5-YEAR REVIEW
Large-fruited sand-verbena (*Abronia macrocarpa*)

1.0 GENERAL INFORMATION

1.1 Listing History

Species: Large-fruited sand-verbena (*Abronia macrocarpa*)
Date listed: September 28, 1988
Federal Register (FR) citation(s): 53 Federal Register (FR) 37975
Classification: Endangered without Critical Habitat.
Critical habitat/4(d) rule/Experimental population designation/Similarity of appearance listing: Not applicable.

1.2 Methodology used to complete the review:

This review considers both new and previously existing information from Federal and State agencies, non-governmental organizations, academia, and the general public. Information used in the preparation of this review includes the information from the previous U.S. Fish and Wildlife Services (Service) November 2010 5-year review, Texas Parks and Wildlife Department (TPWD) Natural Diversity Database (TXNDD), final reports of Traditional Section 6-funded projects, monitoring reports, scientific publications, unpublished documents, personal communications from botanists familiar with the species, consultation records under Section 7 of the Endangered Species Act (Act), and Internet websites. The 5-year review was prepared by personnel of the Service's Texas Coastal Ecological Services Field Office (TCESFO) – Clear Lake, without peer review.

The Service most recently evaluated the biology and status of the large-fruited sand-verbena (LFSV) as part of a status review, conducted on November 19, 2010. We examined if any new information was available and whether that information would alter or affect analyses and conclusions made in the previous 5-year review. Data for this current review were solicited from interested parties through a FR notice announcing the review on February 2, 2022 (87 FR 5834-5838). We also contacted botanist Anna Strong (TPWD); species expert, Dr. Paula Williamson (Texas Status University); Dr. Eric LoPresti (currently at University of South Carolina, research presented in this review was done at Oklahoma State University); curator, Anita Tiller (Mercer Botanic Gardens and Arboretum (Mercer)); and Minnette Marr (Ladybird Johnson Wildflower Center (LBJWC)) to request any data or information we should consider in our review. Additionally, we conducted a literature search and a review of information in our files.

The FR Notice citation announcing the species is under active review:

U.S. Fish and Wildlife Service. 2022. Notice of initiation of reviews; request for information. 87 FR 22, 5834-5838.

1.3 Background

The LFSV was first collected in Leon County, Texas, in 1968 and described by Dr. Leo A. Galloway in 1972 (USFWS 1992, p. 1). Galloway later designated LFSV as a distinct species of *Abronia* (USFWS 1992, p. 7) and the Service continues to accept this taxonomic standing. Recent genomic studies for the family Nyctaginaceae, provide further evidence for the taxonomic classification of the *Abronia* genus and LFSV as a distinct species (Douglas and Manos 2007, pp. 859-861; Flora of North America (FNA) 2022; Integrated Taxonomic Information System (ITIS) 2022).

2.0 REVIEW ANALYSIS

Section 4 of the Act (16 U.S.C. 1533) and its implementing regulations (50 CFR part 424) set forth the procedures for determining whether a species meets the definition of "endangered" or "threatened." The Act defines an "endangered species" as a species that is "in danger of extinction throughout all or a significant portion of its range," and a "threatened species" as a species that is "likely to become an endangered species within the foreseeable future throughout all or a significant portion of its range." The Act requires that we determine whether a species meets the definition of "endangered species" or "threatened species" due to any of the five factors described in Section 4(a) of the Act.

The identification of any threat(s) does not necessarily mean that the species meets the statutory definition of an "endangered species" or a "threatened species." In assessing whether a species meets either definition, we must evaluate all identified threats by considering the expected response of the species, and the effects of the threats—in light of those actions and conditions that will ameliorate the threats—on an individual, population, and species level. We evaluate each threat and its expected effects on the species, then analyze the cumulative effect of all of the threats on the species as a whole. We also consider the cumulative effect of the threats in light of those actions and conditions that will have positive effects on the species—such as any existing regulatory mechanisms or conservation efforts. The Service recommends whether the species meets the definition of an "endangered species" or a "threatened species" only after conducting this cumulative analysis and describing the expected effect on the species now and in the foreseeable future.

2.1 Distinct Population Segment (DPS) policy (1996):

Not applicable; species is a plant.

2.2 Updated Information and Current Species Status

2.2.1 Biology and Habitat:

Since the publication of the previous 2010 5-year review, numerous research studies have been conducted related to the species biology and habitat. Throughout this 5-year review the first use of technical terms are underlined and defined in the glossary of this document.

Known Populations and Habitat Surveys

Wild Populations

Few, if any, efforts to undergo range-wide habitat monitoring for the LFSV have been completed (see Figure 1 and Table 1).

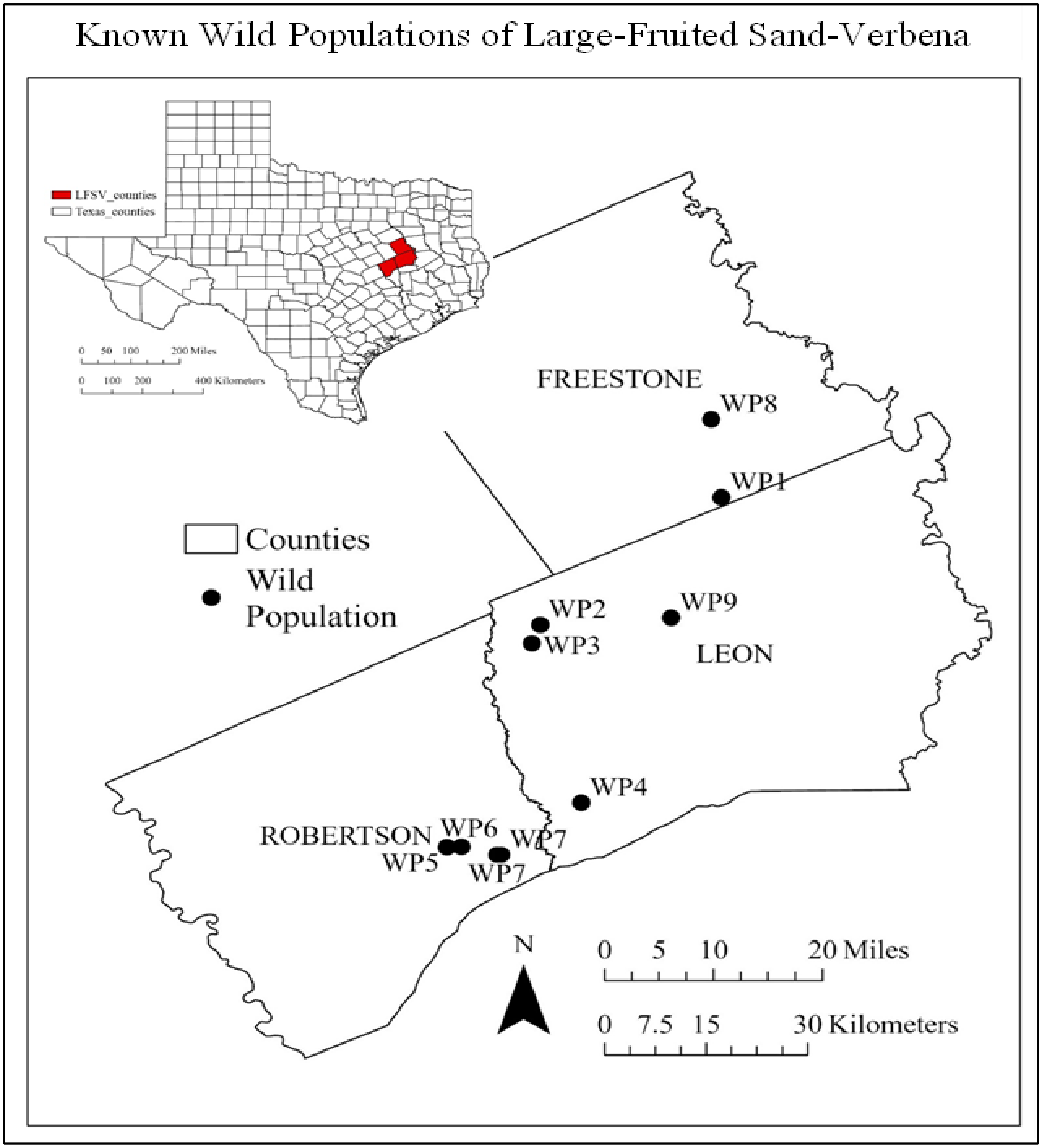

Figure 1. Map of known LFSV wild populations (WPs) as of spring 2022 (TXNDD 2022, entire; P. Williamson, pers. comm. 2022).

Table 1. Documented wild populations of the large-fruited sand-verbena as of spring 2022. Current estimated population abundance data indicates the known number of mature, flowering adult plants at each site. In this column, an asterisk (*) indicates abundance numbers as provided by Williamson (2008) and the Service (2010, p. 13) that are the same.

Population	Element Occurrence (EO) Number	EO ID	County	Current Estimated Population Abundance	Last Observation Date and Observer, if known	Population Notes
WP1	3	4873	Freestone	28,000*	April 2019 (The Nature Conservancy (TNC))	This site is under new ownership (D. Bezanson, pers. comm. 2022).
WP2	n/a	n/a	Leon	6,200*	2008	Communication with the landowner (LO) has occurred (A. Strong, pers. comm. 2019). However, a site visit has not been conducted (P. Williamson, pers. comm. 2022).
WP3	4	1899	Leon	12,000*	2008	No updates are available for this population (P. Williamson, pers. comm. 2022).
WP4	1	5727	Leon	8,000*	Spring 2020 (Service, TNC)	Although this site was not visited after 2020, the Service has anecdotal information that the site persists, and plants are still present. This population was referred to at WP1 in Williamson (1996).
WP5	5	7701	Robertson	61	2022	In 2008, hundreds of plants were estimated (Williamson 2008, p. 7; P. Williamson, pers. comm. 2022). The site was visited by the Botanical Research Institute of Texas (BRIT) in April 2022 and a total of 61 plants were identified with almost 150 observed on the adjacent property (A. Strong, pers. comm. 2022).
WP6	2	3599	Robertson	2,000	April 2016 (TPWD)	A 817 acre (ac) parcel of the site was sold that included the western most portion of habitat, including a large sandy blowout (T. Siegmund, pers. comm. 2022).
WP7	6 & 7	6256 & 2817	Robertson	4,500*	2008	This population is stable and occurs on about 12 ac (Williamson 2008, p. 7; P. Williamson, pers. comm. 2022).

Population	Element Occurrence (EO) Number	EO ID	County	Current Estimated Population Abundance	Last Observation Date and Observer, if known	Population Notes
WP8	n/a	n/a	Freestone	30,000*	2008	This population is stable and occurs on about 30 ac (Williamson 2008, p. 7; P. Williamson, pers. comm. 2022).
WP9	n/a	n/a	Leon	Unknown	2006	This site was identified while visiting an adjacent property. Plants were found on-site however, approval from the LO to access the site has not been received and current population estimates are unknown (P. Williamson, pers. comm. 2022).
WP10	n/a	n/a	Freestone	Unknown	2022	First observed in 1990 (Williamson 1996, p. 24), the status of this population remained unknown until recently. Over time, LO contact was discontinued, and the site had not been observed since 1990. It was recently noted that this population is the same as WP1 (P. Williamson, pers. comm. 2022).

In 1996, a survey conducted of the current wild population 4 (WP4) in Leon County (see Figure 1 and Table 1) by Williamson determined that approximately 25 percent of individuals in each population were in flower at a given time (Williamson 1996 reports the current WP4 as WP1). Surveys of all WPs were conducted by Williamson's lab in 2002, and population estimates were calculated by counting the number of flowering individuals at a site and multiplying that by four (P. Williamson, pers. comm. 2022). All WP sites were visited again in 2008 to collect seeds for various research experiments; however, visual estimates rather than exact population counts were made during this time. Visual estimates were used to determine if the population was stable or declining and/or if a disturbance had occurred. In 2022, BRIT attempted to conduct a range-wide survey but were met with LO access issues (A. Strong, pers. comm. 2022).

It is important to note there are some discrepancies between the population estimates reported in the previous 5-year review (USFWS 2010) and in this current review. The 2010 5-year review, the population numbers listed for 2008 are interpreted as true population survey counts when in fact, these population estimates are based on visual surveys not quantitative count surveys (P. Williamson, pers. comm. 2022). The estimated population abundance of WP1-WP4, WP7, and WP8 are the same values in both the Williamson (2008, pp. 6-7) and the Services 5-year review (USFWS 2010) reports. Further communication with Dr. Williamson and other contractors indicates these populations are stable in recent surveys (see Table 1).

In both the Service's (USFWS 2010, p. 13) and Williamson's report (2008, pp. 6-7), WP5 had an estimated population of 5,000 individuals. However, installation of a fence and development of a natural gas pipeline, both in November 2004, caused soil disturbance such that the population reduced in its abundance to only a few hundred individuals (Williamson 2008, pp. 6-7). Surveys conducted in 2022 by BRIT provided an updated population estimate for WP5 of approximately 61 individuals (see Table 1). Williamson surveyed WP6 in 2005 and discovered a newly established food plot that had caused disturbance to the population resulting in only two plants on-site (Williamson 2008, p. 7). In 2007, Williamson noted the abandonment of the food plot and a notable increase in LFSV flowering plants. Approximately 200 flowering plants were observed which when extrapolated to the full property area of the site (WP6 (10.6 ac)), is approximately 750 individuals (Williamson 2008, p. 7; USFWS 2010, p. 13). Most recently, a survey of WP6 conducted by TPWD in 2016 updated the population estimate to approximately 2,000 individuals (see Table 1). All updates about the species' abundance, status, and last observation date are provided above (see Table 1).

