Florida Statewide Plant Conservation Grant, Annual Report 2010-2011 FDACS Contract 016711

TITLE: Conservation of the critically endangered Florida Torreya (*Torreya taxifolia* Arn.)

PRINCIPLE INVESTIGATOR:

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PROBLEM AND NEED

Florida Torreya *(Torreya taxifolia* Arn.*)* is a critically endangered tree endemic to habitats along the Apalachicola River in Florida and extreme southwest Georgia. In the 1950s natural populations of *T. taxifolia* were all but extirpated as a result of an unidentified fungal pathogen (Schwartz *et al*. 1995, USFWS 1986). There are believed to be fewer than 1000 trees remaining in the wild. There is currently no new seedling recruitment in the wild. In more than 20 years since *T. taxifolia* was added to the endangered species list, conservation research has added important information to what we know about this species and its habitat, but many questions remain about the pathogen, specific habitat requirements, and biology of this species. As a flagship species for the Center for Plant Conservation (CPC) 2,622 stem cuttings were collected from 166 trees from 14 sites in the late 1980s early 90s. These cuttings were sent to 10 institutions for safeguarding. More information is needed about the safeguarded populations and populations of the plant in the wild to effectively conserve this species. The Atlanta Botanical Garden (ABG) is collaborating with the Florida Park Service, University of Florida, and Georgia Institute of Technology to survey safeguarding and natural populations, identify the potential foliar pathogens and develop micropropagation methods for *T. taxifolia*. Recent efforts have resulted in updated maps, revised inventory, new research on potential pathogens, and micropropagation protocols for Torreya.

Florida Torreya is the rarest conifer in North America and possibly one of the rarest in the world. However, *T. taxifolia* was not always this rare as significant populations were present less than a century ago. The damage taking place to this species is irreversible, occurring very rapidly and apparently intensifying in recent years (Schwartz et al. 2000). Damage from a combination of factors including pathogens, herbivory and climate are suspected to have led to not only catastrophic losses in population levels, but also have reduced genetic diversity, size of extant individuals and completely eliminated reproduction in the species. This project aims to address the recovery needs of *T. taxifolia* and increase its chances of survival in the wild.

OBJECTIVES

We are collaborating with the Florida Park Service, the University of Florida and Georgia Institute of Technology to address four overall goals for *Torreya taxifolia* : 1) determine location, size and health condition of current naturally occurring *T. taxifolia,* 2) estimate the level of genetic diversity in natural populations of Florida Torreya, 3) manage existing populations to create optimal growth conditions and provide adequate protection from herbivores, and 4) develop long-term storage techniques for *T. taxifolia.*

EXPECTED RESULTS AND BENEFITS

This project directly addresses the Recovery Plan for *Torreya taxifolia* listed in Part II. B. under Recovery Objectives (USFWS 1986):

1. Control Torreya decline
2. Produce seedlings and cuttings
3. Investigate the ecological requirements population dynamics and life history of Florida Torreya
4. Establish experimental collections of Torreya outside of its native habitat
5. Place seed in long term storage

Within the two year project time frame we expect to complete: 1) Documentation of the location, health condition, and size of naturally occurring *T. taxifolia* 2) Reports on the development of genetic markers and estimation of current levels of genetic diversity in existing populations of *T. taxifolia*, 3) Establishment of ecological experiments for testing optimal conditions for growth of *T. taxifolia* and adequate protection of extant populations from herbivory, drought, competition and edaphic influences within focal areas of Torreya State Park, 4) Documentation of techniques for long-term storage of *T. taxifolia* and tissue culture for safeguarding and maintaining germplasm *ex situ*.

