

**INTERIM REPORT**

As Required by

**THE ENDANGERED SPECIES PROGRAM**

**TEXAS**

Grant No. TX E-110-R-2

Endangered and Threatened Species Conservation

**Reproductive Biology, Genetics and Ecology of South Texas Ambrosia: Implications for the  
Management, Recovery and Reintroduction**

Prepared by:

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Director, Wildlife

13 November 2012

## INTERIM REPORT

STATE: Texas GRANT NUMBER: TX E-110-R-2

GRANT TITLE: Reproductive Biology, Genetics and Ecology of South Texas Ambrosia: Implications for the Management, Recovery and Reintroduction

REPORTING PERIOD: 1 Sep 11 to 30 Sep 12

**OBJECTIVE(S).** To acquire the basic genetic, ecological, and reproductive data currently lacking on *Ambrosia cheiranthifolia* necessary to manage of extant populations scientifically and to write an evidence-based protocol for future reintroduction efforts.

### Segment Objectives:

#### Tasks:

**July 1, 2011-August 30, 2011**

- Visit accessible sites
- Collect samples for preliminary genetic study; extract DNA and start optimizing microsatellites

**September 1, 2011-August 30, 2012**

- Complete preliminary genetic studies
- Begin population monitoring, including physiological ecology studies
- Begin soils study
- Choose sites for management study
- Begin data analyses and manuscript preparation as appropriate

**September 1, 2012-August 30, 2013**

- Begin full genetic diversity study
- Continue population monitoring
- Complete soils study
- Continue data analyses and manuscript preparation as appropriate
- Begin management study

**September 1, 2013-June 30, 2014**

- Complete full genetic diversity study
- Complete population monitoring
- Complete management study
- Complete data analyses and manuscript preparation
- Complete Reintroduction Protocol

### Significant Deviations:

None.

### Summary Of Progress:

Please see Attachment A.

**Location:** Nueces and Kleberg Counties, Texas.

**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:** 13 November 2012

**Approved by:** \_\_\_\_\_



C. Craig Farquhar

**Date:** 13 November 2012

## **ATTACHMENT A**

### **Section 6 Interim Report**

#### **Reproductive Biology, Genetics and Ecology of South Texas Ambrosia: Implications for the Management, Recovery and Reintroduction**

**Principle Investigator: R. Deborah Overath, PhD**

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**Co-Principle Investigator: David J. Grisé, PhD**

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#### **Report Period**

**September 14, 2011-September 30, 2012**

**Report sent November 13, 2012**

## Summary of Progress

Based on the revised timeline (by Fiscal Year) in our proposal, we agreed to do the following in the first year or so of the grant.

July 1, 2011-August 30, 2011

- Visit accessible sites
- Collect samples for preliminary genetic study; extract DNA and start optimizing microsatellites

September 1, 2011-August 30, 2012

- Complete preliminary genetic studies
- Begin population monitoring, including physiological ecology studies
- Begin soils study
- Choose sites for management study
- Begin data analyses and manuscript preparation as appropriate

Note that this timeline assumed a July 1, 2011 start date for the grant, but in fact the grant started September 14, 2011 over two months later.

### 1. Visit accessible sites

Members of the Overath Lab visited accessible sites on The Naval Air Station-Kingsville (NAS-K) as part of the USFWS annual fall survey to learn their methods in November 2011. Collections for a study of *Ambrosia* contracted by the Navy were made at that time. Other known sites that were listed as "Extant" in Hempel's Interim Report of 2009, were visited in July 2012. We also visited several of the sites listed as "Extirpated" in the same report. While we found plants at the Cemetery and at the US77 on the west side of the South bound lane, we did not find plants on the east side of the north-bound lane, which were present in 2009. We also visited the demonstration site in Robstown and while there were still many plants in the demonstration site, we did not find any along the near-by roadside as report by Dr. Hempel in past years (pers. comm. Robyn Cobb, USFWS).

## **2. Collect samples for preliminary genetic studies**

Samples collected for a study of Ambrosia contracted by the Navy in Fall 2010 and Fall 2011 were used for early optimization work. Due to low variation in most of the markers at the Naval Air Station – Kingsville, we mapped and collected from a patch of Ambrosia at the St. James Cemetery near Bishop as a comparison with the permission of the parish priest, Fr. Kozol.

## **3. Complete preliminary genetic studies**

We are currently analyzing data. Early indications are that small patches are made up of one or a few clones. However, there are issues with the genetic data that make it difficult to place a lot of confidence in these results. For example, of the 13 microsatellite markers we optimized and tested, seven gave reliable results in trials on multiple individuals. However, three of these had no variation even when tested on over 100 samples and a fourth was not scorable in these larger trials. Of the other three, one had a very low amount of variation, and the remaining two exhibited signs of polyploidy (up to four “peaks,” indicating the presence of four alleles in an individual sample, in a fragment analysis), making good estimates of clone size and variation difficult. In particular, these latter two markers had to be scored in terms of the presence and absence of bands or peaks or, in other words, as dominant (present) and recessive (absent) data. Dominant markers have inherently less power to detect clones, etc. These difficulties likely stem from the fact that we needed to use markers developed for another species of Ambrosia. Within the last year or so, newer methods using “next-gen” sequencing approaches have become more affordable and possible with non-model species, such as South Texas Ambrosia. It may be time to try these methods to obtain species-specific markers for South Texas Ambrosia. PI Overath has contacts that may be useful in this regard.