Experimental Populations

The creation of reintroduction sites is essential to the recovery of the LFSV (USFWS 1992, pp. 32-33; recovery actions 62 and 23, respectively). The LFSV experimental populations (EPs) were created to determine the species potential success of reintroduction within suitable habitat (Williamson 2008, pp. 29-39). As of 2022, it is unknown how many of the 9 planted EPs remain because no surveys have been conducted on any EP since 2008 (see Figure 2 and Table 2).

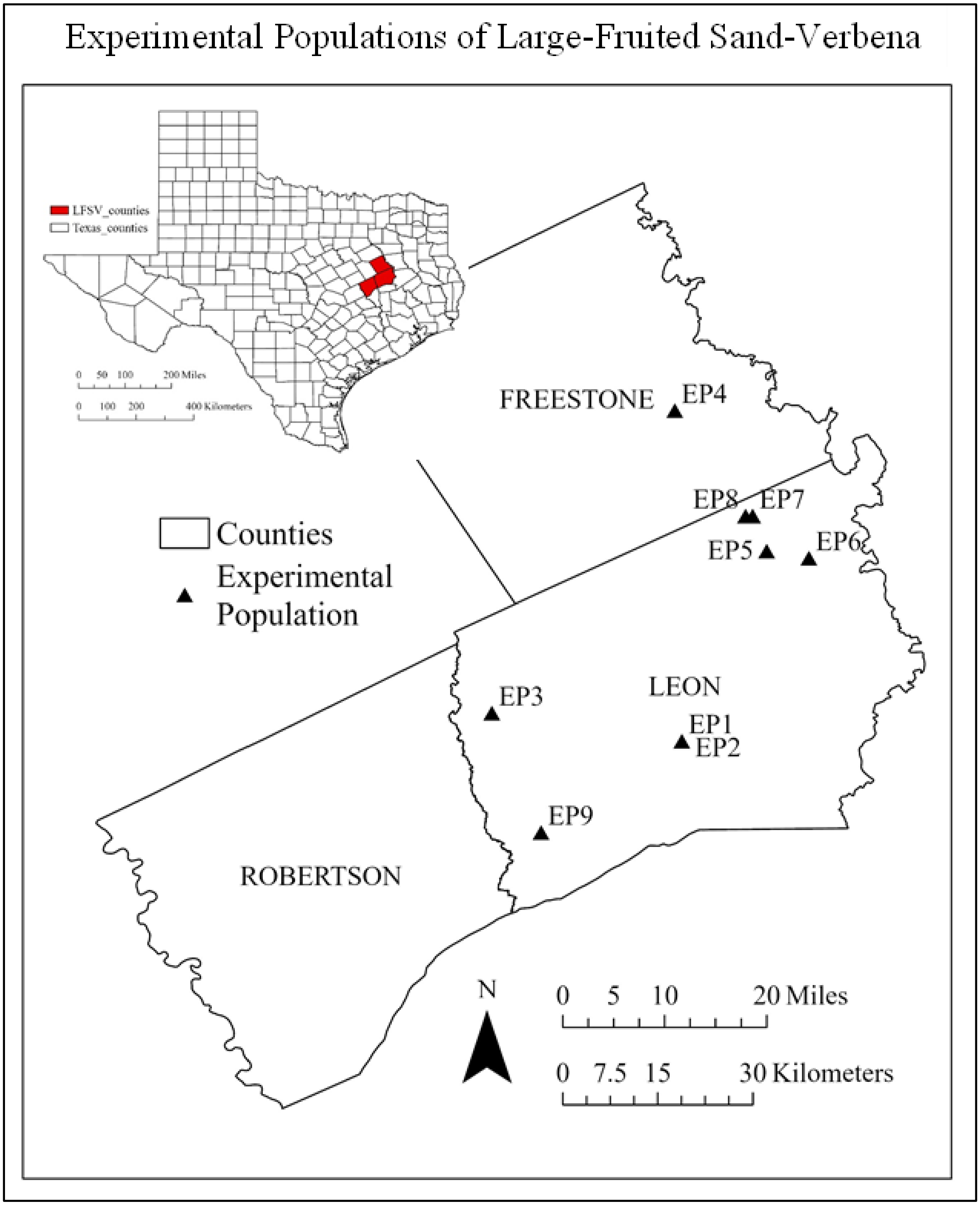

Figure 2. Map of LFSV EPs as of spring 2022 (P. Williamson, pers. comm. 2022).

Table 2. Documented experimental populations of the large-fruited sand-verbena as of spring 2022. Current estimated population abundance data indicates the known number of individual seedlings/plants at each site.

Population	Element Occurrence (EO) Number	EO ID	County	Seed Source and Number of Seeds	Current Estimated Population Abundance	Last Observation Date and Observer	Population Notes
EP1	n/a	n/a	Leon	WP3; n = 360 (2005), n = 240 (2006)	0	2008	The site was established in 2005 (Williamson 2008, pp. 31-34) but no surviving individuals were observed in 2006. Seeds were re-planted in 2007 and no seed germination was observed in 2008 (P. Williamson, pers. comm. 2022).
EP2	n/a	n/a	Leon	WP3; n = 360 (2005), n = 240 (2006)	29	2008	The site was established in 2005 (Williamson 2008, p. 31) and visited during the species' flowering between 2006 and 2008. The site was visited again in 2010 but not during blooming period so abundance data was not recorded (P. Williamson, pers. comm. 2022).
EP3	n/a	n/a	Leon	WP3; n = 240 (2006)	n/a	2007	The site was established in 2006 and germination observed in 2007. Surveys could be not completed in 2008 due to LO health (P. Williamson, pers. comm. 2022).
EP4	n/a	n/a	Freestone	WP8; n = 240 (2007)	90	2022	This site was established in 2006 (Williamson 2006, p. 9) and visited in 2008, 2011, 2012, and 2022. The site visit in 2022 was cut short due to inclement weather so abundance data reported is likely more than the 90 plants reported (P. Williamson, pers. comm. 2022).
EP5	n/a	n/a	Leon	WP1; n = 240 (2007)	2	2008	This site was established in 2007 and surveyed in 2008 (P. Williamson, pers. comm. 2022).
EP6	n/a	n/a	Leon	WP1; n = 120 (2007)	4	2008	This site was established in 2007 and surveyed in 2008 (P. Williamson, pers. comm. 2022).

Population	Element Occurrence (EO) Number	EO ID	County	Seed Source and Number of Seeds	Current Estimated Population Abundance	Last Observation Date and Observer	Population Notes
EP7	n/a	n/a	Leon	WP1; n = 240 (2007)	14	2022	This site was visited by BRIT contractors in April 2022. There were 14 large, flowering plants that were positively identified as the target species. There were also quite a few smaller plants that were suspected to be young LFSV but could not be confirmed (A. Strong, pers. comm. 2022).
EP8	n/a	n/a	Leon	WP1, n = 240 (2007)	10	2008	This site was established in 2007 and surveyed in 2008 (P. Williamson, pers. comm. 2022).
EP9	n/a	n/a	Leon	WP4; n = 120 (2007)	5	2008	This site was established in 2007 and surveyed in 2008 (P. Williamson pers. comm. 2022).

Seeds for EPs were collected in 2005 from all WPs except for WP6 and WP9 (Williamson 2008, p. 11). Selection of which WP would provide the seed source for each EP (see Table 2) was based on genetic analysis of WPs by Williamson and Werth (1999, entire), which found that LFSV has high genetic variability among populations, and that populations closer together were more genetically similar. Therefore, to maintain high genetic variation with the EPs, Williamson measured distance between locations of EPs and WPs, and determined that it would be best to use seeds from WP3 to establish EPs (Williamson 2008, p. 31).

The initial reintroduction experiment began in 2005 and established EP1 and EP2 on two properties in Leon County (see Figure 2 and Table 2). Six plots were created on each EP and half the plots were seeded in either the spring or fall of 2005 (Williamson 2008, p. 31). Subsequent surveys were conducted in 2006 and 2007 and plants were observed; however, no surviving plants were observed during a 2008 survey of EP1 (Williamson 2008, p. 34). Williamson (2008, p. 34) proposed that EP1 declined due to the presence of cutter ants (genus *Atta* or *Acromyrmex*); more research/observation would be needed to determine the species of cutter ant at the site and if there was any herbivory observed, as the species' presence was the only noted observation. The reintroduction of EP2 has persisted and showed both a high survival rate and population abundance during surveys in 2008. Population estimates mimicked those of a natural population, with 10 percent seedlings, 59 percent vegetative plants, and 31 percent plants in anthesis (Williamson 2008, p. 34).

A second reintroduction experiment was conducted in 2006 and created EP3 in Leon County. During the same time, six new plots were added each to EP1 and EP2 in Leon County (see Figure 2 and Table 2) (Williamson 2008, p. 35). Seeds were planted in both spring and fall 2006 at each of these sites and germination was recorded in 2007. Both EP1 and EP2 had spring germination rates of between 16 to 17 percent and fall germination rate of approximately 1 percent; EP3 had 4 percent germination in spring and zero germination in the fall (Williamson 2008, p. 36).

A third reintroduction experiment conducted in 2007, creating six new EPs, five in Leon County and one in Freestone County (see Figure 2 and Table 2) (Williamson 2008, pp. 38-39). Germination data for each EP was collected in March 2008. Seedlings and vegetative plants were observed at EPs 4 (Freestone County), 7, 8, and 9 (Leon County); and one plant was observed in anthesis at EP9 (Williamson 2008, p. 39; Table 7). EPs 5 and 6 only had seedlings present; however, further surveys have not been conducted on any of these EPs so no new updates on population estimates or population structure are known (Williamson 2008, pp. 38-39; P. Williamson, pers. comm. 2022).

Reproductive Biology and Pollination

Reproductive Biology/Mating System

Certain floral characteristics (Doubleday *et al.* 2013, pp. 2280-2281; LoPresti *et al.* 2021, pp. 4-9) and mating systems (including self-incompatibility) have evolved repeatedly over time, particularly in the family Nyctaginaceae (Douglas and Manos 2007, p. 858; Douglas *et al.* 2008, p. 403). The LFSV inflorescence is a capitulum of 25 to 35 flowers (see Figure 3) that open centripetally over 7 to 8 days (Corlies 1991, p. 14).

Figure 3. Image of LFSV inflorescence provided by Dr. Paula Williamson (2017).

Once anthesis is complete, the upper 10-12 millimeter (mm) of the floral tube coils tightly clockwise, leaving the remaining lower 10 mm of the floral tube uncoiled. Self-pollination is facilitated in some members of the Nyctaginaceae (Four O'clock family) by a similar coiling in which the filaments and style become twisted (Corlies 1991, p. 16). The floral tube coiling of the LFSV stops before the stigma and style are reached by the anther sac (Corlies 1991, pp. 15-16), making self-pollination not likely for the species.

Further investigation of LFSV's mating system have been conducted. Hand-pollination experiments where some flowers were unmanipulated ("control") while others were hand pollinated with both self-pollen and cross-pollen were conducted by Corlies (1991, p. 16) and Williamson and Bazeer (1997, p. 410). Corlies (1991, p. 16) found unmanipulated flowers did not produce fruits but did have a high abundance of pollen on the stigma suggesting that LFSV is self-pollinating but self-incompatible. Hand pollinated flowers using cross-pollen produced fruit and seeds consistently, confirming self-incompatibility of LFSV. Williamson and Bazeer (1997, p. 410) analyzed the mating system of LFSV using fluorescent microscopy to observe pollen germination and growth of pollen tubes. Pollen germination and initial pollen tube growth was similar regardless of whether flowers had been hand-pollinated with self or cross-pollen (p. 411);

however, growth of self-pollen tubes was arrested, and no fruit set occurred, suggesting a pre-fertilization barrier and self-incompatibility mating system of LFSV (pp. 411-413).

Research has shown that the mating system across the genus *Abronia* is rather "leaky" with an estimated one percent of ovaries expected to make seed without pollination (E. LoPresti, pers. comm. 2018). While LFSV is self-incompatible, the "leakiness" within the Nyctaginaceae family is supported through mating system variation. Species including Yellowstone sand-verbena (*Abronia ammophila)* and pink sand-verbena (*A. umbellata* var. *umbellata* and *A. umbellata* var. *breviflora)* appear to have a mixed mating system as seed set was observed from flowers with and without pollination (Saunders and Sipes 2006, p. 80; Doubleday *et al.* 2012, p. 32; VanNatto and Alyson 2020, p. 23).

