered species when they have a vested interest in it either economically or personally (Meadows, 2004; Flores-Palacios and Valencia-Diaz, 2007). Terlingua Creek Cat's-eye habitat continues to be mined and developed for bentonite. At the same time, private property owners in Texas have collaborated to allow an assessment of previously unmapped or unknown populations, thus protecting the plants on a larger scale. They have built fences to protect this endangered species and purchased adjacent properties because Terlingua Creek Cat's-eye grows there. Without this level of commitment, the

chances of recovery for this species are considered low.

Acknowledgements.

We wish to thank Texas Parks and Wildlife for awarding the Section 6 grant and for making this research possible. We also wish to thank our collaborating landowners.

Literature Cited.

BARROW, J. R., M. E. LUCERO., I. REYES-VERA, AND K. M. HAVSTAD. 2008. Do symbiotic microbes have a role in plant evolution, performance and response to stress? Communicative & Integrative Biology 1:69-73.

BRISKE, D.D., AND B. J. CAMP. 1982. Water stress increases alkaloid concentrations in Threadleaf Groundsel (Senecio longilobus). Weed Science 30:106-108.

BROWN, C. D. 2009. Study of the ecology, harvest factors, and population data of Ocotillo (*Fouquieria splendens*) in the Trans-Pecos region of Texas. M.S. thesis, Sul Ross State University, Alpine, Texas.

CASPER, B. B., AND T.R. LA PINE. 1984. Changes in corolla color and other floral characteristics in *Cryptantha humilis*: (Boraginaceae): Cues to discourage pollinators? Evolution 38:128-141.

CASPER, B. B. 1985. Self-compatibility in distylous *Cryptantha Flava* (Boraginaceae). New Phytologist 99:149-154.

CRONQUIST, A. A., H. HOLMGREN, N.H. HOLMGREN, J. L. REVEAL, AND P. K. HOLMGREN. 1984. Intermountain flora-vascular plants of the Intermountain West, U.S.A. The New York Botanical Garden, Bronx, New York.

ELBROCH, M. 2003. Mammal tracks and sign. First edition. Stackpole Books, Mechanicsburg.

FLORES-PALACIOS, A., AND S. VALENCIA-DIAZ. 2007. Local illegal trade reveals unknown diversity and involves a high species richness of wild vascular epiphytes. Biological Conservation 136:372-387.

FU, P.P., X. QINSU, G. LIN, AND M. W. CHOU. 2004. Pyrrolizidine alkaloids—genotoxicity, metabolism enzymes, metabolic activation, and mechanisms. Drug Metabolism Reviews 36:1-55.

GEOLOGIC ATLAS OF TEXAS. 1979. Bureau of Economic Geology, Emory Peak-Presidio Sheet. The University of Texas, Austin, TX.

GUERRERO-CAMPO, J., F. ALBERTO, M. MAESTRO, J. HODGSON, AND G. MONTSERRAT-MARTI. 1999. Plant community patterns in a gypsum area of NE Spain

II. Effects of ion washing on topographic distribution of vegetation. Journal of Arid Environments 41:411-419.

HALLMARK, C. T., AND B. L. ALLEN. 1975. The distribution of Creosotebush in West Texas and Eastern New Mexico as affected by selected soil properties. Soil Science Society of America Proceedings 39:120-124.

HANSON, A. D., B. RATHINASABAPATHI, J. RIVOAL, M. BURNET, M. O. DILLON, AND D. A. GAGE. 1994. Osmoprotective compounds in the Plumbaginaceae: A natural experiment in metabolic engineering of stress tolerance. Proceedings of National Academy of Science. 91:306-310.

HIGGINS, L. C. 1971. A revision of Cryptantha subgenus Oreocarya. Brigham Young University Science Bulletin 13:1-63.

HOL, W. H. G., AND J. A. VAN VEEN. 2002. Pyrrolizidine alkaloids from *Senecio jacobaea* affect fungal growth. Journal of Chemical Ecology 28:1763-1772.

HUGHES, B. 1992. In manuscript. Cryptantha crassipes-raw data. Sul Ross State University Herbarium, Alpine, TX.

JOHNSTON, I. M. 1939. Studies in the Boraginaceae, XIII new or otherwise noteworthy species, chiefly from Western United States. Journal of the Arnold Arboretum 20:375-402.

