DEMOGRAPHICS, LIFE CYCLE, HABITAT CHARACTERIZATION AND TRANSPLANT METHODS FOR THE ENDANGERED ORCHID,

Spiranthes parksii CORRELL

A Thesis

by

JONATHAN RYAN HAMMONS

Submitted to the Office of Graduate Studies of Texas A&M University in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

December 2008

Major Subject: Rangeland Ecology and Management

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Approved by:

Chair of Committee,Fred E. SmeinsCommittee Members,William E. RogersCharles T. HallmarkKeven G. Whisenant

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ABSTRACT

Demographics, Life Cycle, Habitat Characterization and Transplant Methods for the Endangered Orchid, *Spiranthes parksii* Correll. (December 2008) Jonathan Ryan Hammons, B.S., Texas A&M University Chair of Advisory Committee: Dr. Fred E. Smeins

Spiranthes parksii Correll is an endemic terrestrial orchid to the Post Oak Savannah of East Texas and is currently listed as federally endangered. The construction of Twin Oaks landfill, approximately 20 km east of College Station, TX, will destroy an estimated 379 *S. parksii* individuals and 44.7 ha of its habitat. Research has been funded to mitigate for this loss and includes documenting demographics, life cycle, local and landscape habitat, and on-site transplantation of *S. parksii*.

Results found that *S. parksii* was highly variable between years at Twin Oaks and might be due to seasonal rainfall in rosette and early flowering growth. It was also found variable in its production of a rosette and influorescence from year to year. Individual plants were found to occur farther from drainages in higher count years, probably due to soil moisture, although further research should be conducted to confirm this hypothesis.

A significant difference (p = 0.026) was found for percent canopy cover > 2 m above 1 m x 1 m quadrats with and without *S. parksii*, with a mean of 55 percent with *S. parksii* and 97 percent without *S. parksii*. A Non-Metric Multidimensional Scaling ordination revealed three different combinations of herbaceous species that occur with *S. parksii*, which were driven by the presence of three dominant bunch grasses of the Post Oak Savannah: *Schizachyrium scoparium, Chasmanthium laxum* var. *sessiliflorum*, and *Andropogon ternarius*. A close to significant difference (p = 0.07) was found for the leaf litter depth between quadrats with and without *S. parksii*, with a fewer number of stacked leaves with *S. parksii*.

Analysis of aerial images indicated woody encroachment on Twin Oaks from 1958 to 2004 in areas that have not been mechanically cleared. Additionally, *S. parksii* was found to persist in an open savannah landscape and likely occurred in the same locations and more widespread in 1958 than are currently found.

Transplantation of *S. parksii* was documented to be successful by a soil-intact method. While a bare-root method showed success with *S. cernua*, no conclusions can be made of its success for *S. parksii* due to a low sample size (n = 10).

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CHAPTER I

INTRODUCTION AND OBJECTIVES

Spiranthes parksii Correll (Navasota ladies'-tresses) is a terrestrial orchid endemic to Central and East Texas and is currently listed as federally endangered (USFWS 1982). The primary threat to *S. parksii* is loss and fragmentation of habitat due to anthropogenic causes, such as urban sprawl, agricultural activities, lignite mining, landfills and other disturbances (USFWS 1982; Wilson 2006b). Little published research exists on this orchid and most information on the species has been derived from observations (Correll 1950; Luer 1975; Catling & McIntosh 1979; USFWS 1980, 1982, 1984, 1993, 2006, 2007; Bridges & Orzell 1989; Parker 2001; Wilson 2006b). To make regulatory and ecological management decisions, scientists and officials require improved scientific data on the species' biological and ecological characteristics. Some data have been published on the genetics of *S. parksii* and a closely related species, *Spiranthes cernua* (L.) Rich., but results have not produced definite data on genetic differences between *S. parksii* and *S. cernua* (Walters 2005; Dueck & Cameron 2007, 2008).

The Brazos Valley Solid Waste Management Agency (BVSWMA) will construct a new landfill (Twin Oaks) and surrounding facilities that will destroy an estimated 379 *S. parksii* plants and 44.7 ha of its habitat (USFWS 2006). As part of the mitigation requirements of the Biological Opinion issued by the United States Fish and Wildlife

This thesis follows the style of Conservation Biology.