Pollination

Large-fruited sand-verbena flowers open from 3:00 or 4:00 pm until 9:00 or 10:00 am and produces a strong sweet aroma resembling honeysuckle that increases until early evening. These floral characteristics are commonly associated with moth pollination (Corlies 1991, p. 12). Williamson *et al.* (1994, p. 339) observed, captured, and identified several floral visitors of LFSV which included Sphynx and Noctuid moths (family Sphingoidea and Noctuidae, respectively) such as the black alder or pawpaw sphynx (*Dolba hyloeus*), lettered sphynx (*Deidamia inscripta*), obscure sphynx (*Erinnyis obscura*), and large necklace moth (*Hypsoroph monilis*i). The proboscі of these moths were dusted with LFSV pollen, making them likely pollinators. Furthermore, the larval food sources for these likely pollinators have been noted at all of the LFSV sites and include yaupon (*Ilex decidua*), grape (*Vitis* spp.), and milkweed (*Asclepias* spp.) (Figure 3, *In* Williamson 2008, p. 18; Table 9, *In* USFWS 2010, pp. 18-22). Among the diurnal floral visitors, some incidental pollination may be due to bees (genus *Bombus* and *Apis*) (Williamson *et al.* 1994, p. 339).

Noted pollinator observations of various other *Abronia* species including Yellowstone sand-verbena, pink sand-verbena, and Ramshaw Meadows sand-verbena (*A. alpina,* RMSV) support the notion that species of *Abronia* are moth pollinated (Saunders and Sipes 2006, p. 80; Jabis *et al.* 2011, p. 1587; Doubleday 2012, p. 23). Pollinator observations included mostly moths including Noctuids and some Sphingids, Geometrids, and Pyralids; however, bumblebees and butterflies were also noted as potential pollinators. While observational studies provide insight into potential pollinators, it is important to determine if those visitations result in plant reproductive output (i.e., the production of fruit and seed set and effective pollination). Doubleday and Eckert (2018, p. 71) found floral visitation of pink sand-verbena was higher during the day (8.67 percent) compared to those visits at night. Yet nocturnal visitation by Sphingid moths resulted in significantly higher seed set, suggesting Sphingid moths are the primary pollinators. Moreover, while honeybees were observed during this study their visitations resulted in no fruit/seed set suggesting that these insects are acting as potential pollen thieves, collecting floral pollen/nectar but not necessarily pollinating the flowers (Doubleday and Eckert 2018, p. 72).

Species' Longevity

Although LFSV is known to be a perennial species (USFWS 2010, p. 16), studies have not been completed to determine the lifespan of LFSV individuals, and population surveys have not been consistent enough to assess the species' demographics. Research on LFSV individuals and seeds has focused on seed germination and reintroduction methodology. Longevity information about the LFSV would help understand the species recovery needs to maintain long-term viability.

Genetics
In general, species with larger geographic ranges compared to those of smaller ranges, tend to have higher dispersal capabilities, increased gene flow, and ultimately higher levels of genetic diversity (Lester *et al.* 2007, p. 746; Moeller *et al*. 2011, pp. 46-47). Isolation and geographic distance play an important factor in a species genetic diversity (Shapcott 2007, p. 447). Reductions in size of the historical range of a species can often lead to population bottlenecks and thus a loss of genetic variation, causing species rarity (Cole 2003, pp. 213-214; Moeller *et al.* 2011, p. 47). However, many species in the genus *Abronia* exhibit high genetic diversity even though there is variation among species population ranges and sizes (see Figure 4).

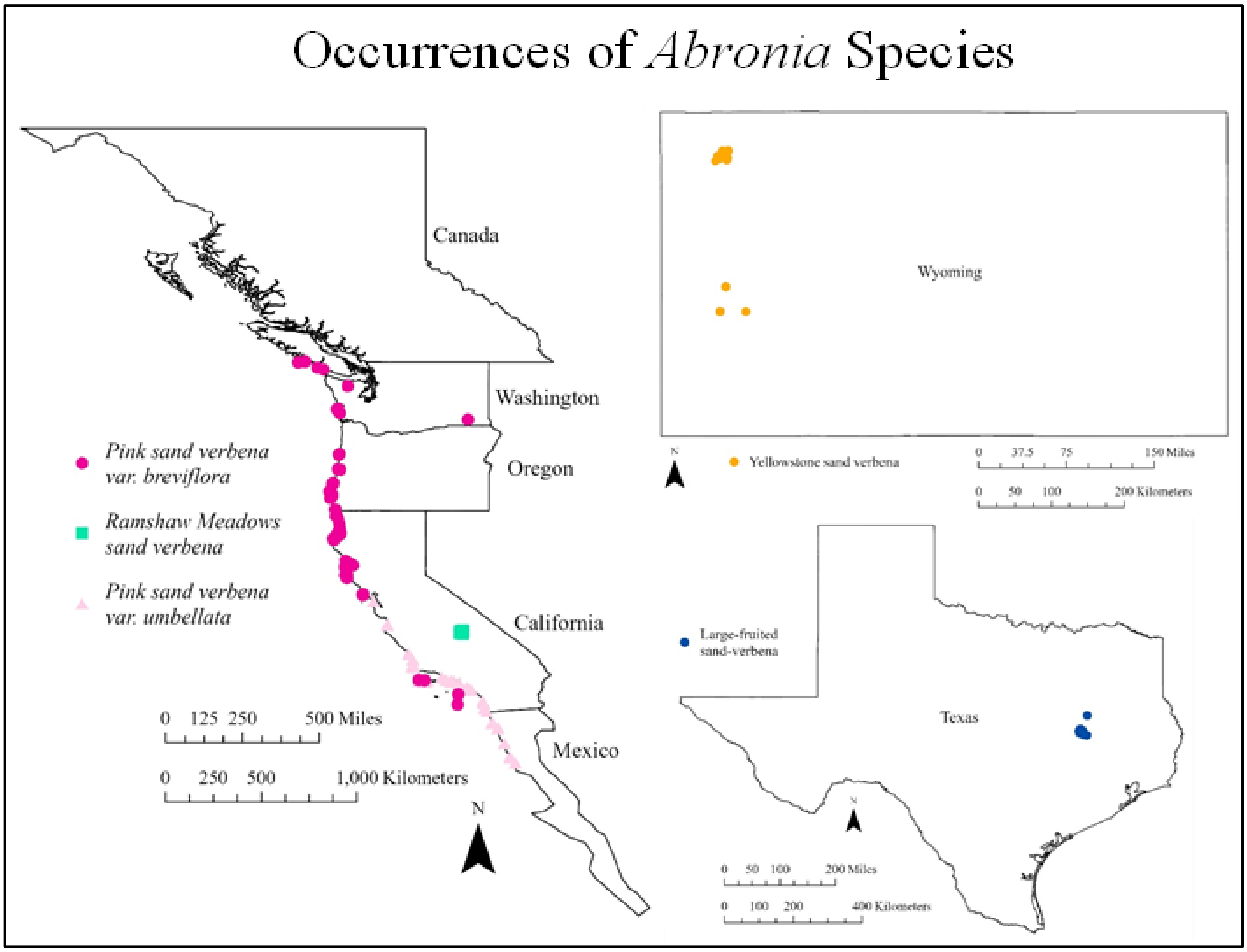

Figure 4. Map of *Abronia* species known to occupy the United States including pink sand-verbena (var. *breviflora;* var. *umbellata)*, Yellowstone sand-verbena, LFSV, and Ramshaw Meadows sand-verbena. Species occurrence data was obtained from the Global Biodiversity Information Facility, https:www.gbif.org, accessed online August 1, 2022.

Population genetic analyses have been conducted on RMSV, pink sand-verbena, and LFSV, but out of these species pink sand-verbena has the broadest species range (see Figure 4). Genetic analysis of RMSV using amplified fragment length polymorphisms (AFLPs), found the species exhibited a high percentage of polymorphic loci and heterozygosity particularly for a rare, endemic species (Jabis *et al.* 2011, pp. 1588, 1590). In addition, RMSV subpopulations appeared to have high rate of gene flow (Jabis *et al.* 2011, pp. 1587-1588). Similar genetic diversity was observed in pink sand-verbena (*A. umbellata* var. *breviflora*), although this species has a larger geographic range, spanning from Canada through southern California (Figure 4; McGlaughlin 2002, p. 412). Canadian populations of pink sand-verbena, those both geographically close and separated, were observed to have similar genetic variation; whereas those populations at the southern end of the range were genetically distinct compared to other populations regardless of geographic distance (VanNatto and Eckert 2022, pp. 261-262).

LFSV had higher genetic diversity than a plant species with a wide geographic range, which is abnormal for a narrow endemic species (Williamson and Werth 1999, pp. 296-299; USFWS 2010, pp. 16-17). More recent genetic analysis of LFSV noted that the largest populations had the lowest levels of genetic variability, whereas the smallest populations had intermediate and high levels of genetic variability, respectively (A. Strong, pers. comm. 2020). Further genetic analysis of LFSV populations will aid in the recovery and future reintroduction efforts for this species.

Seed Biology

Seed Viability and Germination

Results of seed viability testing for both the LFSV and other species within the genus *Abronia* are high. Seed viability testing for LFSV was conducted from one WP in Leon County where results showed that 94 percent of collected seeds were viable (Goodson and Williamson 2011, p. 142). Among the species in the genus *Abronia*, at least 90 percent viability was exhibited in seeds ranging from 0-6 years of age (Drennam 2008, pp. 848-849). Although seed viability is relatively high, seed germination in the field for LFSV is relatively low, between 0 to 40 percent, when planted in the spring and 0 to 5 percent germination when planted in the fall (Goodson 2007, p. 19). In laboratory experiments, seed germination varies based on treatments used to improve germination (Young 1989, p. 1; Goodson 2007, pp. 7-8; Drennam 2008, p. 848; Goodson and Williamson 2011, pp. 142-143; Ferrazzano and Williamson 2013, pp. 123-125).

Scarification, the physical removal of the seed coat surrounding the seed, is one technique used to aid in seed germination. Since the LFSV occurs in a sandy habitat, Goodson (2007, p. 7) postulates that once the dispersed seeds land in the sand, their thin coating is naturally removed by sand movement. Stratification is another technique to aid in seed germination by breaking seed dormancy and mimicking natural habitat conditions. In laboratory experiments, LFSV seeds were determined to have highest germination when scarified and subjected to warm followed by cold stratification (see Figure 5; Goodson 2007, p.15; Drennan 2008, p. 848; Goodson and Williamson 2011, p. 144).

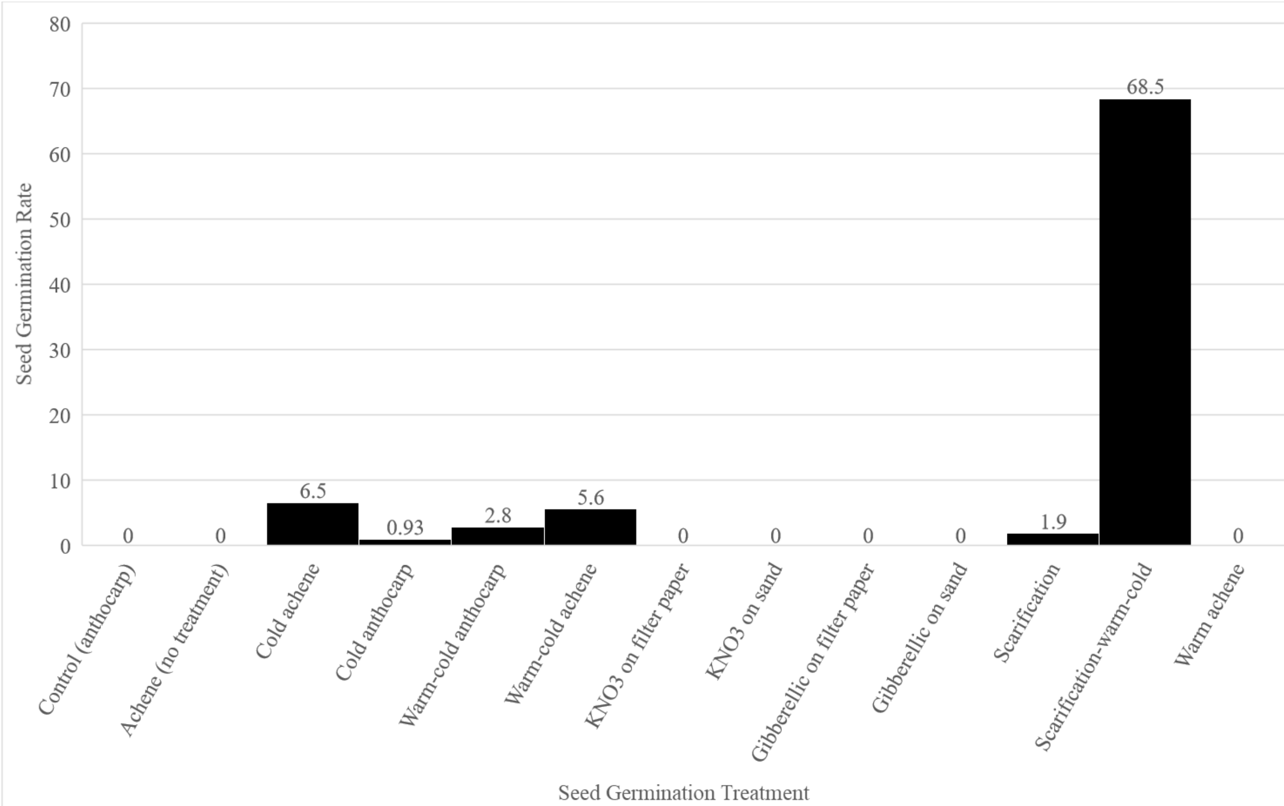

Figure 5. Results of LFSV seed germination experimental treatments as demonstrated by Goodson (2007, p. 15). Seed treatment including scarification with warm then cold stratification had highest germination success (68.5 percent).

