Florida Ziziphus
(Ziziphus celata)

5-Year Review:
Summary and Evaluation

Drawing by Jean C. Putnam Hancock; flower photograph by Steve Shirah.

U.S. Fish and Wildlife Service
Southeast Region
South Florida Ecological Services Office
Vero Beach, Florida

5-YEAR REVIEW
Florida ziziphus/*Ziziphus celata*

I. GENERAL INFORMATION

A. Methodology used to complete the review: This review is based on monitoring reports, surveys, and other scientific and management information, augmented by conversations and comments from biologists familiar with the species. The review was conducted by a biologist with the South Florida Ecological Services Office. Literature and documents on file at the South Florida Ecological Services Office were used for this review. All recommendations resulting from this review are a result of thoroughly reviewing all available information on the Florida ziziphus. Public notice of this review was given in the Federal Register on April 16, 2008, with a 60-day public comment period. No part of the review was contracted to an outside party. See the Appendix for a summary of the peer review.

B. Reviewers

Lead Region: Southeast Regional Office, Nikki Lamp, 404-679-7091

Lead Field Office: David Bender, South Florida Ecological Services Office, 772-562-3909

C. Background

1. FR Notice citation announcing initiation of this review: April 16, 2008. 73 FR 20702.

2. Species status: Stable (2008 Recovery Data Call). Previously only one wild population was known to contain enough mating types to allow for sexual reproduction. In 2008, fruit set and maturation was documented in two additional wild populations (discovered in 2007). In addition to the resiliency conferred by larger numbers of known plants and genotypes, recovery efforts can benefit from the increased genetic diversity and possibly new mating types that will be available for introduction and augmentation projects. As a result, the threat posed by lack of sexual reproduction in wild populations and a limited number of mating types may be reduced. However, the only documented recruitment continues to be vegetative from root sprouting. Survival of transplants from three large-scale experimental introductions was 56 to 79 percent, but little growth has been observed and no transplants have reached maturity (Menges et al. 2008). Although two sites are managed with prescribed fire, fire suppression and habitat conversion are still occurring. Based on survey numbers from comparable populations over the past year and trends in threats, the species status was stable from October 1, 2007 through September 30, 2008.

3. Recovery achieved: 2 (25-50 percent of recovery objectives completed) (2008 Recovery Data Call).

4. Listing history
Original Listing
FR notice: 54 FR 31190
Date listed: July 27, 1989
Entity listed: Species
Classification: Endangered

5. Associated rulemakings: N/A

6. Review History: 5-year review, November 6, 1991 (56 FR 56882). In this review, different species were simultaneously evaluated with no species-specific in-depth assessment of the five factors or threats as they pertained to the species' recovery. The notices summarily listed these species and stated that no changes in the designation of these species were warranted at that time. No changes were proposed for the status of Florida ziziphus**.**
Final Recovery Plan: 1999
Recovery Data Call: 2000, 2001, 2002, 2003, 2004, 2005, 2006, 2007, 2008

7. Species' Recovery Priority Number at start of review (48 FR 43098): 5. A recovery priority number of 5 indicates a high degree of threat and low recovery potential.

8. Recovery Plan or Outline
Name of plan: South Florida Multi-Species Recovery Plan (MSRP)
Date issued: May 18, 1999
Dates of previous revisions: Recovery Plan for nineteen central Florida scrub and high pineland plants June 20, 1996 (revised plan). Recovery plan for eleven Florida scrub plant species January 29, 1990 (original plan).

II. REVIEW ANALYSIS

A. Application of the 1996 Distinct Population Segment (DPS) policy

1. Is the species under review listed as a DPS? No. The Endangered Species Act (ESA) defines species as including any subspecies of fish or wildlife or plants, and any distinct population segment of any species of vertebrate wildlife. This definition limits listing DPS to only vertebrate species of fish and wildlife. Because the species under review is a plant, the DPS policy is not applicable.

B. Recovery Criteria

1. Does the species have a final, approved recovery plan containing objective, measurable criteria? No. No recovery criteria are specified in the MSRP. The

stated recovery objective in the MSRP is to increase existing populations and prevent extinction (Service 1999).

C. Updated Information and Current Species Status

1. Biology and Habitat

a. Abundance, population trends (e.g., increasing, decreasing, stable), demographic features (e.g., age structure, sex ratio, family size, birth rate, age at mortality, mortality rate), or demographic trends: Florida ziziphus was believed to be extinct when it was described in 1984 from a 36 year old herbarium specimen. Between 1987 and 2007, 14 remnant populations were discovered. Nine populations were found between 1987 and 2002. After a 5-year lapse in new discoveries, five populations were found in 2007. All populations are on the Lake Wales Ridge (LWR) of central Florida in Polk and Highlands Counties. Florida ziziphus is perhaps the most well monitored and intensively studied imperiled plant species in Florida. Almost all of the 14 populations have been censused annually since their discovery. The small number, small size, and limited extent of populations has both created interest in the species and facilitated close study. Detailed demographic monitoring, involving tracking of marked plants and numbers of individuals (Level 3 monitoring *sensu* Menges and Gordon 1996) across multiple populations has occurred since 1996.