Service (USFWS), BVSWMA is required to fund research for *S. parksii*. The Biological Opinion indicates several areas of research that need to be addressed on *S. parksii*, including monitoring protocols, habitat characterization and transplantation techniques.

It is the goal of this research to provide improved and more complete information on the biology and ecology of *S. parksii*. Specific objectives are:

- Monitor permanently marked individual flowering and rosette forms of *S. parksii* to determine numbers of plants, degree of year to year variation in flowering and rosette formation of individual plants and characterize the phenological dynamics of the life history of the species from season to season and year to year (Chapter IV).
- Characterize the immediate habitat of individual plants based on biotic and abiotic features and compare that with randomly assessed habitat areas (Chapter V).
- 3) Explore historical and current aerial imagery of the site to determine vegetation cover/land use changes and relate that to the current distribution and abundance of *S. parksii* (Chapter VI).
- 4) Test *in situ* bare root and soil intact transplantation procedures to determine survival success of transplanted individuals (Chapter VII).

CHAPTER II

LITERATURE REVIEW

Morphology/Taxonomy

S. parksii is a perennial terrestrial orchid with fleshy tuberous roots which produce a leaf-less flowering stalk that blooms between mid-October to early December (Parker 2001). A rare occurrence of flowering stalks appearing in January has been reported (S. Sultenfuss, personal communication). The individual flowers are cream white at 0.64 cm long and arranged in a single spiral rank on the upper 2–10 cm of a 10– 40 cm stem in numbers from 1 to 30 (Correll 1950; Parker 2001; USFWS 2006; Wilson 2006b). The stem is slender, glabrous below and glandular-pubescent above with several tubular acuminate sheaths (Correll 1950).

Five taxonomic characteristics are used to differentiate the flower of *S. parksii* from its congener, *S. cernua*: 1) the flowers are shorter and cream white as opposed to longer and bright white on *S. cernua*, 2) the flowers are slanted upwards at approximately a forty-five degree angle as opposed to a ninety degree angle from the stalk on *S. cernua*, 3) the lip of each flower is truncated, pointing downwards as opposed to a more flat lip pointing downwards on *S. cernua*, 4) each vegetative flower bract is white-tipped as opposed to green tipped on *S. cernua* and 5) the lateral sepals are upcurved and extend beyond the lower lip and dorsal sepal as opposed to not up-curved

and not extending beyond the dorsal sepal or lower lip in *S. cernua* (Correll 1950; Parker 2001; Wilson 2006b).

After, or rarely during flowering, *S. parksii* may produce a rosette of 2 to 5 leaves which persist until late March or late June, depending on environmental conditions (Parker 2001; Wilson 2006b). Currently, leaves of *S. parksii* cannot be distinguished from leaves of *S. cernua* based on gross morphology or leaf surface features (Wilson 2006b). Therefore, if a rosette of leaves is found, its identification remains unknown as *S. parksii* or *S. cernua*. The rosette stage of development will form a new root tuber, and if sufficient amounts of photosynthate are stored and favorable environmental conditions persist, it will send up a flowering stalk the following season (Wilson 2006b). *S. parksii* individuals will not necessarily produce a flowering plant and/or rosette of leaves every year (Parker 2001; Wilson 2006b). The amount of time that *S. parksii* can remain vegetatively dormant, while skipping over flowering and/or rosette stages, is unknown.

Life Cycle/Development

Terrestrial orchids begin their life cycle as a dust-like seed adapted for wind dispersal, containing only an embryo and surrounding testa. The testa is an important part of a terrestrial orchid's ability to remain dormant. Time of dormancy is unknown, but with the application of hypochlorite to some terrestrial orchid seeds, it still took six months for the testa to decay completely in soil. Thousands of these seeds are produced per fruit on a plant. Terrestrial orchid seeds have been measured to be 0.11 to 1.97 mm long, making them and epiphytic orchids the smallest known seeds in the plant kingdom (Rasmussen 1995). After a seed falls to the soil, it must then become infected by a mycorhizal fungus, usually *Rhizoctonia* spp., in order to germinate (Wells 1981).