Additional germination methods include ethylene application, which was tested at 0, 10, 100, and 500 micromolar (µ/mol) on *Abronia* species including sweet sand-verbena (*A. fragrans*), red sand-verbena (*A. maritima*), pink sand-verbena, and desert sand-verbena (*A. villosa*) seeds (Drennan 2008, pp. 848-849). Seeds exposed to ethylene at any ethylene treatment had higher germination compared to controls, regardless of species (Drennan 2008, pp. 849-850), and had higher germination with increased ethylene concentration.

Another technique used to enhance seed germination and seedling growth is mycorrhizal fungi inoculation. Mycorrhizal fungi are a naturally occurring organism that have a symbiotic relationship with many plant species, aiding in a plants uptake of nutrients including nitrogen, phosphorus, and potassium, ultimately aiding in plant growth (Ferrazzano 2012, p. 3). Root samples were collected from plants at WP4 (Leon County) found in association with LFSV in its habitat including Texas sandmint (*Rhododon ciliates*), silver croton (*Croton argyranthemus*), and the common lantain *(Plantego major*) (Ferrazzano 2012, p. 11). Roots were analyzed for both the presence of mycorrhizal fungi and to determine if they were naturally occurring in the area. Ferrazzano (2012, p. 26) found that there is relatively strong preexisting presence of mycorrhizal fungi at the field site.

Ferrazzano (2012, p. 12) also tested the benefits of mycorrhizal fungi inoculation on seed germination in a greenhouse setting. Seeds collected at WP4 (Leon County) were tested with and without mycorrhizal fungi inoculation and placed in soil collected from the field site (WP4) where seeds were collected to mimic natural growth conditions. Seed germination rates and growth were monitored for two years. Ferrazzano's findings determined that mycorrhizal fungi inoculation aided in seed germination of LFSV for the first year of growth; however, it provided no benefit in the second year of growth (2012, p. 13). The existing low levels of soil nutrients (nitrogen, phosphorus, and potassium) sampled at the LFSV sites likely benefited from the initial inoculation of mycorrhizal fungi from year one, increasing available soil nutrients and thereby, boosting seedling growth. A lack of growth in the second year likely occurred because mycorrhizal fungi inoculation only occurred once at the beginning of the experiment. Overall, long term improved seedling survival and growth would likely require multiple inoculations of mycorrhizal fungi (Ferrazzano 2012, p. 4). These germination experiments provide valuable knowledge to improve LFSV germination and aid in future reintroduction experiments and conservation efforts of the species.

Seasonality of Germination

Goodson (2007, p. 22) conducted germination experiments in the field to determine if seasonality altered germination rates of LFSV seed reintroductions. Seed germination rates were significantly greater in the spring (4.2 percent - 16.7 percent) compared to the fall (0.0 percent – 0.83) season. In a second study, LFSV seeds planted in the field (Leon County) also exhibited higher germination in the spring compared to the fall with 27.8 percent and 0.80 percent, respectively (Goodson and Williamson 2011, p. 143). Variation in seasonal germination is likely due to seeds planted in autumn not being exposed to immediate period of warmth like the seeds in the spring receive, mimicking what laboratory experiments have observed the temperature-based stratification the species needs for improved germination. It is important to note that while there is a strong difference in germination of seeds planted seasonally, natural seed germination may vary due to microhabitat characteristics including soil nutrients and soil moisture (Goodson and Williamson 2011, p. 145).

2.2.2 Threats Analysis:

Primary threats (or stressors) to the LFSV include destruction and modification of habitat including clearing of vegetation for oil and gas pipeline projects and residential development within habitat (listing factor A and D); conversion of native grassland to improved pastures of introduced grasses (listing factor A and E); conversion of open grassland to woodland or food plots (listing factor A); and over-stocking of grazing animals (listing factor A and C). Additionally, other incompatible land use practices based on the species biology include herbicide application from October to April (listing factor A); mowing from February to April (listing factor A); off-road vehicle (ORV) or off-highway vehicle (OHV) use within populations from October to April (listing factor A); and, broad-scale insecticide use (which could kill pollinators) (listing factor A) (Kennedy *et al.* 1990, p. 10; USFWS 1992, pp. 34-37; Williamson 2008, pp. 1-4; USFWS 2010, pp. 27-31).

Additional threats to the species that have been noted in previous LFSV recovery documents including fire suppression (listing factor A); wildflower collecting (listing factor B); and herbivory associated with deer and/or livestock (listing factors A and C) (USFWS 1992, pp. 6-7; USFWS 2010, p. 27). There are no new updates regarding these additional threats listed above and therefore, they are not discussed further in this report.

2.2.2.1 Listing Factor A. Present or threatened destruction, modification, or curtailment of its habitat or range:

Habitat Loss, Conversion, and Fragmentation

Urban Residential Development
The Service is unaware of any large planned residential development projects at any of the WP or EPs. Site visits were not conducted between September 2015 and 2016, so any new disturbances could not be noted (P. Williamson, pers. comm. 2016). In 2022, part of WP6 was split and sold, and is now under multiple ownership (D. Bezanson, pers. comm. 2022).

Industrial Habitat Loss, Conversion, Fragmentation
Two surface mines exist within the known range of the LFSV, the Jewett and Bremond Mines. Both are lignite mines (coal), operated by the Texas Westmoreland Coal Company and Luminant Mining Company, LLC, respectively. The Jewett mine is located approximately eight miles northwest of Jewett in Leon County. For this mine, the Service recommended that the applicant conduct habitat surveys for the LFSV; the applicant indicated that surveys for the LFSV were conducted and yielded negative results. The Bremond Mine is located six miles east of Bremond, Robertson County, and although within the range of the species, did not directly affect the plant. The Service provided recommendations for both applications under Section 7 of the Act and 404 of the Clean Water Act for each permit application.

Mining operations can have impacts to the species habitat through removal, disturbance, and direct mortality of plants. Both of these mining facilities included other federally-listed species like the Navasota ladies'-tresses (NLT, *Spiranthes parksii*) and only NLT has been observed on these sites. It was estimated in 2014, that the total estimated impact acreage from mining projects within range of the NLT and LFSV was 50,600 acres (20,477 hectares (ha)) as of November 2013 (A. Vale, pers. comm. 2014). Lignite mining is the Railroad Commission of Texas (RCC) and our field office has not received any requests for the development of new lignite mines within the range of the LFSV.

Linear Development
Linear development projects, including transportation and oil and gas activities (i.e., liquid natural gas pipeline development and maintenance), has increased within the known range of the LFSV. Since 2010, the Service has formally consulted under Section 7(a)(1) of the Act (see Table 3) on four consultations that have included LFSV related to these activities, resulting in approximately 175 ac (70.8 hectares (ha)) of effects to suitable habitat.

Table 3. Summary of linear development projects requiring consultation under Section 7 of the Act with the Service regarding the LFSV since 2010. Other species included in these consultation efforts included the Navasota ladies'-tresses (NLT, *Spiranthes parksii*).

Consultation Code	Conclusion Date	Project Name	County	Length of Proposed Project	Species	Project-related Habitat Disturbed and/or Conserved for LFSV
02ETTX00-2021-I-0193	June 2021	Dallas to Houston High speed Rail, permit responsible wetland mitigation	Freestone, Limestone, Montgomery, Walker	240 mile high-speed passenger rail system	LFSV, NLT	0.05 ac (0.20 ha) (Young site, Freestone County) placed in conservation easement, also with NLT.
02ETTX00-2019-F-2135	July 2020	Dallas to Houston High Speed Rail	Dallas, Ellis, Navarro, Freestone, Limestone, Leon, Madison, Grimes, Waller, Harris	240 mile high-speed passenger rail system	LFSV, NLT	116 ac (46.9 ha) of habitat (disturbed)
02ETTX00-2017-F-1748	November 2018	Targa Downstream LLC / Grand Prix South Pipeline	Ellis, Freestone, Hill, Johnson, Leon, Madison, Navarro	275 mile natural gas pipeline	LFSV, NLT	52.8 ac (21.3 ha) of habitat
21450-2011-F-0184	January 2012	Oncor Electric Habitat Conservation Plan	100 Texas counties including Leon, Limestone, Freestone, Grimes		LFSV, NLT	5.5 ac (2.2 ha) of habitat

An additional project (02ETTX00-2021-I-0193) resulted in the conservation of 0.05 ac (0.20 ha) of habitat through implementation of avoidance and/or minimization measures to reduce such effects to insignificant and/or discountable levels under the Act. Both types of projects require consultation with the Service under Section 7 due to a federal nexus (i.e., federal funding, permitting (U.S. Army Corps of Engineers, research/recovery permit with the Service (Section 10(a)(1)(A)), or on federal lands).

Despite some potential consultation and coordination of linear projects, many projects do not require consultation with the Service due a lack of a federal nexus. The RRC regulates the oil and natural gas industry in the state of Texas. The RRC has detailed information on all existing pipelines, but the agency has no way to predict future routes for pending pipelines or wells. Potential pipelines and route locations are not displayed on the RRC online tracker, and therefore, the Service cannot forecast where future pipelines may be developed. Figures 6, 7, and 8 show the extent of linear oil and gas projects (natural gas) within the species' suitable habitat.

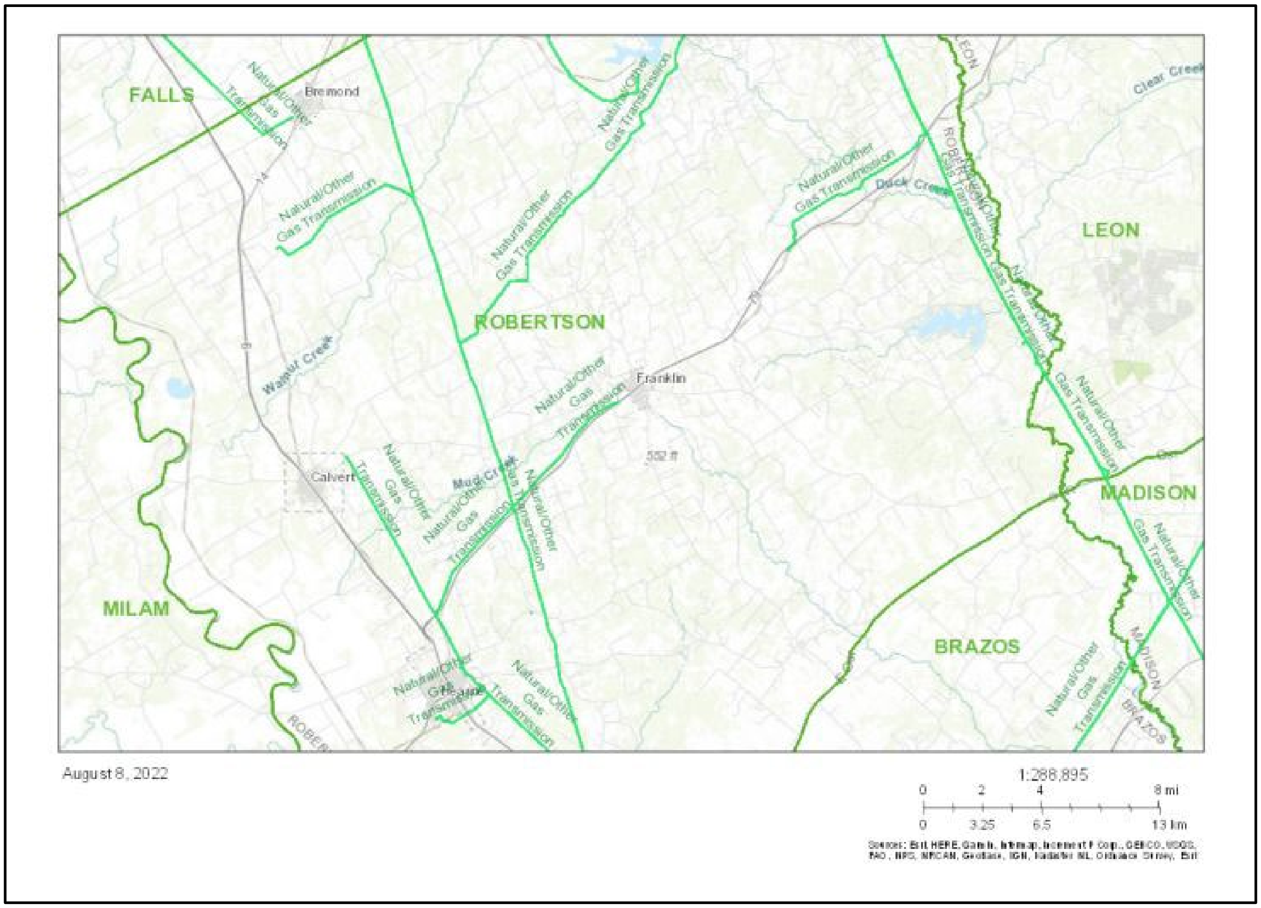

Figure 6. In-service natural gas transmission lines within Robertson County, Texas, according to RRC online mapper as of August 1, 2022.