Florida ziziphus is a hermaphroditic shrub that spreads and reproduces vegetatively through the production of shoots from lateral roots. This presents a difficulty in discerning individual plants because some above-ground stems are connected underground, while others may have fragmented and are physically separate but genetically identical individuals (i.e., clones). Clonal dispersion across a site is the result of repeated episodes of dieback and resprouting, with new root shoots often appearing a meter or more from the plant that gave rise to it. At a few sites this has resulted in clonal populations that spread over hundreds of square meters (Weekley and Menges 2006).

The tendency to reproduce vegetatively is important to understanding the overall status of Florida ziziphus. Although populations may have numerous individual plants (ramets) from a physiological perspective, they contain only a single individual (genet) from a genetic perspective. Nearly all remaining wild populations consist of a single fragmented individual of the same genetic type (genotype). These single genotype populations are referred to as uniclonal (as opposed to multiclonal) in this review.

Florida ziziphus is species with gametophytic self-incompatibility (GSI), meaning plants are incapable of self-fertilization when pollinated with pollen from themselves or a plant with the same S-mating type. While each genet in a population has a different genotype, some may share the same S-allele, of

which there may be as few as three in Florida ziziphus. As a result, each of the nine populations composed of multiple ramets of a single genotype are effectively self-sterile; they cannot reproduce sexually. Compatible crosses that result in seed production are possible only in the populations that contain a minimum of two mating types, and five natural populations meet this requirement. Production of viable fruits has only been confirmed in two of the five multiclonal populations. Multiclonality does not guarantee cross-compatibility because plants belonging to different genotypes may belong to the same mating type.

Extant Populations

Florida Natural Areas Inventory (FNAI), the principle steward of natural heritage data for Florida, contains 10 Element Occurrence Records (EORs) for Florida ziziphus (FNAI 2008). In some cases EORs combine multiple sites and populations under a single record based on their close proximity. Populations are extant at each of the EORs currently in the database, and correlate with the sites discussed in this review (Table 1).

Ten of the fourteen extant populations are located on private land. The two largest and most genetically diverse populations were discovered in 2007 at the Masterpiece Pasture site. The pasture contains a residential development that split the population in two, resulting in 'north' and 'south' populations. Totaling 622 plants, together they represent 57 percent of all extant Florida ziziphus.

Florida ziziphus is poorly represented on public conservation lands in terms of number of wild plants (44), number of populations (4), and genetic diversity (6 genotypes). Two publicly protected sites contain remnants of wild populations. Two small populations (totaling 14 wild plants) are protected within the State-owned Lake Wales Ridge State Forest (LWRSF). Two more small populations (totaling 30 plants) are protected within the State-owned Lake Wales Ridge Wildlife and Environmental Area (LWRWEA). These four small populations, totaling 44 wild plants and six wild genotypes, represent the entirety of publicly protected wild plants and genetic diversity for the species (FNAI 2008, Weekley and Menges 2008).

Introduced populations of Florida ziziphus have been established at The Nature Conservancy's (TNC) Tiger Creek Preserve and the Carter Creek Unit of the Lake Wales Ridge National Wildlife Refuge (LWRNWR). Augmentation of wild populations has also been implemented at two sites (LWRSF Reedy Creek tract and Sullivan Pasture). The introduced and augmented projects utilized multiple genotypes propagated from seed harvested from the Bok Tower Gardens (BTG) *ex situ* collection (FNAI 2008, Weekley and Menges 2008).

Abundance

The current status of all known populations is thoroughly documented (Table 1). For the thirteenth consecutive year, a complete census of Florida ziziphus sites was performed in 2008 by botanists from Archbold Biological Station (ABS). Census numbers for Florida ziziphus are normally based on clusters of separate above-ground stems (ramets). For monitoring purposes, plants are defined as clumps of ramets that fall within a 25 centimeter (cm) radius, with each clump being a potentially physiologically independent plant (Ellis et al. 2007).

In the 2008 annual census, a total of 1,088 plants were counted in the 14 wild populations. The two introduced populations totaled 396 plants. Planted individuals at two augmented wild populations totaled approximately 60 plants (FNAI 2008, Weekley and Menges 2008).

Population Trends

It is too soon to determine trends for the populations discovered in 2007. This discussion will be limited to the populations known prior to 2007, most of which have been censused annually since their discovery. Trends for these populations are stable overall, with variable rates of clonal reproduction (new shoots) among populations and years. A pattern of dieback and re-growth has been observed in some populations. Shoot dieback and re-sprouting show little change year-to-year when averaged across all populations. For example, between the 2006 and 2007 census, 51 shoots died and 72 new shoots were recorded across five populations (Weekley and Menges 2008). Most populations have remained stable over the past decade. There are two notable exceptions. The Avon Pines population has experienced a significant decline from over 200 plants in 2000 to fewer than 50 in 2008. In contrast, the Friedlander Pasture population has experienced a significant increase from fewer than 50 plants in 1996 to more than 150 in 2008 (Weekley and Menges 2008). The factors driving these two exceptions are not well understood.

Trends for the introduced populations can be characterized in terms of percent survival since planting. Cumulative survival for transplants was 70.8 percent after 6 years at Carter Creek, and 64.7 percent after 3 years at Tiger Creek Preserve (Weekley and Menges 2008; C. Weekley, ABS, pers. comm. 2009). All of the plants in introduction and augmentation efforts are currently small, vegetative individuals. Until these plants mature and become active participants in pollen and ovule contribution to the reproductive pool it is not possible to completely evaluate the effectiveness of these efforts in achieving the goal of creating reproductively viable populations.