A terrestrial orchid's life cycle can be thought of in three main stages of development: protocorm, mycorhizome and adult plant. The protocorm stage is the time from germination until the seedling has a shoot tip with primordial leaves, but no roots. The mycorhizome stage occurs when the apical meristem elongates and the first roots are formed. Mycotrophy in the protocorm stage is obligate, while all other stages of development have differing degrees of mycotrophy depending on the species (Rasmussen 1995). S. parksii's time spent in the protocorm, mycorhizome and adult plant stages are unknown. S. cernua is thought to remain as a protocorm and mycorhizome for as little as one year and to flower the year following the first appearance of a rosette of leaves (Ames 1921). S. spiralis is thought to remain underground in the protocorm and mycorhizome stages for eight years, at which point the mycorhizome dies and forms a root tuber, then produces a rosette in the 11th vear and inflorescence in the 13th to 14th year (Wells 1967). While S. spiralis is thought to become independent of mycorrhizae when the root tubers are formed, root tubers of S. cernua were found to be infected at all stages of growth, including flowering (Wells 1967; Pileri 1998).

Studies on terrestrial orchid populations have documented high variation in number of flowering individuals from year to year and an inconsistent production of rosette and influorescence each season by individuals from year to year (Tamm 1972, 1991; Wells 1981; Willems 1989; Farrell 1991; Wells & Cox 1991; Willems & Bik 1991; Antlfinger & Wendel 1997; Parker 2001). Low numbers can be attributed to weather, herbivory, or age of plants, but often it is unknown what causes a decline or increase in plant numbers (Willems 1989; Wells & Cox 1991; Willems & Bik 1991; Antlfinger & Wendel 1997). *S. spiralis* was documented as being vegetatively dormant for one year before re-appearing as a flower or rosette and *Dactylorchis incarnata* and *Listera ovata* can remain vegetatively dormant for 3 years before reemerging (Wells 1967; Tamm 1972). Longevity of *S. parksii* is unknown and is a pressing concern to preserve existing individuals. Carl Olof Tamm (1972) documented some individuals of the terrestrial orchid *Dactylorchis sambucina* (L.) Vermln. to live at least thirty years.

Two cohorts of *S. parksii* individuals, followed 5 and 6 years, have been monitored and both cohorts showed individual plants flowering once or twice, followed by a decline in rosette and flower production of those individual plants (USFWS 1993). These data might suggest that *S. parksii* is not likely to persist much longer after flowering. However, this finding cannot be considered conclusive given the short duration of the study and an overall low productivity on the site in low count years (USFWS 1993). Antlfinger and Wendel (1997) reported 18 of 40 *S. cernua* individuals flowered at least 5 out of 9 years. This *S. cernua* cohort was reported as having five main categories of flowering: 1) almost every year, 2) every other year, 3) every 3 years, 4) 4 to 5 years and 5) rarely (Antlfinger & Wendel 1997). Additional data in this study suggests that the size of *S. cernua* individuals correlate to its re-appearance in influorescence and rosette stages as plants of *S. cernua* with 16 – 30 flowers produced a rosette the following season at a higher frequency than those with 31 or more flowers, while those plants with 31 or more flowers were more likely to reproduce in subsequent years (Antlfinger & Wendel 1997). *S. cernua* plants with more flowers were found to be more likely to produce another inflorescence in subsequent years than those with fewer flowers (Antlfinger & Wendel 1997). This suggests that plants with fewer flowers have a lower amount of reserves for flowering and must become vegetative in subsequent years to compensate for reproduction, while those with more flowers have sufficient reserves to cover the cost for subsequent years of reproduction. By comparing rosette sizes before and after a flowering season, rosette sizes of *S. cernua* plants after the flowering season that did flower had a smaller increase or larger decrease in size from the previous rosette season than those rosettes that did not flower, suggesting a cost of reproduction (Antlfinger & Wendel 1997).

Other terrestrial orchids such as *Cordula* spp. and *Pogonia verticillata* (Willd.) Nutt. (*Isotria verticillata* Ref.) will take time to establish a well developed and/or maximum rosette size before producing an influorescence. In subsequent years the size of these species' rosette and influorescence remain relatively the same (Ames 1921). *S. cernua* was seen to produce an influorescence before its rosette reached a maximum size, as the rosette and influorescence became significantly larger in subsequent years (Ames 1921; Antlfinger & Wendel 1997). Therefore, an influorescence of *S. cernua* is thought to contribute to the maturation process of an individual plant. Further information indicates an inflorescence of *S. cernua* can contribute up to fifty percent of its own carbon requirement and 8.4% of the total plant annual carbon requirement (Wendel 1994). Even though the size of a *S. cernua* rosette would not indicate inflorescence production the following season, a minimum rosette size for flowering in was found (Antlfinger & Wendel 1997).