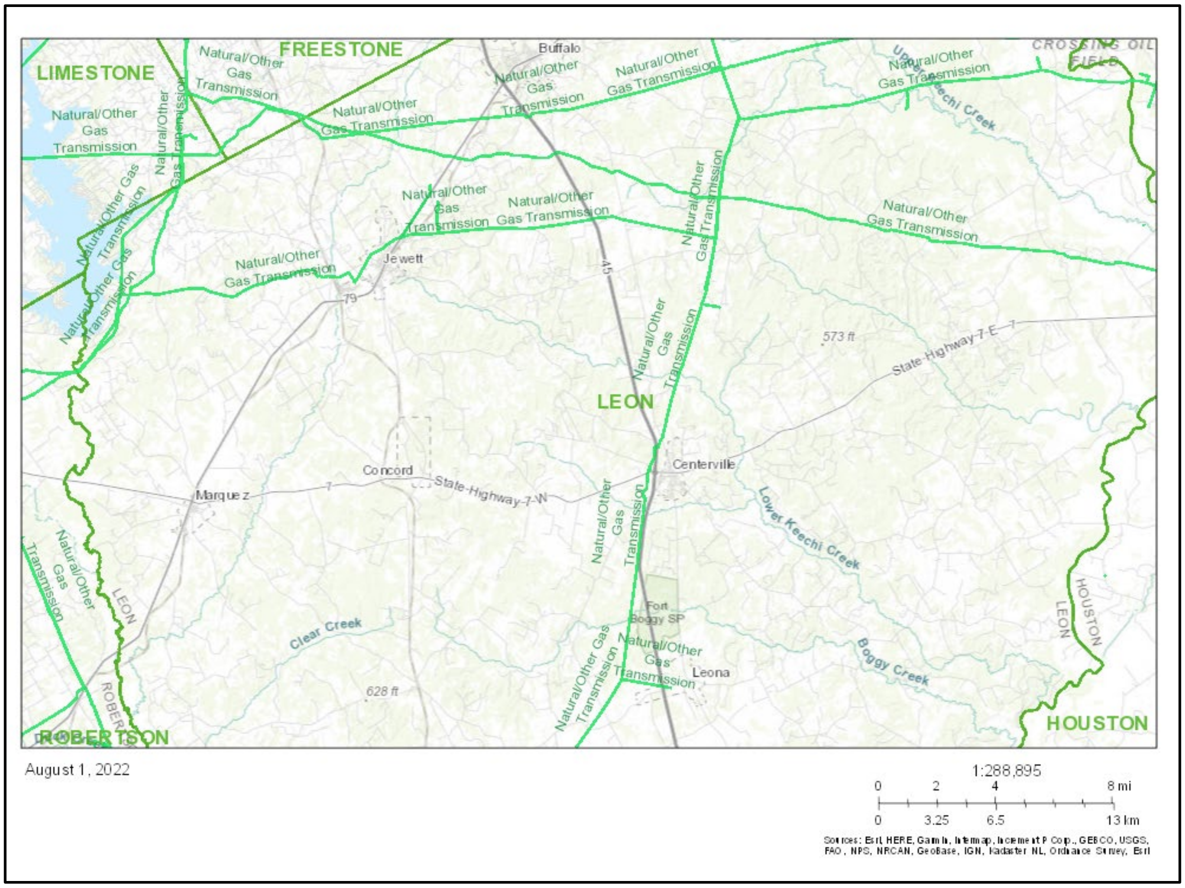

Figure 7. In-service natural gas transmission lines within Leon County, Texas, according to RRC online mapper as of August 1, 2022.

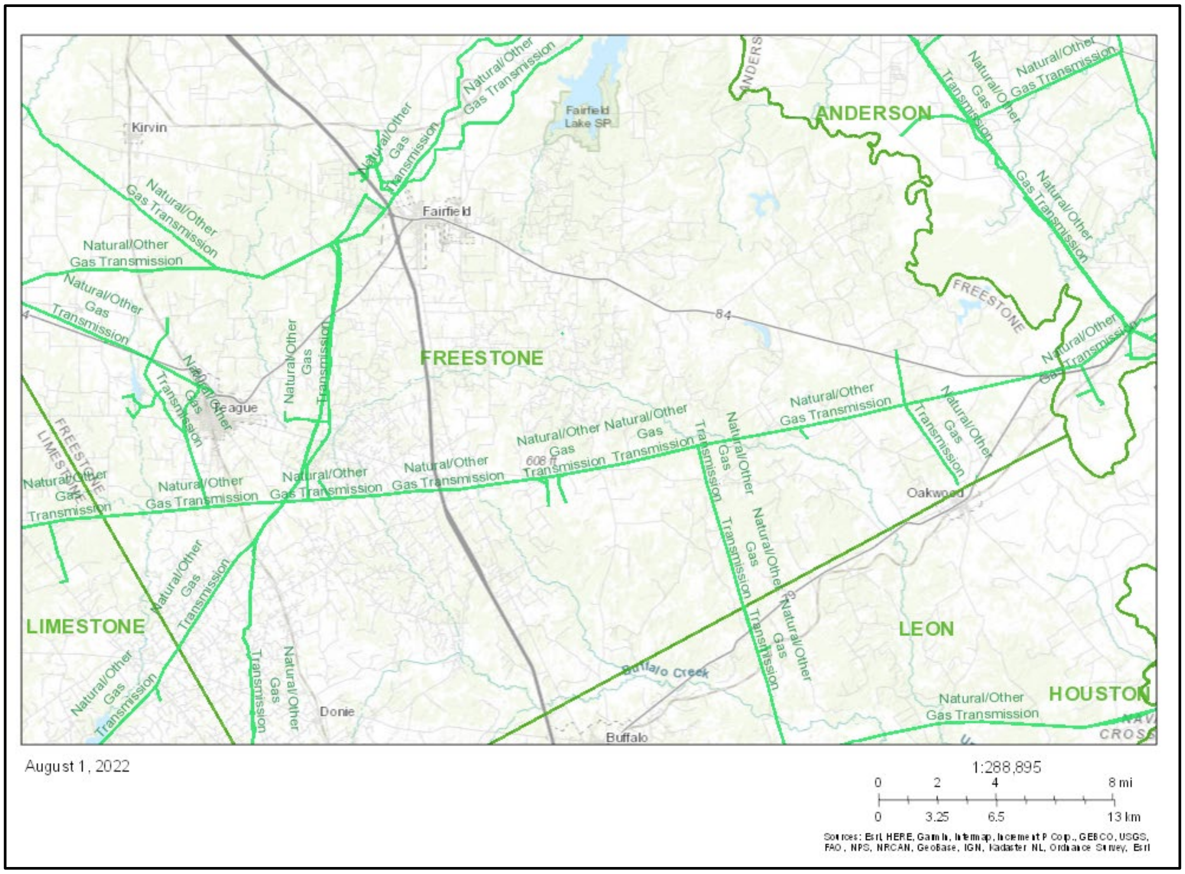

Figure 8. In-service natural gas transmission lines within Freestone County, Texas, according to RRC online mapper as of August 1, 2022.

In addition, linear transportation projects are currently underway and/or planned by the Texas Department of Transportation (TxDOT) within the counties occupied by the LFSV. TxDOT maps projects currently underway; those that are to be started within the next four years; those to begin construction in 5 to 10 years, and projects planned for 10 or more years from now (see Figure 9). The map below shows the location of transportation projects that could be conducted in LFSV suitable habitat.

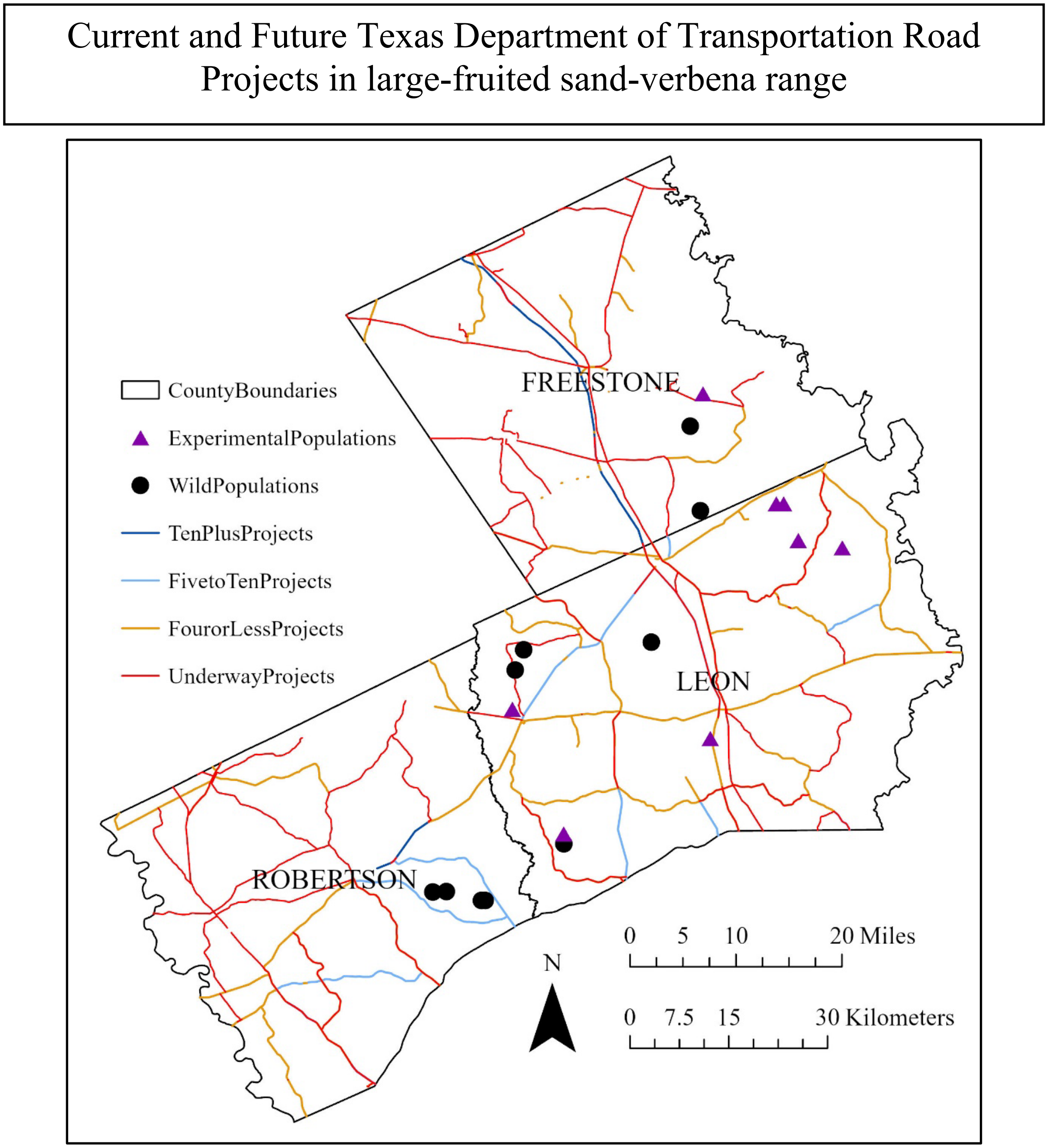

Figure 9. Map of TxDOT road project to occur within the known county range of the LFSV in Freestone, Leon, and Robertson counties, Texas, as of August 2022. Red lines indicate projects currently underway; orange lines depict projects to begin construction in less than four years;

light blue lines depict projects to being in five to ten years; and dark blue lines indicate projects being discussed and to being in ten or more years.

All types of linear projects can include the potential direct loss of and fragmentation of LFSV habitat. The use of heavy equipment and machinery may reduce habitat quality and quantity (acres) through soil disturbance, soil compaction, introduction of nonnative species, effects to canopy cover, reduction in pollinator resources (host plants), and effect deep sandy soils and slopes. Additionally, the loss of plant individuals could occur due to land clearing and grubbing. The direct killing of individual flowering plants or rosettes could impact LFSV across its range by reducing the number of populations; the number of individuals in a population; the future migration of individuals to new areas within suitable habitat; foraging opportunities for known pollinators; and any genetic variation of individual plants within or among a population. Direct loss resulting from linear projects could affect the resiliency of the LFSV to future stochastic events, reducing future reproductive opportunities, and impacting the representation across its range. Indirect effects from linear projects and resulting land clearing include soil disturbance and introducing foreign material/seed from other sites which could result in an increase in nonnative invasive species within LFSV suitable habitat and adjacent habitats. The colonization and spread of nonnative species could out-compete LFSV for water, light, and space resources and alter suitable habitat variables.