Demographic Features

The primary demographic features of Florida ziziphus are high annual survival rates, variable rates of clonal reproduction, and lack of recruitment from seed (Ellis et al. 2007, Weekley and Menges 2008). The factor with the greatest influence on the long-term viability of Florida ziziphus and that which has received the most research attention is the failure of sexual reproduction. All single genotype populations are self-sterile and incapable of producing seeds. Three of the wild populations with multiple genotypes have produced seeds, but no recruitment from seed has been observed at these sites (C. Weekley, pers. comm. 2008). While mature plants are observed to produce flowers in most years, only three *in situ* populations have been observed to produce fruits (an *ex situ* collection at BTG has produced fruits annually since 1994). Prior to the discovery of new populations in 2007, fruit set had only been observed in a single population (Avon Pines). The two Masterpiece Pasture populations (discovered in 2007) produced fruit in 2008. Most fruiting plants produced fewer than 100 fruits each, but six plants produced several hundred fruits each (Weekley and Menges 2008). This is the first time wild *in situ* plants have been observed to produce fruit at this magnitude. Even when fruits are produced, Florida ziziphus typically exhibits a low level of seed production. Germination trials demonstrate that 75 percent of fruits lack viable seeds. The production of seedless fruits and seed abortion apparently account for the low level of germination despite the production of fruits (Weekley et al. 2002).

Experimental introductions have provided insights into seedling ecology. All three introduction attempts have included a direct seeding component utilizing over 1,000 seeds per trial (Weekley and Menges 2008). *In situ* seed germination rate is low, for example the seed germination rate was 2.75 percent for the Tiger Creek 2007 introduction site (33 seedlings resulted from 1,200 seeds). At the LWRNWR Carter Creek introduction site seed germination rates were less than 5 percent (Weekley and Menges 2008). Of the seeds that germinated, survival was 32.4 percent after 3 years at Tiger Creek Preserve and less then 10 percent after 6 years at LWRNWR Carter Creek (Weekley and Menges 2008).

Taken together, these factors (self-sterile uniclonal populations, low seed set in multi-genotype populations, low germination rates, and high seedling mortality) explain in large part why recruitment from seed has not been observed in any wild populations. Recruitment from seed has been observed in the *ex situ* plantings at BTG, and as a result of experimental direct seed trials as described above.

Demographic Trends

Ellis et al. (2007) evaluated the role of clonal reproduction in the long-term population dynamics of Florida ziziphus using 9 years of data from two single genotype (and thus sterile) populations. The population viability analysis (PVA) model defined plants as clumps of ramets within a 25 cm radius. Seedling recruitment is not present in the uniclonal study populations and was not included in the model. Results of the PVA suggested that study populations would experience long-term population declines and up to 20 percent extinction risk within 50 years. The study found that the greatest influence on population growth rate was the production of new ramets. However, all study populations were predicted to experience protracted declines due to the lack of recruitment from seed. These results led Ellis et al. (2007) to recommend that augmenting populations and protecting new plants were important short-term objectives to offset the pattern of long-term decline, but the translocation of cross-compatible genotypes to augment single genotype populations is necessary for long-term persistence.

b. Genetics, genetic variation, or trends in genetic variation (e.g., loss of genetic variation, genetic drift, inbreeding): Florida ziziphus has exceedingly low genetic diversity (Godt et al. 1997, Weekley et al. 2002, Weekley et al. 2007). However, there have been considerable advances in the understanding of its genetics. Genetic analyses conducted at the Laboratory of Molecular Systematics and Evolutionary Genetics at the Florida Museum of Natural History used four microsatellite markers to identify genotypes and assess genetic variation in all pre-2007 *in situ*, introduced, and augmented populations, and plants in the Bok living collection. Based on the 4-loci microsatellite genotypes, their data support the hypothesis that Florida ziziphus was once more genetically diverse and has lost diversity as wild populations were fragmented, gene flow was halted, and sexual reproduction ceased (Weekley et al. 2007).

Several research efforts have focused on identification of the genotype(s) and mating type(s) present within populations. Godt et al. (1997) used allozyme studies and identified four populations each representing a single genotype, and a fifth population consisting of multiple genotypes. Weekley et al. (2002) used genetic markers to analyze the five populations studied by Godt et al. (1997), and confirmed that four of the five populations consisted of a single genotype and determined that the fifth consisted of four genotypes (Table 1). Microsatellite genotyping is currently underway for the populations discovered in 2007. One of the new populations on State land is small (5 plants) and uniclonal, but the other two each contain at least two genotypes (Weekley and Menges 2008). The two populations at Masterpiece pasture are far more numerous and spread out over a larger area than other populations. Genetic analysis is incomplete for these populations, but 21 genotypes have been identified to date (C. Weekley pers. comm. 2009). The fecundity

observed in these two populations in 2008 confirms that compatible mating types must be present. The five new populations together comprise at least 26 new genotypes, 60 percent of all known wild genotypes (Weekley and Menges 2008, C. Weekley pers. comm. 2009).

Further genetic studies are needed at a finer scale to better understand and document the identity of Florida ziziphus genotypes. Subsequent studies should utilize at least eight microsatellite loci to resolve uncertainties arising from genotyping efforts using four loci (C. Weekley pers. comm. 2009).