JOOSTEN, L., P. P. J. MULDER, P. G. L. KLINKHAMER, AND J. A. VAN VEEN. 2009. Soil-borne microorganisms and soil-type affect pyrrolizidine alkaloids in *Jacobaea vulgaris*. Plant Soil 325:133-143.

KELLER, D.W. 2005. Below the Escondido Rim: A history of the O2 ranch in the Texas Big Bend. Center for Big Bend Studies, Alpine, Texas.

LUCERO, M. E., J. R. BARROW, P. OSUNA, AND I. REYES. 2006. Plant-fungal interactions in arid and semi-arid ecosystems: Large-scale impacts from microscale processes. Journal of Arid Environments 65:276-284.

MCLAUGHLIN, S. P. 1986. Floristic analysis of the Southwestern United States. The Great Basin Naturalist 46:47-63.

MEADOWS, R. 2004. Conservation incentives do work. Conservation in Practice. 5:5-11.

MEYER, S. E. 1986. The ecology of gypsophile endemism in the eastern Mojave desert. Ecology 67:1303-1313.

MEYER, S. E. AND E. GARCIA-MOYA. 1986. Gypsum grasslands of northern San Luis Postosi, Mexico: patterns of vegetation, soil, and seasonal moisture availability. Occasional Papers of the Chihuahuan Desert Research Institute, Alpine, Texas.

MILFORD, M. H. 1997. Soils and soil science. Fourth edition. Kendall/Hunt publishing Company, Dubuque.

MOON, C. G. 1957. Geology of Agua Fria quadrangle, Brewster County, Texas. Bulletin of the Geological Society of America 64:151-196.

NUMATA, I., F. F. SAMPAIO, F. C. LEONIDAS, J. V. SOARES, O. A. CHADWICK, D. A. ROBERTS, AND J. P. SCHIMEL. 2007. Temporal nutrient variation in soil and vegetation of post-forest pastures as a function of soil order, pasture age, and management, Rondonia, Brazil. Agriculture, ecosystems and environment 118:159-172.

POOLE, J. M. 1987. Status Report on Cryptantha crassipes. Texas Parks and Wildlife Department, Austin.

POOLE, J. M. 1994. Terlingua Creek Cat's–eye (*Cryptantha crassipes*) Recovery Plan. U.S. Fish and Wildlife Service, Austin, TX.

POOLE, J. M., W. R. CARR, D. M. PRICE, AND J. R. SINGHURST. 2007. Rare plants of Texas. Texas A & M University Press, College Station.

POWELL, A. M., AND B. L. TURNER. 1977. Aspects of the plant biology of the gypsum outcrops of the Chihuahuan Desert. Pages 315-325 in Transactions of the symposium on the biological resources of the Chihuahuan Desert region, United States and Mexico (R. H. Wauer and D. H. Riskind, editors). National Park Service Transactions and Proceedings Series, Number 3. U.S. Department of the Interior, Washington, D.C., USA.

REVEAL, J. L. 2006. *Cryptantha gypsophila* (Boraginaceae: Boraginoideae), a new species from western Colorado. Brittonia 58:178-181.

ROBERTS, M. 2005. Organic compatible solutes of halotolerant and halophilic microorganisms. Saline Systems 1:5.

ROTH, G. A., W. G. WHITFORD, AND Y. STEINBERGER. 2007. Jackrabbit (*Lepus californicus*) herbivory changes dominance in desertified Chihuahuan Desert ecosystems. Journal of Arid Environments 70:418-426.

SCHEMSKE, D.W., B. C. HUSBAND, M. H. RUCKELSHAUS, C. GOODWILLIE, I. M. PARKER, AND J. G. BISHOP. 1994. Evaluating approaches to the conservation of rare and endangered plants. Ecology 75:585-606.

STERMITZ, F. R., M. A. PASS, R. B. KELLEY, AND J. R. LIDDELL. 1993. Pyrrolizidine alkaloids from *Cryptantha* species. Phytochemistry 33:383-387.

UNGAR, I. A. 1974. Inland halophytes of the United States. Pages 235-253 in Ecology of halophytes (R.J. Reimold and W. H. Queen, editors). Academic Press Inc., New York.