Furthermore, the activities associated with the ongoing operation and maintenance of linear projects could further threaten LFSV individuals and populations. Maintenance such as mowing and/or herbicide use could kill plants and therefore, the timing and frequency of mowing is particularly impactful should these activities occur during the species blooming period (i.e., reducing its reproductive output potential). Herbicides may occasionally be needed to control woody growth in project vicinities, which would likely directly affect LFSV individuals and populations as aerial application could cause direct mortality to plants, habitat quality, and kill known pollinating and visiting insect species if applied during the LFSV blooming period.

Off Road Vehicle/ Off Highway Vehicle (ORV/OHV) use, Mowing, Clearing, Herbicides, and Wildlife Food Plots

Other threats noted to impact the LFSV include the loss and fragmentation of habitat from the use of ORV/OHVs, mowing, clearing of habitat, herbicide use, and the development of food plots. Habitat disturbance from ORV and OHV use has been frequently documented as a potential threat to the LFSV. Prior to 2010, vehicular disturbance activities have been documented at WP4. This type of disturbance may be continuing at WP4 as its effects were captured during site visits in 2019 and 2021 (A. Strong, pers. comm. 2021; see Figure 10).

Figure 10. Images provided by Anna Strong (TPWD) at WP4 from observations of LFSV population/habitat in 2019 and 2021. The image in 2021 depicts potential ORV use and disturbance to the LFSV population.

Effects to flowering plants and suitable habitat from the suspected ORV/OHV activities recorded in 2021, caused disturbance of the sandy blowouts where plants within the population are found as well as a noticeable reduction in the number of flowering plants. WP4 does have signs posted about restricting ORV use however, it has been suggested that activities may be related to frequent mowing rather than ORV use. The status of ORV/OHV use at other sites is not known.

Mowing
Mowing is a direct threat to LFSV, particularly during the blooming season (February through April). As discussed above mowing could be a potential threat for WP4 although the direct disturbance mechanism at that site isn't clear. As discussed in Williamson (2008, p. 5), and USFWS (2010, p. 25) to best preserve known populations it is recommended to LO's that mowing be done outside the flowering period of the plant.

Herbicide Use
Herbicide use is a direct threat to germinating seeds, seedlings, and flowering plants. It is recommended to LO's delay herbicide application until LFSV seeds have dispersed and plants have become dormant during June-September (Williamson 2008, p. 5; USFWS 2010, p. 25). We lack data about the use and/or effects of herbicide on populations as minimal surveys have been conducted.

Listing Factor B: Overutilization for commercial, recreational, scientific, or educational purposes:
There is no new information related to this threat to report since the publication of the 2010 5-year review.

Listing Factor C: Disease or predation:
There are no known diseases that impact LFSV; however, with anticipated changes in temperature and precipitation associated with climate change (see the Climate Change section below), the number of diseases (pathogens and parasites) impacting plants and wildlife globally

could increase, impacting population dynamics of these species (Harvell *et al*. 2002, p. 2158; Altizer *et al.* 2006, p. 467; Burdon and Zhan 2020, pp. 1-3). Future research of LFSV focusing on disease and predation of LFSV could be conducted when more information about this threat is known.

Herbivory

Minimal herbivory from deer, domesticated grazing animals, or insects have been noted in the past (USFWS 2010, p. 29) likely due to LFSV being psammophonious thus creating a sand armor to avoid herbivory (LoPresti 2018, p. 826). Experiments have been conducted to determine if levels of herbivory differed between psammophonious and non-psammophonious plants. LoPresti and Karbor (2016, pp. 826-832) compared yellow sand-verbena (*A. latifolia*), and honey-scented pincushion plant (*Navarretia mellita*, non-psammophonious) by removing sand from stems, petioles, and leaves of yellow sand-verbena and adding sand to soils of honey-scented pincushion plants, and then exposing both species to leaf-mining caterpillars (*Lithariapteryx abroniaeella*). Leaves with sand present resulted in less caterpillar herbivory, consistent with a physical defense function, than those without sand (LoPresti and Karbor 2016, p. 831).

In a similar experiment, LoPresti (2018, p. 6) found that approximately 50 percent of plants with sand removed had feeding damage on leaves compared to intact (sand present) leaves which exhibited only 25 percent herbivory. Despite these results, the ecological function behind psammophony is not clear. LoPresti and Karbor (2016, pp. 826-827) postulated that plants exhibiting crypsis were also psammophonious. To test this hypothesis, sand was removed from 90 stems of the yellow sand-verbena; tan or green sand was added to 30 plants each to mimic the sand habitat or leaf color, and the remaining 30 plants had no sand added to act as controls (LoPresti 2018, pp. 829-830). Herbivory was highest on stems with no sand (15 percent) than those stems with sand. Overall, there was no difference in caterpillar herbivory due to sand color suggesting that sand armor functions as a defense mechanism is not a result of crypsis (LoPresti 2018, pp. 5-6).

Listing Factor D: Inadequacy of existing regulatory mechanisms:

There is no new information related to this threat to report since the publication of the 2010 5-year review.

Listing Factor E: Other natural or manmade factors affecting its continued existence:

Climate Change

Climate change may be a factor affecting LFSV however, we do not know whether changes in climate that have already occurred have affected populations and/or its distribution. While future climate projections indicate an increase in average annual temperature and decrease in average annual precipitation (Climate Mapper 2022; see Table 4), the Service is not able to predict how the species might be affected by the type (i.e., temperature or precipitation) and degree of future climate change under different greenhouse gas emission scenarios (representative concentration pathways (RCPs)).

Table 4. Projected change in annual temperature or annual precipitation through the University of California MERCED Climate Mapper tool within Freestone, Leon, and Robertson counties,

Texas. Since the species seedling germination is dependent on spring temperatures, future climate projections for annual and spring seasonal variation were chosen.

County	Season	Climate Variable	RCP 4.5 (2010-2039)	RCP 4.5 (2040-2069)	RCP 8.5 (2010-2039)	RCP 8.5 (2040-2069)
Freestone	Annual	Mean Annual Temperature	2.3	4.0	2.5	5.3
Freestone	Annual	Mean Annual Precipitation	-0.1	-0.1	0.0	-1.2
Leon	Annual	Temperature	2.3	4.0	2.4	5.2
Leon	Annual	Precipitation	-0.3	-0.3	-0.3	-1.6
Robertson	Annual	Temperature	2.3	3.9	2.4	5.2
Robertson	Annual	Precipitation	-0.4	-0.3	-0.3	-1.4
Freestone	Spring	Temperature	2.2	3.9	2.2	5.1
Freestone	Spring	Precipitation	-0.1	0.1	0.0	-0.3
Leon	Spring	Temperature	2.2	3.8	2.2	5.0
Leon	Spring	Precipitation	0.0	0.0	0.0	-0.4
Robertson	Spring	Temperature	2.2	3.8	2.2	5.0
Robertson	Spring	Precipitation	-0.1	-0.1	-0.1	-0.4

The future climate projections in Table 4 are made by a multi-model mean derived from 20 downscaled CMIP5 models. The RCPs indicate the amount of greenhouse gases expected to be emitted in each climate change model. RCP 4.5 represents a "status quo" emission scenario where emissions stabilize over time, and RCP 8.5 represents a high emissions scenario where emissions are constantly increasing over time. The timeframes of 2010-2039 and 2040-2069 were selected based on our understanding of plausible near-term and long-term future planning timeframes for the LFSV based on its basic biology as a perennial plant.

Plant species vary drastically in their response to climate change, based on numerous factors including life history, topographic complexity, geographic range, species rarity, among others (Anacker *et al.* 2013, p. 207). Due to LFSV's endemism and adaptation to the post-oak savannah ecoregion, changes in climate change as a result of rising temperatures, and variations in frequency and amount of precipitation, the species may become further restricted across its known geographic range (Climate Mapper 2022). Furthermore, climate change can lead to degraded habitat and increased species invasion. Endemic species, like LFSV, are commonly outcompeted by other species within their range due to increased competition for light, nutrients, and water resources (Jabis *et al.* 2014, p. 186), and the effects of climate change could impact LFSV persistence. However, more research is needed to fully understand how climate change could affect the LFSV.

In addition, multiple studies document the effects of climate change on species that require pollinators. Changes in a species phenology can cause known pollinators to become "out of sync" with the species flowering period, resulting in the reduction in the species overall viability as a result of a reduced reproductive output (Forrest 2014, pp. 5-6; Kudo and Cooper 2019, p. 4;

Gerard 2020, pp. 77-78). Further research is needed on the phenology of LFSV and associated moth pollinators to determine the impacts, if any, as a result of climate change.

There are no known diseases that impact LFSV. However, with increasing temperatures and precipitation associated with climate change, the number of diseases (pathogens and parasites) globally is likely to increase, and moreover increase the ability for diseases to spread and impact population dynamics of affected species (Harvell *et al.* 2002, p. 2158; Altizer *et al.* 2006, p. 467; Burdon and Zhan 2020, pp. 1-3). As a result of climate change, LFSV could become more susceptible to pathogens and parasites. Since little is known about the species and effects from parasites and pathogens, any compounding effects from this threat and climate change are also unknown.

Conservation Measures

Seed Banking

Creating and maintaining a reserve seed bank is an important recovery criterion of LFSV (USFWS 1992). Seed banking is important for species recovery because it provides long-term storage of genetic material and can be an important tool for species reintroductions as part of species recovery and conservation. Currently, seeds are banked at Mercer and accessions have been moved to newer storage repositories to protect from potential flooding events in the area (Kennedy 2012, p. 10; M. Marr, pers. comm. 2022). In addition, Williamson provided updates on existing seed banking efforts, confirming that as of 2022, LBJWC has six collections of LFSV seeds being stored in a -17 °C freezer (Williamson 2002, p. 10; P. Williamson, pers. comm. 2022). Further duplication of seed storage at additional facilities ensures protection of the seed bank for the species in the event of flooding and other stochastic events that could occur.

1n 1995, Mercer created a seed banking plan in collaboration with Dr. Williamson, to collect and preserve seeds of LFSV for current and future conservation needs of the species (Wieland 1995, entire). Williamson collected 242 seeds from approximately 50 plants at WP4 in 1995 (referred to as WP1 in the Williamson 1996 report). Seeds were cold preserved and further testing was planned to better understand and perfect seed preservation methods for the LFSV (Wieland 1995, entire). In 2002, seeds were collected from all WPs except WP6 and WP8 and banked at Mercer. A portion of these seeds were to be sent to the National Seed Storage Laboratory (U. S. Department of Agriculture (USDA)) in Ft. Collins, Colorado (Williamson 2002, p. 10; Oxley 2002, p. 1); however, there are no records of LFSV seeds at this facility (A. Tiller, pers. comm. 2022; P. Williamson, pers. comm. 2022).

Research Efforts

Phylogenetics

Research on LFSV phylogenetics are being conducted by Dr. Eric LoPresti and associated students. Sonia Nosratinia, a prior graduate student of the LoPresti lab, investigated the phylogeny of *Abronia*, including the species *macrocarpa* although her data and that of Dr. LoPresti is unpublished and pending. Nosratinia's phylogenetic research findings confirm that LFSV is a genetically distinct species from others in the *Abronia* genus (E. LoPresti, pers. comm. 2022; see Figure 11). Further research into the *Abronia* phylogeny will improve the current understanding of plant characteristics, adaptations, and mating system variation in related *Abronia* species.