Studies have identified the genetic basis of the species' breeding system that is the primary factor in the failure of sexual reproduction. Florida ziziphus has a gametophytic self-incompatibility system (GSI) whereby plants sharing the same self-incompatibility alleles (S-alleles) are cross-incompatible. S-alleles are genes that prevent self-fertilization by controlling the growth of the pollen tube, thus causing male sterility and preventing inbreeding depression in monoecious plants (Allaby 1998). Thus, plants belonging to different genotypes may be cross-incompatible. To be cross-compatible, a flowering plant must have at least one S-allele not shared by its mate. Plants that share no S-alleles have complete cross-compatibility; plants that share one S-allele are semi-compatible (C. Weekley pers. comm. 2009). Based on breeding system experiments (Weekley and Race 2001, Weekley et al. 2002), all pre-2007 Florida ziziphus populations fall into two mating types and there may be as few as three S-locus alleles within wild populations, the minimum number a GSI species can have and still be capable of sexual reproduction. Populations of Florida ziziphus that have failed to reproduce sexually are of two types, clonal populations arising from fragmentation of a single genet over time, or multiple genotype populations in which all plants carry the same S-alleles. In either case, these populations are effectively sterile and doomed to eventual extirpation unless they are augmented with individuals from cross-compatible genotypes (Weller 1994, Weekley et al. 2002).

In plant species that have experienced genetic bottlenecks, been reduced to rarity, and persist as remnant isolated populations, the number of remaining S-alleles can become greatly reduced, as is apparently the case with Florida ziziphus. Based on hand pollination trials with plants of all known genotypes Weekley et al. (2002) assigned mating self-incompatibility (SI) types to each genotype and concluded that the species contained as few as three S-alleles and three compatible mating types. Of critical importance for the continued survival of Florida ziziphus is the fact that three S-alleles is the minimum number for successful reproduction in a species with GSI. Loss of any of these alleles would end sexual reproduction in the species (Weekley et al. 2007).

The Laboratory of Molecular Systematics and Evolutionary Genetics at the Florida Museum of Natural History used several state of the art techniques in

an attempt to identify the S-locus, but were unsuccessful (Weekley et al. 2007). A molecular technique for assaying the S-locus would be helpful to the design of translocation efforts for the recovery of Florida ziziphus. Assessment of seedlings would help ensure that mixtures of compatible mating types are adequately represented in introduction efforts. As populations mature and reproduce, this technique would allow for the ongoing monitoring of the genetic structure of populations. This is particularly important for Florida ziziphus because the maintenance of genetic diversity is the key to ensuring that populations are capable of sexual reproduction. Identifying the elusive S-locus remains a high priority for genetic management, which is a tool for recovery of the species. Until then mating types must be inferred through controlled pollination of genotypes to test for cross-compatibility, which is a very time-consuming process.

Inbreeding depression may be responsible for some limitations on sexual reproduction in Florida ziziphus, such as fruit abortion, deficiencies in pollen viability, and germination success (Weekley et al. 2008).

c. Taxonomic classification or changes in nomenclature: None at this time. The Integrated Taxonomic Information System (2008) was checked while conducting this review. The taxon *Ziziphus celata* Judd & Hall is accepted and current (ITIS 2009).

Judd and Hall (1984) placed *Ziziphus celata* within the Condaliopsis group of the genus *Ziziphus*. More recently, Islam and Simmons (2006) determined that Condialopsis is not a monophyletic group and recommended that the placement of *Z. celata* within the group be re-examined. Research is ongoing to clarify the taxonomic position within the Rhamnaceae. The Service will wait to make any determinations related to the taxonomy of the species until a taxonomic change is published.

d. Spatial distribution, trends in spatial distribution (e.g., increasingly fragmented, increased numbers of corridors), or historic range (e.g., corrections to the historical range, change in distribution of the species' within its historic range): The historical distribution, range, and abundance of Florida ziziphus populations are unknown (Weekley and Race 2001). The species may always have been rare. Field botanists such as J. K. Small (1869-1938) and R. M. Harper (1878-1966), both of whom worked on the LWR, did not report the species (Weekley and Race 2001).

Known populations range along a roughly north-south axis running from near Lake Wales in Polk County to near Sebring in Highlands County, an area about 50 kilometers (km) north to south by 20 km east to west. All populations occur on the LWR (Weekley and Menges 2006). Discovery of two small populations at Carter Creek LWRWEA extended the current range south by 9 km.

Habitat fragmentation has likely played a large role in the current abundance and distribution of Florida ziziphus. Large-scale destruction of Florida ziziphus habitat on the LWR began in the 1880s. The loss and fragmentation of habitat that has taken place over the last few decades has resulted in scattered remnant, genetically depauperate, and largely sterile populations persisting on degraded sites.

e. Habitat or ecosystem conditions (e.g., amount, distribution, and suitability of the habitat or ecosystem): A recent analysis of land conversion on the LWR suggests that about 78 percent of upland habitats were lost by about 1990 (Turner et al. 2006). By the early 2000s, this increased to about 87 percent of upland habitat lost (Weekley et al. 2008). The few hundred acres of remaining sandhill on the LWR are generally degraded due to a history of logging, fragmentation, and fire-suppression. All Florida ziziphus populations occupy yellow sand sites that historically supported longleaf pine (*Pinus palustris*) / wiregrass (*Aristida stricta* var. *beyrichiana*) sandhills or oak (*Quercus myrtifolia)* / hickory (*Carya floridana*) scrub, but most have been converted to pastures or other uses. Citrus growers favored yellow sands and many sites potentially supporting Florida ziziphus populations were converted to citrus production in the early decades of the 20th century (Weekley and Menges 2006).