APPROACH and ACTIVITIES

Job 1) Documentation of the location, health condition, and size of naturally occurring *T. taxifolia.*

For the reporting period Nov 1, 2010 to Oct 31, 2011, we have continued surveys for *T. taxifolia* across its native range. These surveys have focused on areas within Torreya State Park, but have also targeted The Nature Conservancy owned Bluffs and Ravines Reserve, Gohlson Park in Chattahoochee FL, the Army Corps of Engineers owned bluffs in Georgia at the Woodruff Seminole\Site Office and a variety of privately owned properties. Hand drawn maps were digitized and added to survey information collected from the late 1990s and early 2000s. Working from the maps of previous surveys, we searched bluffs and ravines where *T. taxifolia* was known to occur. The surveys were conducted by partners during five multiday field trips and by field technicians, Raya Pruner and Marvin Friel. Pruner and Friel also surveyed for *T. taxifolia* widely across its range as they also set up ecological experiments described under Job 3. When trees were located we recorded GPS location, tree height, basal diameter, presence of disease or deer damage, as well as environmental characteristics including slope aspect, canopy cover, depth of humus layer and associated species. Each tree also received a unique ID number and a tag.

During the recent surveys, 205 new trees were found. A total of 242 trees were visited, including 41 re-visits of trees that were found and tagged in previous years. Of these trees, 61 were caged to protect them from deer damage (see below).

Data collected during the most recent surveys was added to data collected since the project started in 2008. The following measurements were documented for trees in the naturally occurring *T. taxifolia* population based on 473 observations:

- Average stem length: 127.61 cm
- Average stem diameter of main stems: 1.90 cm
- 53.3% of trees had cambium rub by deer (252 trees)
- Of the trees that were rubbed by deer, the average percent of stem that was rubbed was 46.1%
- 60% of trees had deer browse
- Of the trees that were browsed an average 3.41% of each stem was browsed
- 83.2% of the trees had sign of canker disease present
- Average number of live stems per tree was 4.01
- Average dead stems per tree was 1.63
- 71.5% of the total number of stems surveyed were alive
- 25.5% of stems (sprouts) were dead

During these surveys approximately 244 trees were sampled for cuttings to be propagated at the Atlanta Botanical Garden for *ex situ* conservation. These activities will continue during the second year of the project.

Job 2) Development of genetic markers and documentation of current levels of genetic diversity in existing populations of *Torreya taxifolia* in Florida.

During March and April, 2011, more than 331 trees in the Atlanta Botanical Garden Torreya collection were sampled for population genetic analysis using allozymes. This work was conducted in collaboration with Dr. Jim Hamrick at the University of Georgia. The results of this part of the study are still being analyzed. They will provide information on genetic diversity in remaining populations in the wild and estimates of the level of diversity held in *ex situ* collections of *T. taxifolia* at the Atlanta Botanical Garden. The results will also be compared with the published allozyme study for Torreya taxifolia from the early 1990s (Schwartz, 1993).

Additionally work in Dr. Jason Smith's laboratory at the University of Florida tested amplification of microsatellites originally developed for the rare congener *Torreya jackii*, endemic to China (Li et al., 2007). This work and analysis of allozyme data will continue during the second year of the study.

Job 3) Establishment of optimal conditions for growth of *T. taxifolia* and adequate protection of extant populations from herbivory, drought, competition and edaphic influences within focal areas of Torreya State Park.

Deer rubs are causing significant cambial bark tissue damage to young trees. In addition, deer are browsing on young shoots from each branch thus further stressing trees. Trunk protectors or cages are recommended to prevent deer rubs and damage due to herbivory. Small exclosures, designed by John Bente of the Florida Park Service, that prevent deer from being able to browse or rub on seeding or sapling trees have been constructed around 61 trees in Torreya State Park. These exclosures are rechecked for performance and maintenance purposes.

A subset of 25 caged trees (5 treatments x 5 replicates) are used in ecological experiments to establish the best management conditions for remaining trees and provide minimum condition guidelines for successfully reintroducing trees into the park. The experimental treatments were set up during February-April 2011. The treatments included 1) Control (caged tree), 2) application of mulch and cage, 3) application of lime and cage, 4) experimental opening of canopy over the Torreya to allow increased morning sunlight and caging, and 5) a combination of all treatments. These treatments will be monitored during 2012 to measure the effect of ecological manipulation. An additional 100 trees will be located and caged to protect from herbivory during 2012.