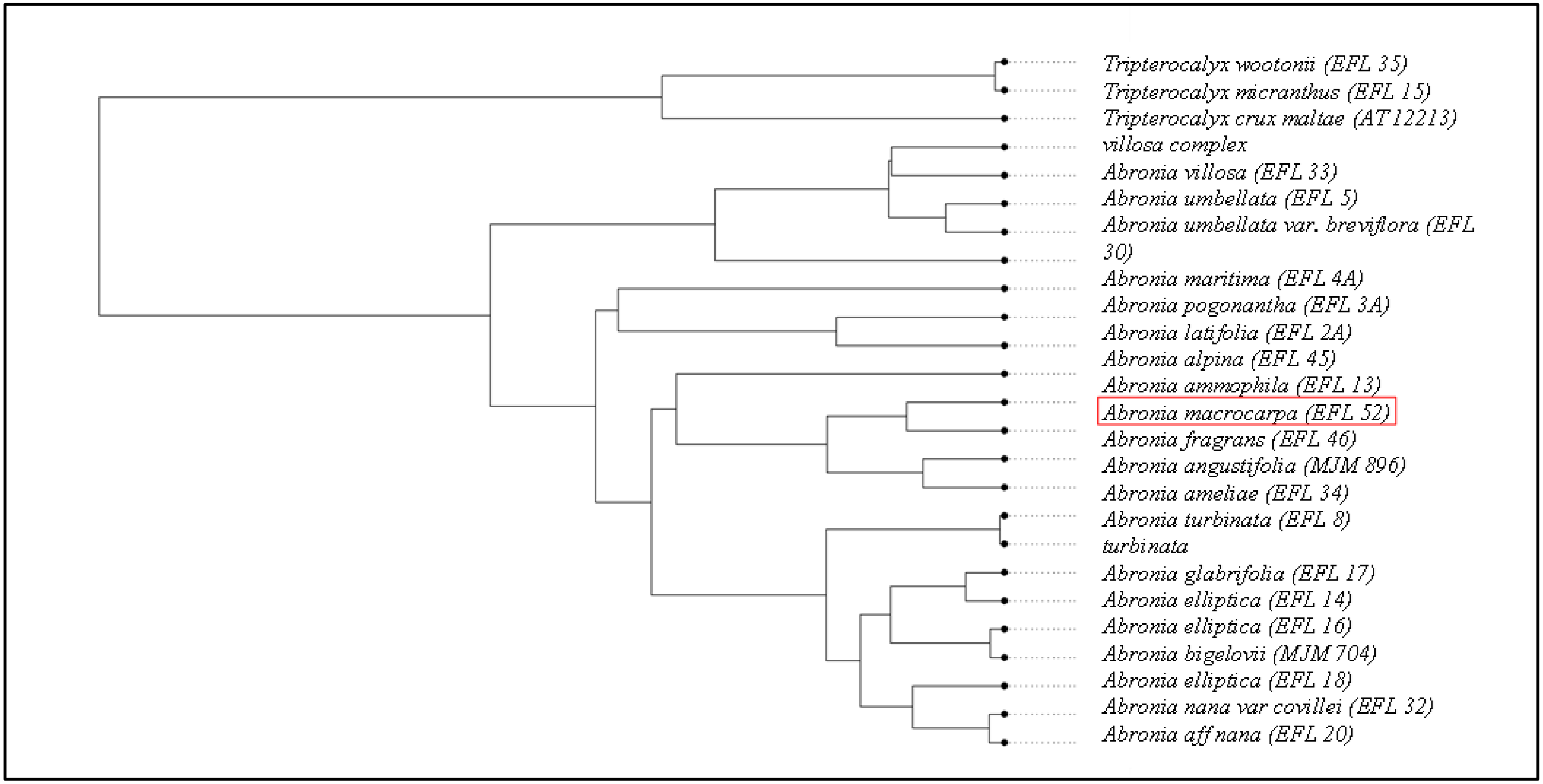

Figure 11. Research findings from Sonia Nosratinia and LoPresti on *Abronia* phylogenetics (unpublished; E. LoPresti, pers. comm. 2022). The LFSV (*A. macrocarpa*) is highlighted, and findings support the species as distinct.

Floral Fragrance

Research on LFSV floral fragrance composition is being conducted by Dr. LoPresti's lab and associated students, however much of their work is unpublished. Floral fragrance is known to be a key factor in plant-pollinator relationships as being a known characteristic in attracting pollinators to a plant often through a floral nectar or food reward. Major groups of pollinators (i.e., bees, butterflies, moths, bats) are generally attracted to different floral fragrance compounds; therefore, understanding floral fragrance composition of *Abronia* species can aid in understanding variation in primary pollinators in the genus. Primary components of LFSV floral fragrance include organic and chemical compounds such as sesquiterpenes, benzenoids, and phenylpropanoids (see Figure 12). Closely related sweet sand-verbena has similar floral fragrance composition to LFSV although they vary in floral pigmentation (see Figure 13). Further research into the *Abronia* phylogeny in relationship to floral fragrance, characteristics, and pollinators will improve the current understanding of *Abronia* demographics and particularly for LFSV, investigation of its pollination biology (recovery action 3232).

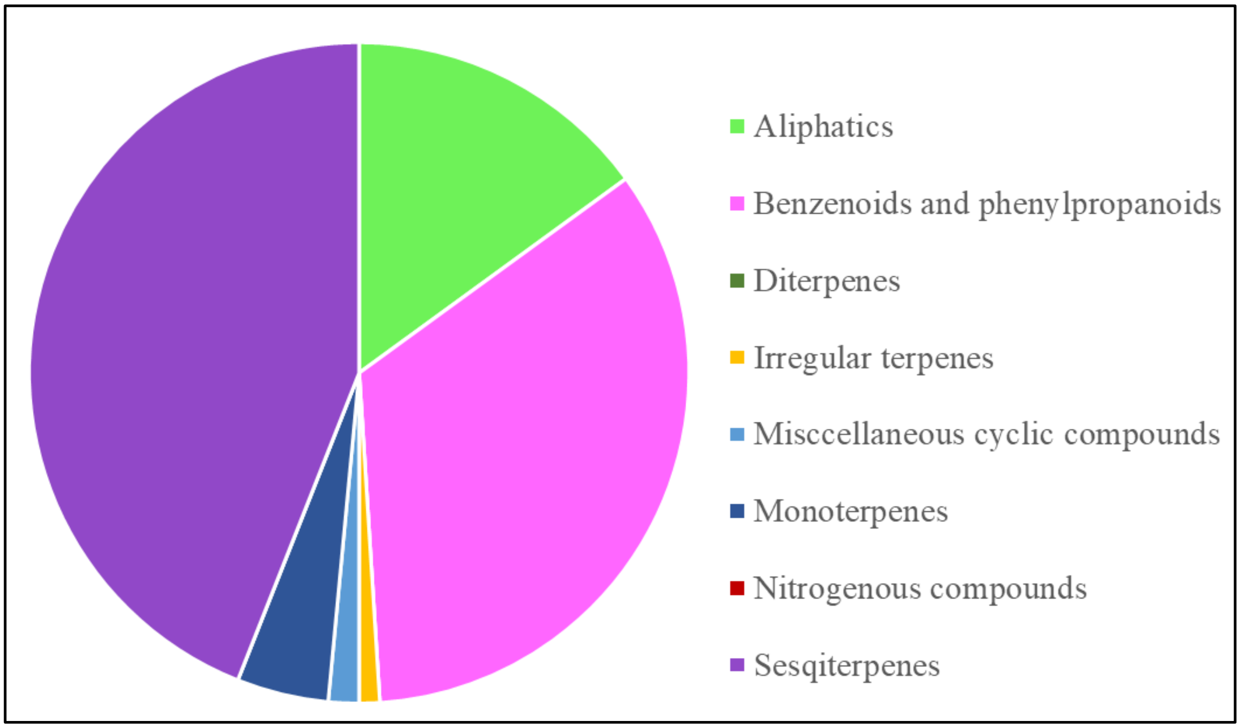

Figure 12. Current floral fragrance composition of LFSV as determined by LoPresti (pers. comm. 2022; *unpublished* – shared with permission).

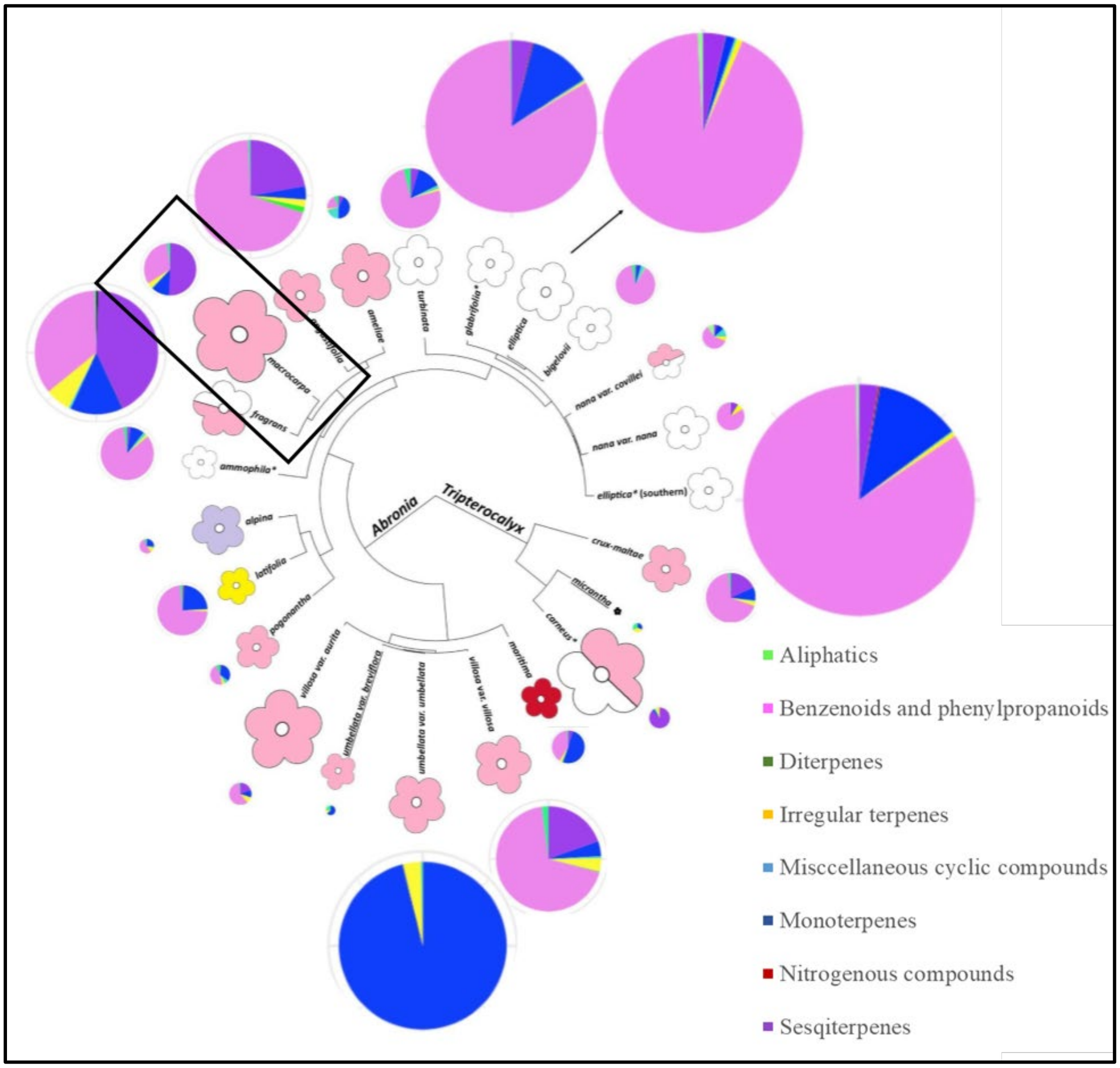

Figure 13. Results of floral scent production research across species of the genus *Abronia* in relationship to a species phylogenetic relatedness and floral pigmentation (E. LoPresti, pers. comm. 2022; *unpublished* – shared with permission). The LFSV is highlighted with a black box.

2.3 Synthesis:

Large-fruited sand-verbena is a perennial forb in the family Nyctaginaceae, with taxonomic classification still accepted from Galloway (1972), FNA (2022), and ITIS (2022). Nine documented WPs remain extant, with plant abundance ranging from an estimated 61 to 30,000 individuals across Freestone, Leon, and Robertson counties, Texas. As of 2022, two EPs (EP4 and EP7) were surveyed and confirmed to be persistent populations; while seven other EPs have been created it is not clear if they currently persist. The last survey of EPs shows populations range in plant abundance from two to 90 individuals. However, surveys are needed to confirm if the EPs still exist as the most current range-wide survey efforts were conducted in 2008. Ownership of many of the WPs has changed, therefore establishing and developing a collaborative relationship with existing and new LOs is vital. Outreach and monitoring efforts are the means by which on the ground conservation, habitat preservation, and long-term stewardship are possible for the recovery of LFSV.

Research efforts to understand the species' genetic structure and phylogeny, seed germination, and pollination mechanisms have been completed and/or are underway. Genetic analysis indicates that LFSV has high heterozygosity within and among its WPs however, more research is needed to understand how genetic variation equates to population stability, floral characteristics, and mating system. Seed germination experiments have shown that scarification followed by warm and cold stratification leads to the highest percentage of germination within the species. In addition, research to understand the timing of plantings has been completed. Seeds of LFSV planted in the spring versus the fall had a higher rate of germination. Information from seed germination studies will aid in reintroduction efforts as well as development of additional experimental populations (i.e., introductions). Several studies on the mating system and pollination of LFSV have determined that the species is self-incompatible and therefore relies on pollinators for reproduction. LFSV relies on primarily Noctuid, Sphingid, Geometrid, and Pyralid moths for pollination, although some butterflies and bees have been observed as well. Although both mating system and pollinators of LFSV have been studied, the mating system of *Abronia* varies and further studies would be beneficial to ensure the stability of LFSV self-incompatibility. In addition, climate change could alter the LFSV or pollinator phenology so additional analysis of LFSV pollination would provide a better understanding of plant-pollinator relationship under changing environmental conditions.

Prominent threats to the LFSV still largely stem from listing factor A covered above (destruction, modification, or curtailment of its habitat or range). Additional threats to the species include effects from herbivory, herbicide use, and climate change. Impacts from many of these threats remain unknown as consistent monitoring of populations has not been conducted to ascertain their status and/or effects to the LFSV. Properties are being sold and fragmentated, as is the case with WP6. The LFSV exhibits sand armor (psammophony), which acts as a defense mechanism against herbivory from caterpillars, however cutter ants may be the cause for decline of one LFSV population. It is unclear the herbivory agent on the species and the effects from this activity. Potential effects from climate change remain a concern for the species and future research should focus on how expected increases in temperature and decreases in precipitation could impact LFSV geographic range, pollination, phenology, and seed germination.