WARNOCK, B. J. 2009. Population biology, habitat description and delineation and conservation of Terlingua Creek Cat's-eye (*Cryptantha crassipes*). Traditional section 6 project statement form. Texas Parks and Wildlife, Austin, TX.

WILLIAMS, M. T., B. J. WARNOCK, J. M. BETZ, J. J. BECK, D. R. GARDNER, S. T. LEE, R. J. MOLYNEUX, AND S. M. COLEGATE. 2011. Detection of high levels of pyrrolizidine-N-oxides in the endangered plant *Cryptantha crassipes* (Terlingua Creek Cat's-eye) Using HPLC-ESI-MS. Phytochemical Analysis. Article first published online: 23 MAR 2011 DOI: 10.1002/pca.1314.

YANCEY, P. H. 1994. Compatible and counteracting solutes. Pages 81-109 in Cellular and molecular physiology of cell volume regulation (S. K. Strange,editor). CRC Press, Boca Raton.

Significant Deviations: Our first research year (2010) yielded an abundance of blooms while the mapping and delineating of populations occurred. The objective to complete a pollination study the following year was not completed due to an inadequate number of blooming Terlingua Creek Cat's-eye plants in 2011. The field season this year was extremely dry (http://drought.unl.edu/dm) which appeared to limit the amount of blooming plants. Although we were able to verify heterostylistic Terlingua Creek Cat's-eye flowers, these were from the herbarium specimens (SRSU herbarium) and were not from our research populations. This is an important aspect of recovery for this endangered species as each floral type in heterostylistic flowers requires the pollen of the other floral type for fertilization to occur. Casper (1985) found that *Cryptantha flava*, a close relative of Terlingua Creek Cat's-eye to be capable of self-pollination. We do not know whether this is the case with

Terlingua Creek Cat's-eye. We documented floral visitor observations but not under controlled phases nor were we able to collect pollinators and examine their proboscis to identify pollen. We also noted that some of the Terlingua Creek Cat's-eye salverform tube corollas were white while others had the corolla with a yellow throat. The yellow faucal appendages on the mouth of the corolla may identify the opening for pollinators and Casper (1984) hypothesizes that the corolla turning white in another *Cryptantha* species signifies that the nectar source has ended. We have unresolved Terlingua Creek Cat's-eye pollination questions that require further analysis.

Progress Report on Isolation and Chemical Investigation of fungal strains from *Cryptantha crassipes*

(Based on work carried out by Dr. E. M. Kithsiri Wijeratne of A. A. Leslie Gunatilaka lab at the SW Center for Natural Products Research, University of Arizona)

Following studies were carried out with the infected samples of Terlingua Creek Cat's Eye (*Cryptantha crassipes*) procured by Maria Williams from Flaggy Limestone Hills of Terlingua Ranch, Brewster County, Texas (N 3269643.01/E 636519.82), on April 25, 2010.

Fungi from internal tissues and surface of plant parts were isolated using surface sterilized and non-surface sterilized samples of stems and leaves. Nine fungal strains were isolated and stored in 80% glycerol at -80 ^{o}C. Culturing of individual fungal strains, extraction of culture media and thin-layer chromatographic (TLC) analysis of resulting extracts suggested that these fungal strains are different from each other.

1. *Isolation of fungal strains from surface sterilized stems*

A piece of stem (ca 5 cm) of *Cryptantha crassipes* was surface sterilized with 70% aqueous EtOH and vortexed with sterile distilled water (10 mL) for 3 minutes. 1 mL of this suspension was placed onto a Petri dish (100 x 15 mm) containing a layer (5 mm) of Potato Dextrose Agar (PDA), excess suspension was removed by sterile pipette (after evenly spread on the PDA surface) and incubated at 28 ^{o}C. After 9 days three fungal colonies (based on their morphology, KW-155-79-1 to KW-155-79-3) were transferred to three Petri dishes (100 x 15 mm) containing a layer of PDA and incubated at 28 ^{o}C for one week.