Scrub and sandhill differ in community structure, species composition, and natural fire return interval. Oak-hickory scrub is a shrubland system typically dominated by oaks, while sandhill is a savanna-like system with an open canopy, relatively few shrubs and an extensive ground cover of grasses and herbs. The natural fire return interval in oak-hickory scrub is on the order of 6-12 years, while in sandhill more frequent fire (2 to 8 years) is favored by grasses and other fine fuels (Weekley and Menges 2006).

Multiple lines of evidence suggest that sandhill was the historically preferred habitat of Florida ziziphus. Ten of the 14 natural populations occur on sites that support degraded or former sandhill habitat. Weekley and Menges (2006) observed that Florida ziziphus populations occurring in full sun are robust and dynamic, whereas the single extant population beneath a closed canopy appears stressed and has stems that appear to be spindly as they stretch for light and have sparse foliage. They noted that the episodic dieback-and-resprouting that is characteristic of Florida ziziphus also suggests a species adapted to sites with an open canopy (Weekley and Menges 2006). Plants at LWRSF were heavily shaded, covered in lichens, and appeared stressed in 1995, yet resprouted quickly after a prescribed fire. However, no subsequent growth has been observed and the plants have yet to flower (C. Weekley, pers. comm. 2009). Plants at the more open sandhill site and open pasture sites grow vigorously in full sun or light canopy. This combination of a need for open canopy and quick regeneration suggests that this species is adapted to the

frequent fire regime that historically maintained the sandhill ecosystem (Service 1999).

Habitat management is ongoing at all publicly protected sites. State, Federal, and private landowners apply prescribed fire and mechanical treatments to restore and maintain fire-dependent plant communities. However, many land managers on the LWR are behind schedule with their prescribed fire programs. Since 1996, prescribed fire has been applied three times at the LWRSF and the Mountain Lake sandhill sites. Fire was also used to prepare sites at LWRNWR Carter Creek and at Tiger Creek Preserve prior to the introductions at those sites. The Service conducted a second prescribed fire at Carter Creek in 2007. Occasionally, ABS has conducted small-scale burns at a few private pasture sites as part of the effort to control weeds and regenerate plants undergoing diebacks. Management of these populations would not be possible without the cooperation of private landowners (Weekley and Menges 2006).

f. Other:

Fire Ecology

The fire ecology of Florida ziziphus is not fully understood. However, fire may be important for this species. Research by ABS is ongoing at the LWRNWR Carter Creek where an introduced population has been included in a prescribed burn. Numerous small-scale burns have been conducted on pasture populations. Florida ziziphus can survive and regenerate after fire by resprouting. Ongoing monitoring of burned populations aims to provide insight into the post-fire survival and growth of Florida ziziphus (Weekley and Menges 2006).

Pollinators

The fragrant nectar producing flowers of Florida ziziphus attract insect visitors of at least three orders (Diptera, Hymenoptera, Lepidoptera). Potential pollinators include flower flies (Serphidae), honey bees (*Apis mellifera*) and butterflies (Weekley and Menges 2006).

Ad Hoc Recovery Team

Established in 1997, the Florida Ziziphus Ad Hoc Recovery Team meets every 2 to 3 years to review the current management status, research findings, threats, and any other pertinent issues. A broad spectrum of Federal, State, and non-governmental partners is usually represented at the meetings. The team coordinates yearly searches for new populations, discusses monitoring and management of populations, plans reintroduction projects, and identifies

research priorities. ABS also maintains a website that provides information about Florida ziziphus.

Ex situ conservation

BTG is the major *ex situ* repository for genetic diversity of Florida ziziphus. As part of the Center for Plant Conservation National Collection of Endangered Species, Bok maintains a living collection of plants representing clones of all pre-2007 genotypes. Work is currently underway to propagate clones of all genotypes in populations discovered in 2007. Seeds and clones from the *ex situ* plants at BTG have provided propagules for all introduction and augmentation efforts. BTG is developing a collection in suitable sandhill habitat within their Pine Ridge Preserve site that will eventually represent all genotypes. BTG is also developing protocols for seed storage and they maintain a store of wild seeds and thousands of seeds generated from *ex situ* crosses at BTG. Seeds from a proportion of each harvest are placed in short-, mid-, and long-term storage. BTG also provides seeds to the National Seed Storage Laboratory in Fort Collins, Colorado for long-term storage in liquid nitrogen.

2. Five-Factor Analysis

a. Present or threatened destruction, modification or curtailment of its habitat or range: Continued conversion of Florida scrub and sandhill to agriculture, housing, and other developments is undoubtedly affecting the habitat of Florida ziziphus. The small area of remaining sandhill habitat on the LWR limits the prospects for protection of additional habitat. Although there has been considerable progress in acquiring habitat on the LWR for other listed species, only two publicly protected sites support wild populations of Florida ziziphus. These populations are small and non-reproductive due to lack of mating type diversity. The largest and most genetically diverse populations are located on private land at sites that have been converted to pasture. While some of these populations are temporarily safe from imminent destruction due to cooperative landowners, they have no legal protection from destruction by the landowner and a transfer of ownership could result in a loss of the populations. For example, the Masterpiece pasture site has been on the market since 2007. A change in ownership of this parcel could result in loss of a population considered critical to the recovery of the species. The Masterpiece pasture site also illustrates ongoing habitat loss and fragmentation. The north and south populations constituted a single large population prior to construction of a housing development. The development in all probability resulted in the destruction of plants and habitat, and resulted in a fragmented population. The breeding system of Florida ziziphus amplifies the consequences of habitat fragmentation because the remaining populations are isolated from others that contain compatible mates.