Job 4) Documentation of techniques for long-term storage of *T. taxifolia* and tissue culture for safeguarding and maintaining germplasm *ex situ.*

Building on research begun in 2009, we have developed a somatic embryogenesis system for *T. taxifolia* that includes culture initiation, embryo growth, embryo germination and establishment in the soil, and methods to cryogenically store cultures. The process uses natural seeds from *T. taxifolia* to start embryogenic cultures. Initiation of embryogenic tissue occurred on a medium with salt formulation originally developed for Douglas fir and with 0.25% activated carbon (AC), maltose (41.6 mM), 2,4-D (0.5 mM) BAP (0.2 mM), Kinetin (0.2 mM), brassinolide (0.1 µM), ABA (3.8 µM), biotin (20.5 µM), folic acid (1.13 µM), MES (1.28 mM) and pyruvic acid (0.69 mM). Initiation from immature embryos ranged from 60% to 100% across six seed sources tested over two years, and the initiation medium was able to maintain all started cultures. Cotyledonary somatic embryo yields of approximately 20 per ml or g of embryogenic tissue developed on medium containing the same salts, maltose (41.6 mM), 1% AC, ABA (37.8 µM), biotin (20.5 µM), brassinolide (0.1 µM), folic acid (0.205 mM), MES (1.28 mM) and pyruvic acid (0.69 mM). Germination for two genotypes tested ranged from 64 to 82%. The measured water potential (-MPa) of *T. taxifolia* megagametophyte tissue rises greatly, in contrast to many other coniferous tree seeds, during seed after-ripening. Mimicry of this rise *in vitro* was necessary to continue development of somatic embryos to a cotyledonary stage.

As of October, 2011 twenty-five *T. taxifolia* embryogenic cultures, representing five mother trees have been placed into liquid nitrogen for long-term storage and confirmed to survive after retrieval from liquid nitrogen (Table 1).

Tree	Seed Year	Number of cultures showing survival after liquid nitrogen storage
19912211	2008	9
19912205	2009	6
19912214	2009	4
19912252	2009	5
19912286	2009	1
19912301	2009	0
Total		25

Tree	Seed Year	# Seeds	# Embryos	# Initiations[1]
1991-2208	2011	11	10	1
1991-2209	2011	2	2	2
1991-2239	2011	1	1	0 (still waiting)
1991-2173	2011	21	18	12
Unknown	2011	12	10	10

Culture (From Tree 1991-2211)	Seedlings with roots	Shoots without roots
D2	10	10

Table 1. *Torreya taxifolia* cryostorage survival summary as of October, 2011

An undergraduate student in Dr. Pullman's Project Lab Class (Georgia Tech BIOL 4590) is working to develop new embryogenic cultures from 2011 seed. New cultures, once grown to suitable amounts of tissue will be placed in cryogenic storage and tested for survival after culture retrieval. Forty-nine seed were collected from ABG safekeeping populations representing five mother trees. Seeds were cleaned, sterilized and dissected to remove the immature embryo.

Tree	Seed Year	Number of cultures showing survival after liquid nitrogen storage
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19912214	2009	4
19912252	2009	5
19912286	2009	1

Forty-three immature embryos were found and placed on embryogenic culture initiation medium (medium 2207).

Table 2. Early evaluations for embryogenic tissue initiation for 2011 *Torreya taxifolia* seed.

[1]Eight embryos from 1991-2208 are showing germination rather than initiation. These seeds have been placed on new media to test for initiation after the addition of hormones.

Torreya taxifolia somatic embryos were grown from four clones that were initiated from Tree 1991-2211 in 2008. These embryos were, germinated, and the resulting seedlings were sent to Dr. Jason Smith (University of Florida) for use in research studies on disease of *T. taxifolia* (Table 3).

Table 3. Material developed in tissue culture that was shared with Dr. Smith's lab for disease studies.

Tree	Seed Year	Number of cultures showing survival after liquid nitrogen storage
19912211	2008	9
19912205	2009	6
19912214	2009	4
19912252	2009	5
19912286	2009	1

GEOGRAPHIC LOCATION

Field work for this project has been conducted throughout the natural range of *T. taxifolia*, including the eastern drainages of the Apalachicola River from Bristol, FL to Chatahoochee, FL in Liberty and Gadsden Counties. Parcels include Torreya State Park, The Nature Conservancy Apalachicola Bluffs and Ravines Preserve, and privately owned lands in Township 1, 2 and 3 in Range 7 and Townships 2, 3 and 4 in Range 6.