Distributional Habitat/Ecology

S. parksii occurs primarily in the Post Oak Savannah region of Texas, but has also been found in the Pineywoods region (Diggs et al. 1999). Current populations are known to occur in thirteen Central and East Texas counties including Bastrop, Brazos, Burleson, Fayette, Freestone, Grimes, Jasper, Leon, Limestone, Madison, Milam, Robertson, and Washington (TPWD 2006). Care should be taken in labeling some of these counties since the identification of the plant has not been confirmed by an expert in some of them. The associated vegetation within the Pineywoods' occurrences are very similar to other places of *S. parksii*, containing post oak (*Quercus stellata*), farkle-berry (*Vaccinium arboreum*), yaupon (*Ilex vomitoria*), and longleaf woodoats (*Chasmanthium sessiliflorum*) (Bridges & Orzell 1989).

Tree and shrub encroachment in a temperate savannah system has been documented as having negative effects on other terrestrial orchids, including *S. spiralis* (Farrell 1991; Tamm 1972, 1991). Over 1000 individuals of *S. parksii* have been documented after bull dozing events in a forested area (J. Thomas, personal communication). *S. parksii* was rare or scarce in this area prior to bull dozing. This observation has led to the hypothesis that *S. parksii* was once well established in many areas, but as a result of woody encroachment does not persist aboveground in a flowering stage.

S. parksii is highly correlated with areas of patchy openings in savannahs and woodlands and exposed soil along upland ephemeral and intermittent streams in the Post Oak Savannah (Parker 2001; USFWS 1993). *S. parksii* is seldom found within floodplains or large open grasslands (USFWS 1993; Parker 2006). Moist sandy soils with a shallow claypan close to upland drainages on slopes are thought to provide more plentiful moisture as subsurface flow occurs after rain (USFWS 2006). Seasonal rainfall patterns are thought to influence populations, especially in the spring and fall seasons, although this does not fully explain the number of flowering plants in a given year (USFWS 1993; Wilson 2006b). Rainfall eight to ten weeks before flowering has been suggested as critical for the flowering of *S. parksii* (Parker 2001).

Transplantation

Terrestrial orchids are well known for their association with mycorrhizae for germination, growth and survivability (Rasmussen 1995). Due to this association, transplantation methods should be chosen to incorporate a microenvironment of soil and mycorrhizae. Mature plants of *S. cernua*, *S. gracilis* and *S. beckii* were all noted as not containing any mycorrhizal fungi except for a small area at the base of the stem (Ames 1921). However, recent work has shown that mycorrhizae were present in low levels of the mature roots and higher levels in the bud root of *S. cernua* plants (Pileri 1998). Pileri (1998) noted that after excavating *S. cernua* plants to analyze the root tubers for mycorrhizal infection, all but one plant that was destroyed by a small mammal survived transplantation by reappearing the next year. She also noted that they were better able to survive when transplanted during the vegetative or early reproductive phases (Pileri 1998). *S. parksii* showed positive responses to soil-intact transplanting by using 15.2 or 20.3 cm aluminum irrigation pipe (K. Parker, personal communication, Parker 2001). *S. parksii* grown *in vitro* and transplanted to areas of *S. parksii* occurrences were not successful (K. Parker, personal communication). The seedlings were noted as having irregular large root tubers due to optimal greenhouse conditions, and for this reason were unable to adapt to harsher native field conditions (K. Parker, personal communication). It was suggested that a more successful strategy to grow *S. parksii* in the greenhouse would be to establish plants in native soil and impose a dry period to mimic field conditions (K. Parker, personal communication).

CHAPTER III

STUDY AREA

The Brazos Valley Solid Waste Management Agency (BVSWMA) of College Station, TX will construct the new Twin Oaks landfill. The construction of this landfill will destroy an estimated 379 *S. parksii* plants and 44.7 ha of its habitat. Research has been funded by BVSWMA to conduct scientific research to off-set the impacts of *S. parksii*, as issued by the USFWS in the Biological Opinion. The 246 ha Twin Oaks landfill site is located in Grimes County, TX on the south side of SH 30, approximately 6.4 km west of Carlos, TX and 20 km east of the center of College Station (96°8'51.86''W, 30°35'47.25''N) (Fig. 3.1) (HDR 2002a). Surrounding the 86 ha landfill footprint are 56 ha of deed restricted areas that are set aside for the conservation of *S. parksii* (USFWS 2006). Within these 56 ha, 45 ha is believed to be *S. parksii* habitat (USFWS 2006).