Four of the seven pasture populations have been fenced to prevent damage by cattle. The degree to which the three unfenced populations are impacted by cattle is not known. Two populations are located at an overgrown sandhill site on private property where they are surrounded by cherry laurel (*Prunus caroliniana*) and non-native camphor (*Cinnamomum camphora*) trees. Part of the area is used as a yard waste dumpsite. This site has no protection and ATV use is a concern (Weekley and Menges 2006).

Land acquisition to date has placed nearly half (87.4 square kilometers [sq km] or 48.9 percent) of the remaining 178.7 sq km of scrub and sandhill habitat on the LWR within protected areas (Turner et al. 2006). A recent analysis of Florida scrub conservation progress based on land acquisition included Florida ziziphus among the 36 rare species of the LWR. Turner et al. (2006) calculated protection indices for each species and for three time periods (past, present, future) based on number of locations, extent of population, and area of occupancy. The overall protection index of less than 2 identified Florida ziziphus as a species of high conservation concern. In addition, the analysis identified Florida ziziphus as one of at least eight LWR species in which translocation and/or captive propagation may be necessary to ensure their survival due to inadequate representation on conservation lands (Turner et al. 2006).

Increasing pressure from population growth will likely result in further loss of LWR habitats. Zwick and Carr (2006) analyzed existing land use and landscape patterns to identify the areas most likely to be developed to accommodate a growing human population (e.g., not a wetland, near major roads, near other development, on the coast thus desirable) and estimated relative losses to agriculture, open space, and conservation to other land uses. They predicted central Florida will experience "explosive" growth, with continuous urban development from Ocala to Sebring, the area encompassing the entire range of Florida ziziphus. They estimated 2.7 million acres of native habitat and 630,000 acres of land currently under consideration for conservation purchase will be lost. Also of significance, they state that "more than 2,000,000 acres within 1 mile of existing conservation lands will be converted to an urban use, complicating management and isolating some conservation holdings in a sea of urbanization" (Zwick and Carr 2006).

The discovery of five populations as recently as 2007 suggests the possibility of additional undiscovered populations. Considering the rapid pace of development in central Florida, it is possible that unknown populations will be destroyed before they are discovered. Florida ziziphus continues to be threatened by habitat loss, modification, and fragmentation.

b. Overutilization for commercial, recreational, scientific, or educational purposes: Overutilization for commercial, recreational, scientific, or

educational purposes was not identified as a potential threat in the original listing package. Since listing, no evidence of overutilization has been found.

c. Disease or predation: Disease has not been observed as a limiting factor in Florida ziziphus. Based on seedlings that resulted from introduction efforts, there is limited evidence to suggest that herbivory can impact seedlings. In one study, browsed seedlings did re-sprout once their protective cages were replaced (Weekley and Menges 2008). Some evidence of herbivory has also been observed on mature plants (Service 1999). There is insufficient data to evaluate predation by herbivores.

d. Inadequacy of existing regulatory mechanisms: Florida ziziphus is listed as endangered by the State of Florida on the Regulated Plant Index (Florida Department of Agriculture and Consumer Services Rule 5B-40). This law regulates the taking, transport, and sale of listed plants. The law does not prohibit private property owners from destroying listed plants nor does it require them to manage habitats to maintain populations.

Existing Federal and State regulations prohibit the removal or destruction of listed plant species on public lands. However, they afford no protection to listed plants on private lands. Under section 7 of the ESA Florida ziziphus is afforded some protection in instances where it occurs on Federal lands or where there is Federal nexus. In addition, State regulations are less stringent than Federal regulations on land management practices that may adversely affect populations of listed plants. Therefore, existing regulatory mechanisms are inadequate to protect this species.

e. Other natural or manmade factors affecting its continued existence:

Fire suppression or lack of adequate fire regime

Fire suppression is a continuing threat to the habitat of Florida ziziphus. In studies of Florida ziziphus introduced to a sandhill site at LWRNWR Carter Creek, Menges et al. (2008) found that resprouting oaks encroached with negative effects on plant survival. The dominance of oaks increases under fire suppressed conditions. Weekley and Menges (2006) suggested a fire return interval of 2 to 8 years for sandhill habitat on the LWR to reduce cover of oaks and produce an open understory. Private property owners are unlikely to apply prescribed fire at this frequency. Fire suppressed conditions will likely continue on these sites, to the detriment of potential Florida ziziphus habitat. Prescribed fire implementation on most public lands is behind schedule due to insufficient resources coupled with logistical obstacles and tentative public support. Smoke, public safety, and property liability issues, along with public perceptions relating to aesthetics still pose obstacles to implementing prescribed fire. Populations on unmanaged private land or inadequately managed public lands will likely decline for this reason.