2. *Isolation of fungal strains from surface sterilized leaves*

A piece of leaf of *Cryptantha crassipes* was surface sterilized with 70 % aqueous EtOH and vortexed with sterile distilled water (10 mL) for 3 minutes. 1 mL of this suspension was placed onto a Petri dish (100 x 15 mm) containing a layer (5 mm) of Potato Dextrose Agar (PDA), excess suspension was removed by sterile pipette (after evenly spread on the PDA surface) and incubated at 28 ^{o}C. After 9 days four fungal colonies (based on their morphology, KW-155-79-4 to KW-155-79-7) were transferred to four Petri dishes (100 x 15 mm) containing a layer of PDA and incubated at 28 ^{o}C for one week.

3. *Isolation of fungal strains from non surface sterile leaves*

A piece of leaf of *Cryptantha crassipes* was placed in a Petri dish (100 x 15 mm) containing a layer (5 mm) of Potato Dextrose Agar (PDA) and incubated at 28 ^{o}C. After 9 days five fungal colonies (based on their morphology, KW-155-79-8 to KW-155-79-12) were transferred to five Petri dishes (100 x 15 mm) containing a layer of PDA and incubated at 28 ^{o}C for one week.

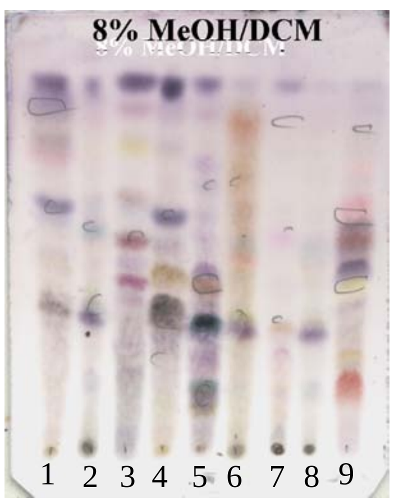
4. *Isolation of fungal strains from non surface sterile stems*

A piece of stem (ca 5 cm) of *Cryptantha crassipes* was placed in a Petri dish (100 x 15 mm) containing a layer (5 mm) of Potato Dextrose Agar (PDA) and incubated at 28 ^{o}C. After 9 days five fungal colonies (based on their morphology, KW-155-79-1 3 to KW-155-79-17) were transferred to five Petri dishes (100 x 15 mm) containing a layer of PDA and incubated at 28 ^{o}C for one week.

Fungal Culture, Extraction, and TLC Investigation

Out of 17 colonies transferred to Petri dishes, only 9 were found to be pure. Those were cultured in Potato Dextrose Broth (PDB, 1L) as described below. Seed cultures of the fungal strains grown on PDA for one week were used for inoculation. Mycelia were scraped out and vortexed with sterile distilled water (20 mL) and filtered through 100 μm filter to separate spores from the mycelia. Absorbance of the spore solutions were measured and adjusted to between 0.3 and 0.5. These spore solutions (5 mL) were used to inoculate 2 L Erlenmeyer flasks holding 1 L of the PDB medium and incubated at 160 rpm and 28 ^oC. Glucose level in the culture mediums were monitored using glucose strips (URISCAN glucose strip), and on day the, strip gave blue color for the glucose test, indicating the absence of glucose in the medium. Mycelia were separated from the culture broths by filtering through Whatman No.1 filter paper. The filtrates were neutralized and extracted separately with EtOAc (3 x 600 mL). Combined EtOAc layers from each fungal strain were washed with distilled water (3 x 500 mL) and evaporated separately under reduced pressure to give EtOAc extracts.

Fungal ID	pH of the filtrate	Wt. of EtOAc extract/mg	Chemical code
KW-155-79-13	2.66	21.0	KW-155-80-1
KW-155-79-9	6.25	17.0	KW-155-80-2
KW-155-79-5	8.05	119.0	KW-155-80-3
KW-155-79-16	2.04	8.7	KW-155-80-4
KW-155-79-4	7.43	30.9	KW-155-80-5
KW-155-79-11	5.29	56.7	KW-155-80-6
KW-155-79-7	6.85	60.7	KW-155-80-7
KW-155-79-2	5.84	13.7	KW-155-80-8
KW-155-79-3	6.43	146.3	KW-155-80-9



1. KW-155-80-1
2. KW-155-80-2
3. KW-155-80-3
4. KW-155-80-4
5. KW-155-80-5
6. KW-155-80-6
7. KW-155-80-7
8. KW-155-80-8
9. KW-155-80-9

TLC profiles of the EtOAc extracts

All isolates were stored in 80% glycerol at -80 ^oC.