Cuttings are safeguarded at the Atlanta Botanical Gardens, Atlanta, GA.

DNA research will take place at the Atlanta Botanical Garden, Atlanta, GA in collaboration with the University of Georgia, Athens, GA and University of Florida, School of Forest Resources and Conservation, Gainesville, Alachua County, FL.

Tissue culture and long term storage research will be conducted at the Atlanta Botanical Garden and Georgia Institute of Technology, Atlanta, GA.

RELATED FEDERAL PROJECTS

Title: Genetic diversity, status assessment and conservation of critically endangered *Torreya taxifolia*. Jennifer Cruse-Sanders, Atlanta Botanical Garden. Activities include:
1. Determine genetic diversity and reproductive biology of seedlings and trees in *ex situ* collections at the Atlanta Botanical Garden, 2. Determine location, health condition, and size of naturally occurring *Torreya taxifolia* in Georgia and the adjacent Florida population and provide adequate protection of extant populations (including safeguarding populations) in Georgia from herbivory, drought, competition and edaphic influences, 3. Ensure the long-term sustainability of *ex situ* collections of *T. taxifolia* for conservation and safeguarding of material. Provide a status survey of all safeguarded populations of *T. taxifolia*. ($9,250 funded with USFW Section 6 funds through the Georgia Department of Natural Resources, 10/1/2009-9/30/2011)

Title: Examining soil-bourne as casual agents of *Torreya taxifolia*'s decline. Vivian Negron-Ortiz, US Fish and Wildlife Service, Lydia Rivera Vargas, University of Puerto Rico Funding: US Fish and Wildlife Service Title: Identifying Primary Micorrhizal symbionts of *Torreya taxifolia*. Vivian Negron-Ortiz, US Fish and Wildlife Service, Melissa McCormick, Smithsonian Environmental Research Center Funding: Us Fish and Wildlife Service

OTHER RELATED PROJECTS

Tree	Seed Year	Number of cultures showing survival after storage
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Identifying pathogens involved in decline of *T. taxifolia* to determine etiology of disease. Work conducted with our collaborator at University of Florida has focused on identifying pathogens responsible for the decline in natural populations of Torreya. a.) *Pathogen survey methods* – Small samples of diseased tissues are used for isolation of pathogens on selective media and microbial molecular profiling using cloning of taxon specific DNA fragments. This research has resulted in the identification of a new species of Fusarium, Fsp1, as the stem canker causing agent in Torreya (Smith et al., 2010). b.) Disease etiology studies – Based upon completed Koch's postulates at the University of Florida with pathogens isolated from wild *T. taxifolia*, potential pathogens were tested with greenhouse-grown plants from ABG. These studies will allow for recommendations on control strategies to reduce future damage to *T. taxifolia*. An added benefit of this line of inquiry is that undergraduate and graduate students are trained in plant pathogen studies and molecular techniques, thereby creating a pipeline of trained scientists available to the workforce. (Funded through the University of Florida).

Safeguard and protect conservation collections of *T. taxifolia* – The Atlanta Botanical Garden is propagating material in the *ex situ* collection, documenting material and backing up the collection at the Garden and other locations. We are also monitoring safeguarded material at sites that have been established outside the natural range of Florida Torreya. A survey of the plants at Smithgall safeguarding population in Georgia in January 2009 showed that almost all of the plants in full sun are producing either male or female cones. This is potentially a source of novel genetic variation in the next generation of *T. taxifolia* that will be maintained in *ex situ* conservation collections. (Funded through ABG, and Section 6 funding through the Georgia Department of Natural Resources (see above))

COST SCHEDULE

We were granted $10,000 for the first year (FY2010-2011) to conduct the work outlined in this proposal. The funding provided during the first year was used to initiate the genetic work and conduct the tissue culture propagation of Torreya. The other activities were supported through in-kind support from the Florida Park Service, District 1 and Atlanta Botanical Garden (ABG).

*The Atlanta Botanical Garden and the Florida Park Service have provided more than the amounts listed in the table as match to the project activities. The amounts listed are all that were required for this funding period.

Budget for the second year of the project:

REFERENCES

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