Invasive plants

Non-native invasive plant species are a threat to Florida ziziphus at sites converted to pasture. At these sites the dominant groundcover of pasture grasses may be a factor in the lack of recruitment from seeds in the few reproductive populations, but this has not been investigated. In addition, non-native vines have grown up and over Florida ziziphus at a few sites. These vines compete for sunlight, space, water, and nutrients and may cause a decrease in the vigor of Florida ziziphus. Invasive plants are managed at the protected sites and are not a significant threat to Florida ziziphus at these sites.

Paucity of compatible mating types

The consequences of fragmented and reduced populations are a threat to Florida ziziphus. The breeding system alone would not be a detriment to its continued existence, but coupled with the reduction, fragmentation, and isolation of the remaining populations, it is perhaps the most significant threat. The destruction of unprotected populations on private land could result in the loss of genotypes critical to conservation and recovery of the species. In particular, if the number of remaining available S-allele types drops below three, sexual reproduction will become impossible and there will be no chance for recovery of the species in the wild.

D. Synthesis. Florida ziziphus populations exist in remnants in a few scattered and mostly degraded sites across a limited geographic area. Half of the populations are found in areas converted to pasture. The number of populations is few and their size is limited in number of plants and the area they occupy. Four populations, totaling 44 plants and six genotypes, represent the entirety of publicly protected wild plants and genetic diversity. Ten of the 14 natural populations are on private land and their protected status is tentative and dependent on landowner cooperation. Most of the habitat for Florida ziziphus has been converted to citrus groves, pastures, and housing developments. The small area of remaining sandhill habitat on the LWR limits the prospects for protection of large areas of additional habitat. Remaining habitat has been degraded by decades of fire suppression and more recently by invasion of non-native plant species.

In modeling the population dynamics of Florida ziziphus, extinction probability was as high as 20 percent in 50 years for one of two study populations (Ellis et al. 2007). Ellis et al. (2007) predicted that populations that fail to reproduce sexually would experience protracted declines without efforts to promote sexual reproduction. The self-incompatible breeding system of Florida ziziphus exacerbates the threat of extinction already posed by small population size and limited number of populations and complicates recovery efforts. The historic destruction and fragmentation of habitat has resulted in genetically depauperate populations that are incapable of sexual reproduction. Only 3 of 14 populations have been observed to set fruit; other populations persist only through vegetative reproduction. None of the four natural populations protected on public land have been observed to set fruit.

Restoring the long-term viability of the non-reproductive populations may be dependent on genetic material present on the unprotected private sites. The protected populations are isolated from compatible mates; human-assisted translocation of cross-compatible genotypes is necessary to make these populations viable. Recovery will require augmentation of existing populations with compatible genotypes and the establishment of new populations in protected areas of suitable habitat. The horticultural and genetic dimensions of these efforts have been the subject of extensive research and many of the elements needed to design reproductively viable populations are now understood. Introductions and augmentations have been carried out at four sites, but the success of these efforts will not be known until the plants reach maturity.

Threats from destruction and degradation of habitat are continuing, and existing regulatory mechanisms are inadequate to protect the species. Due to the small number of populations and individuals overall, small number of populations on protected lands, the lack of sexual reproduction in most populations, and an uncertainty as to the effectiveness of recovery efforts, the species continues to meet the definition of endangered under the ESA.

III. RESULTS

A. Recommended Classification:

__X__ **No change is needed**

IV. RECOMMENDATIONS FOR FUTURE ACTIONS

- Continue searches for additional populations of Florida ziziphus in areas of current or former suitable habitat.
- Continue rescue of genetic material from unprotected sites for banking at BTG and use in ongoing augmentation and (re)introduction efforts.
- Acquire land with existing populations from willing sellers and restore it to sandhill habitat.
- Continue genotyping recently discovered populations and assign mating types when possible. Subsequent genetic analyses should utilize more genetic markers (eight as opposed to four) to improve results.
- Continue research to identify the S-locus to facilitate design of translocation projects.
- Design and implement additional projects to establish reproductively viable populations on protected sites of suitable habitat within the species' historic range.
- Advocate for the application of prescribed fire to restore sandhill habitat at sites with populations of Florida ziziphus.
- Continue annual demographic monitoring of wild and translocated populations.
- Develop recovery criteria for Florida ziziphus based on current understanding of the species' biology and status.

Table 1. Summary of all Florida ziziphus populations (Data from FNAI 2008; Weekley and Menges 2006, 2008).

FNAI EOR Number	ABS Designation	Location / Site Name	Population Type	Owner	Wild Genotypes	Year Discovered / Introduced / (Augmented)	2008 Census Total
2	H01-1	Avon Pines Pasture - 1	wild	private	1	1988	159
2	H01-2	Avon Pines Pasture - 2	wild	private	1	1988	
2	H01-3	Avon Pines Pasture - 3	wild	private	1	1988	
2	H01-4	Avon Pines Pasture - 4	wild	private	4	2001	
6	H02-1	LWRWEA / Carter Creek North / West	wild	State	2	2007	23
7	H02-2	LWRWEA / Carter Creek North / East	wild	State	2	2007	7
1	P01-1	LWRSF / Reedy Creek 11	wild (aug)	State	1 (multiple)	1987 (1998)	9 (28)
9	P01-2	LWRSF/ Arbuckle Tract	wild	State	1	2007	5
3	P02	Sullivan Pasture	wild (aug)	private	1 (multiple)	1988 (2003)	1 (27)
5	P03	Mountain Lake, Lake Wales	wild	private	1	1995	19
4	P04	Friedlander Road	wild	private	1	1995	166
5	P05	Mountain Lake, Lake Wales	wild	private	1	2001	11
10	P06	Masterpiece Road South	wild	private	* 16+	2007	384
10	P07/ AC	Masterpiece Road North	wild	private	* 3-4	2007	238
6	CCS02	LWRNWR Carter Creek South	intro	Federal	multiple	2002	82
8	TCP05	TNC Tiger Creek Preserve	intro	private	multiple	2005	194
8	TCP07	TNC Tiger Creek Preserve	intro	private	multiple	2007	110