## **4. Begin population monitoring, including physiological studies**

We began population monitoring at St. James Cemetery, but have not begun elsewhere. As part of the monitoring, we are also mapping patch locations using GIS (Figure 1) with the assistance of one of the graduate students in the Overath Lab who has extensive GIS experience.

## **5. Begin soils study**

We have not started this yet; however, we requested and received confirmation that we would not need additional permits to do this work.

## **6. Choose sites for management study**

Planning discussions with Environmental staff at NAS-K for areas that are regularly mowed vs. not mowed as well as other regular treatments that the Navy imposes, such as fire, on areas containing

Ambrosia. In addition, the St. James Cemetery site may also be suitable because Ambrosia occurs in some of the area currently being used for a study of brush clearing on slender rush pea, another endangered species, by scientists at Texas A&M University – Kingsville.

#### **7. Begin data analyses and manuscript preparations as appropriate**

Preliminary genetic data are currently being analyzed. Manuscript preparation is not yet appropriate.

### **Significant Deviations**

#### **1. Visit accessible sites**

No significant deviations.

#### **2. Collect samples for preliminary genetic studies**

We had planned to also collect samples along US 77 but had to wait for a state permit, which was recently granted. We will make these collections this fall.

#### **3. Complete preliminary genetic studies**

No significant deviations. Currently analyzing data from preliminary study, but plan to add US 77 collections for comparison with collection from St. James Cemetery.

#### **4. Begin population monitoring, including physiological studies**

Difficulties with genetic studies (see examples under #3 in the previous section) focused our attention there. We began some population monitoring at St. James Cemetery in July. Members of the Overath lab, including PI Overath, will be assisting USFWS staff with their annual monitoring this fall and then use the same methods at other sites. We also need to follow up on another potential site that has recently been reported in Kingsville and include that population in the monitoring plans (pers. comm. Robyn Cobb and Pat Clements, USFWS).

#### **5. Begin soils study**

Difficulties with genetic studies (see an example under #3 in the previous section) focused our attention there. We will begin soil collections this fall while collecting population data.

**6. Choose sites for management study**

Choices will be made soon.

**7. Begin data analyses and manuscript preparations as appropriate**

No other significant deviation except as noted above.

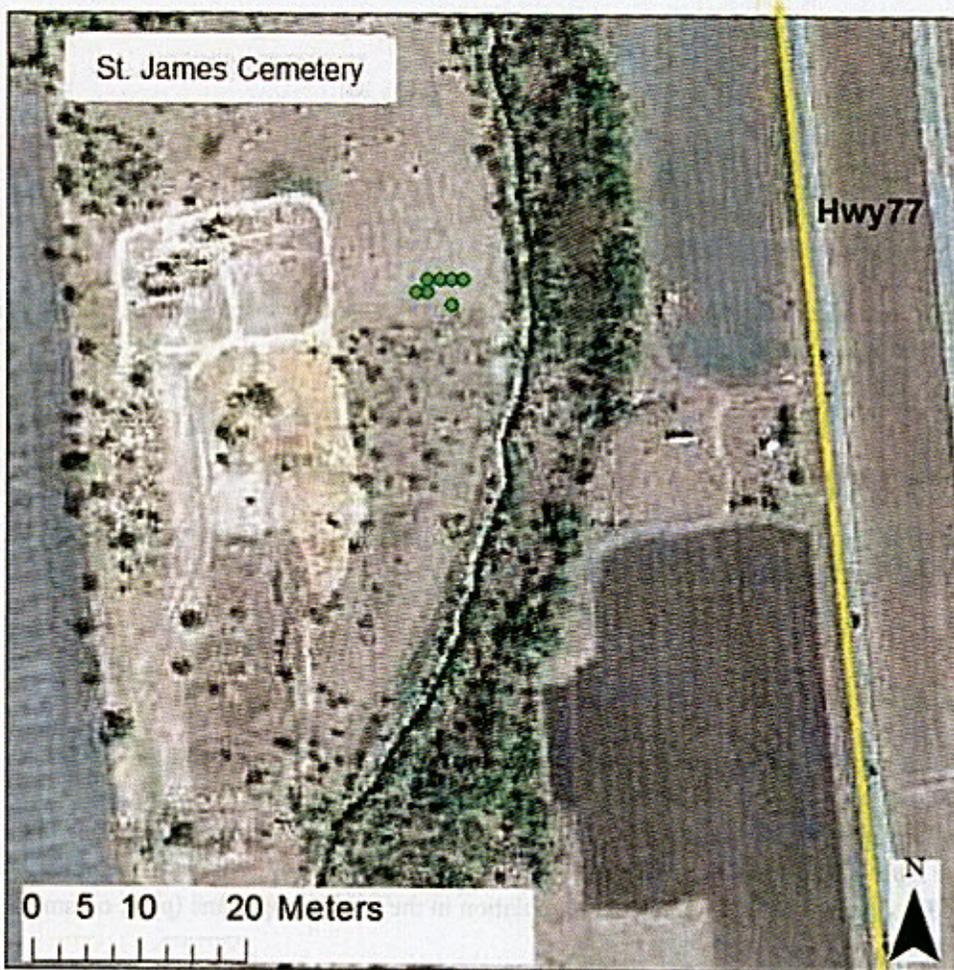


Figure 1. Map of South Texas Ambrosia patches at St. James Cemetery, Bishop, TX located in July 2012. Map courtesy of Alin Gonzalez.