Twin Oaks is located within the Subtropical Humid zone of Texas (Larkin & Bomar 1983). College Station, TX receives approximately 100 cm of annual precipitation (SRCC accessed 2008). The bulk of this rainfall is concentrated in spring and fall with the lowest average in July (SRCC accessed 2008). The coldest temperatures generally occur in January (average minimum 4.32°C (39.78°F)) and steadily increase to the hottest temperatures in August (average maximum 35.67°C (96.2°F)) (SRCC accessed 2008). Occurrences of snow and ice are rare (SRCC accessed 2008).

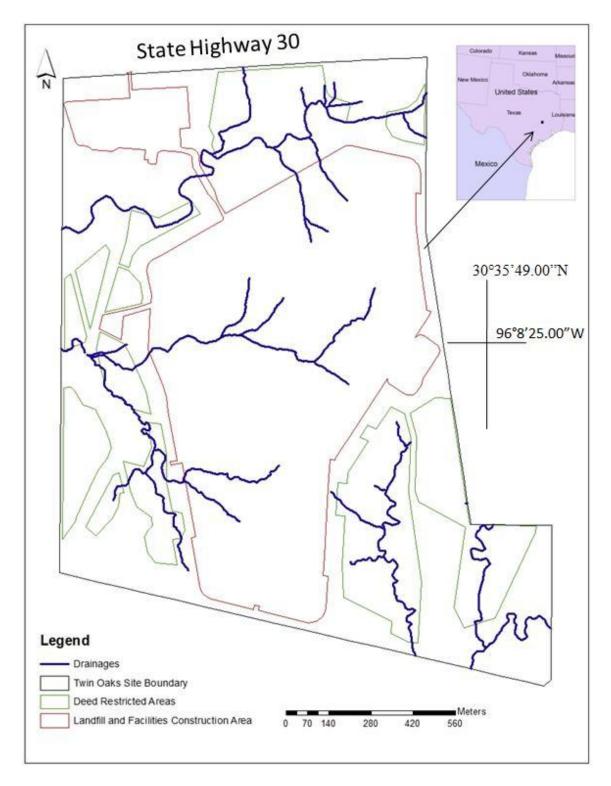


Figure 3.1. Location and layout of the Twin Oaks landfill.

Twin Oaks is located within the Navasota River watershed. There are 10 intermittent and 17 upland ephemeral stream channels (10,306 m), 1 wetland (0.004 ha) and 1 on-channel pond (0.15 ha) within the 246 ha site (HDR 2002b).

Most of the site is currently an open to dense savannah woodland with post oak (*Quercus stellata*¹), yaupon (*Ilex vomitoria*), American beautyberry (*Callicarpa americana*), farkle-berry (*Vaccinium arboreum*), cedar elm (*Ulmus crassifolia*), little bluestem (*Schizachyrium scoparium*) and longleaf woodoats (*Chasmanthium laxum* var. *sessiliflorum*). The flora is diverse with large numbers of grasses and forbs. Some parts of the site consist of grasslands formed from bulldozing to promote grass growth for cattle and the installation and maintenance of transmission lines and one gas pipeline. Most of the dense woodland is concentrated around drainages, although some can be found in the upland areas. The tree/shrub species in the upland thickets are very similar to those in the savannah, whereas, the tree/shrub species around the drainages include water oak (*Quercus nigra*), willow oak (*Quercus phellos*) and hickory species (*Carya spp.*) (HDR 2002a).

Twin Oaks' geology consists of the Wellborn Formation (Eocene) and Alluvium (Holocene) (BEG 2007). The Wellborn Formation is predominantly sandstone and clay with a thickness of ± 50 meters (BEG 2007). Most of the intermittent and ephemeral streams contain small amounts of alluvium, but, Alum Creek, running east and west in the northern portion, is the only portion on the site that has alluvial soils mapped (NCSS 2007).

^{$\overline{1}$} Follows the taxonomic nomenclature of Diggs et al. 1999.