( ) entries in parentheses refer to augmentation efforts
* genetic typing incomplete at time of this review
LWRWEA = Lake Wales Ridge Wildlife and Environmental Area
LWRSF = Lake Wales Ridge State Forest
LWRNWR = Lake Wales Ridge National Wildlife Refuge
TNC = The Nature Conservancy
ABS Designation = Archbold Biological Station Plant Ecology Lab's name for the occurrence
FNAI EOR = Florida Natural Areas Inventory Element Occurrence Record

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U.S. FISH AND WILDLIFE SERVICE
5-YEAR REVIEW of Florida ziziphus (*Ziziphus celata*)

Current Classification Endangered

Recommendation resulting from the 5-Year Review

____ **Downlist to Threatened**
____ **Uplist to Endangered**
____ **Delist**
_X_ **No change is needed**

Appropriate Listing/Reclassification Priority Number, if applicable ____

Review Conducted By David Bender, Botanist

FIELD OFFICE APPROVAL:

for Lead Field Supervisor, Fish and Wildlife Service

Approve _____ Date 6/3/09

The lead Field Office must ensure that other offices within the range of the species have been provided adequate opportunity to review and comment prior to the review's completion. The lead field office should document this coordination in the agency record.

REGIONAL OFFICE APPROVAL:

The Regional Director or the Assistant Regional Director, if authority has been delegated to the Assistant Regional Director, must sign all 5-year reviews.

Lead Regional Director, Fish and Wildlife Service

for Approve _____ Date 8/27/09

Appendix. Summary of peer review for the 5-year review of Florida ziziphus (*Ziziphus celata*)

A. Peer Review Method: The Service conducted a peer review for this species. Three peer reviewers were selected by the Service. Individual responses were requested and received from each of the peer reviewers.

B. Peer Review Charge: See attached guidance.

C. Summary of Peer Review Comments/Report: The reviewers found the 5-year review to be thorough and all agreed with the biological conclusions of the review. One peer reviewer was concerned that there are no recovery criteria established for the species. The reviewer stated that recovery criteria should be delineated in this 5-year status review. A second reviewer provided a source for information on the number of genotypes that have been catalogued. A third reviewer supported the conclusion that low genetic diversity and the self-incompatible breeding system was the primary threat to Florida ziziphus. The same reviewer stated that acquisition of the populations on private land should be a high priority.

D. Response to Peer Review: The Service agrees that recovery criteria can now be delineated for Florida ziziphus. However, delineation of recovery criteria is outside the scope of the 5-year review. The need to establish recovery criteria is included in the 'Recommendations for Future Actions' section of the review. The additional information on genotypes was already included in the review therefore no change was needed. The Service agrees that the acquisition of private sites would be helpful to the recovery of the species. A recommendation to acquire these parcels if available from willing sellers is included in the 'Recommendations for Future Actions' section of the review. Until such time, the Service believes that collection of seeds and cuttings and continued development of an index collection of all genotypes is an important backup strategy to protect against further losses of genetic diversity in the species.

Guidance for Peer Reviewers of Five-Year Status Reviews

U.S. Fish and Wildlife Service, South Florida Ecological Services Office

February 20, 2007

As a peer reviewer, you are asked to adhere to the following guidance to ensure your review complies with U.S. Fish and Wildlife Service (Service) policy.

Peer reviewers should:

1. Review all materials provided by the Service.

2. Identify, review, and provide other relevant data apparently not used by the Service.

3. Not provide recommendations on the Endangered Species Act classification (e.g., endangered, threatened) of the species.

4. Provide written comments on:
   - Validity of any models, data, or analyses used or relied on in the review.
   - Adequacy of the data (e.g., are the data sufficient to support the biological conclusions reached). If data are inadequate, identify additional data or studies that are needed to adequately justify biological conclusions.
   - Oversights, omissions, and inconsistencies.
   - Reasonableness of judgments made from the scientific evidence.
   - Scientific uncertainties by ensuring that they are clearly identified and characterized, and that potential implications of uncertainties for the technical conclusions drawn are clear.
   - Strengths and limitation of the overall product.

5. Keep in mind the requirement that the Service must use the best available scientific data in determining the species' status. This does not mean the Service must have statistically significant data on population trends or data from all known populations.

All peer reviews and comments will be public documents and portions may be incorporated verbatim into the Service's final decision document with appropriate credit given to the author of the review.

Questions regarding this guidance, the peer review process, or other aspects of the Service's recovery planning process should be referred to Paula Halupa, Acting Endangered Species Supervisor, South Florida Ecological Services Office, at 772-562-3909, extension 257, email: Paula_Halupa@fws.gov.