Despite some of the current research efforts for the LFSV, gains to acquire and protect the species and its habitat long-term have not been accomplished. Currently, no populations are under any binding conservation agreement for legal protection and the species still faces moderate threats. Efforts to re-establish and maintain LO contact are a primary need to consistently monitor populations, assess and manage threats, and gain an understanding of the species' population dynamics. Monitoring of EPs to assess strategies for success of future introduction efforts are needed. Other recovery efforts should focus on seed banking and expanding to additional repositories to protect seed storage from stochastic events like flooding and to preserve genetic variation from the existing populations.

3.0 RESULTS

3.1 Recommended Classification:

____Downlist to Threatened
____Uplist to Endangered
____Delist (*Indicate reasons for delisting per 50 CFR 424.11):*
____The species is extinct
____The species does not meet the definition of an endangered species or a threatened species (i.e., is recovered, or new information on status and threats indicate species does not meet definitions)
____The listed entity does not meet the statutory definition of a species.
__x__**No change is needed**

3.2 New Recovery Priority Number:

No change in recovery priority number.

Brief Rationale:

Since the previous 5-year review, new populations of LFSV have not been discovered. There are nine total WPs and nine total EPs thought to be extant, totaling approximately 91,000 and 150 mature individual plants, respectively. Some urban or residential development is occurring within the species range however, the Service is unaware of effects from development on any extant property (both WP and EP). Changes in ownership have occurred as WP6 was split in 2022 and is now owned by several LO's (D. Bezanson, pers. comm. 2022). Threats continue to stem from observed ORV and/or mowing disturbance as documented at WP4 (Figure 4). All known populations occur on privately owned land, and LO contacts need to be re-established to continue population monitoring and reintroduction efforts. We have determined the degree of threat has not changed since the previous 5-year review and remains at moderate. An increased understanding of the species' pollination biology, germination methodology, and seed banking requirements have been conducted, providing valuable information to aid in the species recovery. Efforts to monitor and assess both WP and EPs are needed, as only two populations were surveyed in 2022. Despite this, LOs are likely to be amenable to future survey efforts with continued collaboration and outreach efforts by the Service and partners and therefore, the recovery potential of LFSV remains high. The LFSV continues to be recognized as a valid and distinct species. Since the appropriate conservation measures are generally compatible with most current land uses, recovery is not likely to conflict with economic activity, therefore the recovery priority number remains an 8.

3.3 Listing and Reclassification Priority Number: Not applicable.

Reclassification (from Threatened to Endangered) Priority Number: ____
Reclassification (from Endangered to Threatened) Priority Number: ____
Delisting (Removal from list regardless of current classification) Priority Number: ___

4.0 RECOMMENDATIONS FOR FUTURE ACTIONS

Future actions for LFSV should primarily focus on highest priority needs of the species and its habitat in order to reduce threats and ultimately aid in its recovery. Future actions are dependent on current Service workload, and will require collaboration from LOs, partners, other nongovernmental organizations, and others to carry out such actions. The following actions are not listed in any order and may change as new information becomes available for the species:

- <u>Establish protected sites</u> (recovery action 111; priority 1): Currently, no known occupied LFSV sites are protected by a conservation easement or other binding mechanism. Efforts between the Service, TNC, and LO to protect WP4 with a conservation easement began in 2019, however no such agreement has been secured. LO outreach and cooperation are vital to the success of these securements and should be a priority for future work with the species. Funding mechanisms should be developed and presented in concert with outreach efforts to the LO to implement protection and management of sites.
- <u>Work with LO to manage sites for existing populations</u> (recovery actions 112 and 113; priority actions 1 and 2, respectively): Both short-term and long-term reduction in threats and management should be explored for each known site once LO contact and coordination has been established and maintained. Once LO contact is established, management plans should be created and revised, as needed (recovery action 14, priority 2).
- <u>Monitor known populations for general condition and reproductive success</u> (recovery action 13; priority 2): Monitoring methods should be developed and used at all sites. Monitoring should occur annually at each known population (both WPs and EPs), where access is allowed, to track demographics and current threats.
- <u>Maintain a reserve germ bank/seed bank and cultivated populations</u> (recovery action 21 and 22; priority 1 and 2, respectively): Currently seeds are banked with CPC partners at Mercer and LBJWC. Seeds should also be banked at USDA's National Laboratory for Genetic Resources Preservation in Ft. Collins, Colorado. Additional seed banking could alleviate pressures should any stochastic event occur. Testing of seed viability needs to be conducted and seeds from current populations should be collected in the near future (to have preservation of current population genetics/flowering plant populations). Formal seed collection and management guidelines should be developed and coordinated amongst the Service and partners that includes consideration of the species' needs, genetic representation of the species, and built-in redundancy for species/population preservation from stochastic events. Collection guidelines and purposes for reintroduction should be outlined in a species-specific plan and should adhere to the Service's 2000 Controlled Propagation and Reintroduction Policy (recovery action 62; priority 3).
- <u>Continue with cultivation of LFSV for the purposes of restoration and reintroduction</u> (recovery action 23; priority 2): Seed germination studies (Goodson 2007, p.15; Drennan 2008, p. 848; Williamson 2008, pp. 35-39; Goodson and Williamson 2011, p. 144) and early reintroduction experiments (Williamson 2008, pp. 29-39) have shown LFSV can be successfully cultivated in both the greenhouse and field. However, surveys of reintroduced LFSV have not been consistent and are needed for species recovery to determine the need for more restoration and reintroduction. Further research in species

cultivation, restoration, and reintroduction would help determine the amount of successful germination/per seeds plants and how microhabitat plays a role in successful reintroduction. Continuing efforts to create and monitor additional reintroduced populations could help achieve the species recovery goal of downlisting as least 20 populations are needed.

- Determine habitat requirements (recovery actions 3111, 3112, and 3113; each priority 2): Species geology, soils, and microclimate should be investigated at a site-specific and landscape scale. Habitat mapping will help identify areas with suitable habitat to focus survey efforts for additional populations; and identify areas of suitable habitat to focus potential reintroduction efforts (recovery actions 41 and 42; priority 1 and 2, respectively).
- Assess the demographic needs and conditions for populations (recovery action 3211; priority 1): Analyses completed show variation among soil composition and characteristics across populations, although the plant species composition (i.e., associated species) at each population seem similar. Further analysis is necessary to monitor site condition changes (i.e., changes in soil composition, pH, etc.) and subsequent changes to LFSV populations (i.e., number of flowering plants).
- Characterize phenology (recovery action 322; priority 2): Investigate the phenological response to variations in timing and frequency of important climatic conditions (i.e., precipitation, temperature).
- Investigate the pollination biology (recovery action 3232; priority 2): Several pollinators have been identified in association with LFSV. While some experiments point to a self-incompatibility mating system (i.e., relying on pollinators for seed/fruit set), confirming if these are effective pollinators is important. Pollination biology research will improve management efforts of both LFSV and associated pollinators, and improve the overall long-term viability of the species.
- Investigate seed biology (recovery actions 331 and 332; each priority 2): Germination techniques have been investigated by Williamson and others using various germination methods including scarification, stratification, and mycorrhizal fungi inoculation. Best methodology, from current research knowledge, includes warm then cold seed stratification with the anthocarp removed. Further investigation is needed to understand the longevity and viability of stored seeds and the best germination methods for stored seed for later reintroduction.
- Conducting searches for existing populations (recovery action 41; priority 1): Efforts to search for additional LFSV populations should be coordinated between the Service, TPWD, and other partners. Working with other agencies, such as NRCS, the Service's Partner's for Fish and Wildlife (PFW) program, that conduct on-the-ground management and LO consultation is important to gaining LO trust and access to LFSV populations. Efforts will need to focus on educating LO's and partner organizations. Another important component of this recovery action should include the identification and refinement of suitable habitat requirements as outlined in recovery actions 3111, 3112, and 3113 above.
- Monitor existing reintroduction populations to understand effectiveness and feasibility of techniques (recovery action 51; priority 2): Several reintroduction efforts occurred by Williamson between 2006 and 2008, creating nine EPs. These efforts demonstrated that spring reintroductions are more successful than fall reintroductions however, this was

ultimately dependent on site-specific characteristics (i.e., presence of cutter ants, soil characteristics, precipitation events). Since the initial creation of the EPs, sites have not been surveyed or monitored. Consistent monitoring of EPs is necessary to provide accurate reintroduction methods and management actions in the future. The genetic structure of LFSV has been observed to exhibit high heterozygosity in and among populations, and was used to improve genetic structure of EPs; however, no population genetic research has been conducted since the creation of EPs. Genetic structure research is needed to verify genetic variation was improved with created EPs, and ensure high heterozygosity among LFSV populations to protect the species from stochastic events.

- Continue to promote public support for the conservation and recovery of the species (recovery action 7; priority 2): Efforts should focus on providing the public with knowledge of the species needs and the importance of its conservation. In addition, the public should be made aware of potentially deleterious land management practices (i.e., mowing, ORV use, etc.); how to alter such land management practices so they provide dual benefits to the species and the LO/land manager (i.e., timing and frequency of mowing); other strategies to aid in species recovery (i.e., providing access for monitoring, allowing reintroductions, etc.); and necessary information about plant regulations under the Act.
- Determine response to disturbance, agricultural practices, and other land uses (recovery action 3133; priority 1): Efforts should focus on determining the impact of threats to LFSV including but not limited to ORV use, fire (prescribed burning), herbivory, and climate change. ORV use or mowing was observed at WP4, however, consistent surveys/observations are needed to better understand the types of disturbance occurring and their impact. Little to no information is known about the impacts of prescribed burning on LFSV. This needs to be investigated to determine if it could be a suitable restoration management strategy for the species. Herbivory by caterpillar was investigated by Dr. LoPresti, however LFSV exhibits psammophony (i.e., sand armor) which acts as defense against herbivory. Williamson observed population loss in an EP that was thought to be impacted by cutter ants however, these findings need further investigation.

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U.S. FISH AND WILDLIFE SERVICE

5-YEAR REVIEW of Large-fruited sand-verbena (*Abronia macrocarpa*)

Current Classification:

Recommendation resulting from the 5-Year Review:

____ Downlist to Threatened
____ Uplist to Endangered
____ Delist
__X_ No change needed

Appropriate Listing/Reclassification Priority Number, if applicable:

FIELD OFFICE APPROVAL:

Lead Field Supervisor, Fish and Wildlife Service, Texas Coastal Ecological Services Field Office – Clear Lake

Approve CHARLES ARDIZZONE Digitally signed by CHARLES ARDIZZONE Date: 2022.08.29 12:40:09 -05'00'

REGIONAL OFFICE APPROVAL:

Assistant Regional Director, Ecological Services, U.S. Fish and Wildlife Service, Region 2

Approve ____

GLOSSARY

Amplified Fragment Length Polymorphisms (AFLP): a polymerase chain reaction based technique that uses selective amplification of a subset of deoxyribonucleic acid fragments to generate and compare unique fingerprints for genomes of interest. This technique aids in the study of genetic diversity within a species and/or populations.

Anthesis: the flowering period of a plant, from the opening of the flower bud.

Anthocarp: any of various forms of fruits in which part of the perianth tissue remains attached to the fruit, as part of the mechanism of dispersal. In LFSV this is papery to aid in wind dispersal.

Capitulum: a compact head of a structure, particularly a dense flat cluster of small flowers or florets.

Centripetally: proceeding or acting in a direction toward a center or axis.

Cross-pollination: the process of applying pollen from one flower to the pistils of anther flower, can be vector (pollinator) mediated as in LFSV.

Crypsis: the ability of an organism to conceal itself especially from a predator/herbivore by having a color/pattern/and/or shape that allows it to blend into the surrounding environment.

Heterozygosity: the condition of having two different alleles at a locus and is fundamental to the study of genetic variation in populations.

Petioles: the stalk that joins a leaf to a stem.

Phylogeny: a description, in words or diagrams, of the evolutionary history of a group of related species. It depicts a sequence of branching events and may also identify the characteristic features that mark various lineages.

Proboscis: an elongated mouthpart in many insects that is typically tubular and flexible.

Psammophony: a plant characteristic known as “sand-armor”. Species with this characteristic have glandular-hairy surfaces.

Resiliency: describes the ability of populations to withstand stochastic events (arising from random factors). Resiliency can be measured based on metrics of population health, for example, birth versus death rates and population size. Highly resilient populations are better able to withstand disturbances such as random fluctuations in birth rates (demographic stochasticity), variations in rainfall (environmental stochasticity), or the effects of anthropogenic activities.

Representation: describes the ability of a species to adapt to changing environmental conditions. It can be measured by the breadth of genetic or environmental diversity within and among populations and gauges the probability that a species is capable of adapting to environmental changes. The more representation, or diversity, a species has, the more it is capable of adapting to changes (natural or human caused) in its environment. In the absence of species-specific genetic and ecological diversity information, we evaluate representation based on the extent and variability of habitat characteristics across the geographical range.

Rosette: a circular arrangement of leaves, petals, or flowers.

Scarification: physical based methodology used to break seed dormancy and aid in seed germination.

Stratification: temperature-based methodology used to break seed dormancy and aid in seed germination.

Self-compatibility: a mechanism that allows pollen from one flower to fertilize another flower on the same plant.

Self-incompatibility: a mechanism that prevents pollen from one flower from fertilizing another flower on the same plant.

Self-pollination: the pollination of a flower by pollen from the same flower or from another flower on the same plant.