Soils of the Twin Oaks site are primarily mapped as Burlewash fine sandy loam at 1 to 5 and 5 to 12 percent slope (NCSS 2007). Other soil series are Boonville, Hatliff, Koether-Rock, Padina, Robco, Shiro and Tabor (NCSS 2007). These soil series are mostly loamy fine sand and fine sandy loams, that are moderately to well-drained, have low to moderate available water capacity, and moderate to severe hazard of erosion (USDA 1996). Most are suited to range, pasture and wildlife use, while only a few could be used for cropland.

Bobby J. Trant (personal communication) is the previous owner of the property and provided the following account of land use on the area. Twin Oaks was previously used for moderate grazing (an estimated 1 animal unit per 8 or 9 acres) and wild game hunting in the last 50 to 60 years. An estimated 1 or 2 white-tail deer (*Odocoileus virginianus*) and 20 feral hogs (*Sus scrofa*) were taken from the site each year in the last 50 or 60 years. Cotton farming most likely occurred in the 1920s on a small portion of the site where an old homestead was located since a cotton gin used to exist just south of Twin Oaks around the same time. Prescribed burning has occurred on selected areas once every three years during the last 50 to 60 years. Tree ringing occurred in the 1940s and 1950s to increase pasture for cattle grazing. As a result, dead trees would remain on the ground for about two years and be burned during prescribed burning practices. Bull dozing has occurred in portions of the site and seeded with bahiagrass (*Paspalum notatum*).

CHAPTER IV

LIFE HISTORY AND DEMOGRAPHICS

Introduction

S. parksii is perennial and has a unique annual growth cycle. It produces an influorescence for a short time (approximately 1 month) in the fall, followed by a basal rosette from mid-winter to summer and is subterranean during the summer. Appearance and disappearance of these have not been evaluated in detail. Additionally, little data exist on the seasonal and annual variation in plants that flower and produce rosettes, as well as its habitat association. It is the purpose of this chapter to provide documentation of these features of the species.

Results from this can give a better insight into the longevity of *S. parksii*. Also, these results, which are on undisturbed individuals, can be compared to transplanted individuals to better determine the success of those transplanted individuals. It has been stated that *S. parksii*'s vegetative stage can be dormant for 5 years, before reemerging (Parker 2001). The source of this information was further looked into and found to be invalid. Even if an individual plant did not appear for five years, but then re-appeared from the same spot in the soil, it would be hard to determine if it was the same individual, since another individual could have established in the same spot (H. Wilson, personal communication).

Methods

To address Objective 1, surveys were conducted during peak flowering (mid-October to mid-November) and rosette stages (March – April) of *S. parksii* at Twin Oaks to locate individuals. Much of the surveying was conducted in areas where HDR Inc., who found 774 *S. parksii* in 2001, found *S. parksii*, while areas in between were also haphazardly surveyed. Individual plants were located and marked with a 38.1 cm aluminum stake flag placed exactly 30.5 cm north of the plant, or at any other compass direction and distance if the stake flag would possibly pierce a root tuber of another *Spiranthes* spp. or land at any other unsuitable site that would jeopardize the ability to re-locate the plant from the stake flag. Most individuals were logged into a GPS. Some individuals were only marked with a stake flag marker and thought to be easily relocated by landscape reference points to later log into a GPS, however, they were unable to be re-located, and therefore were not logged into a GPS. Individual plants initially found in rosette form were unknown as *S. parksii* or *S. cernua* since they cannot be differentiated from one another, except when in full flower.

The following data were recorded on *S. parksii* and *S. cernua* individuals in 2006: date found in full flower, date fruits began to dehisce, number of plants eaten, number of plants producing a rosette on 21 November 2006, plant height, stem diameter at 5 cm from the soil surface, number of flowers, number presenting a rosette in 2007 (*S. cernua* not recorded), number which flowered again in 2007, and number presenting a rosette in 2008. An independent samples t-test was run to determine differences

between plant height, stem diameter at 5 cm, and number of flowers per plant. Observations were made on individuals as time permitted, therefore, not all individuals in each cohort had an observation recorded for each parameter.

For all other individuals found in rosette and/or flowering stages, presence and absence data were recorded, as well as data on herbivory, size of rosettes, canopy cover relationships, and other pertinent observations. Herbivory was estimated to the nearest percent, the size of rosettes was recorded as the sum of all leaf lengths of the rosette, and canopy cover relationships were determined by the percent shade to the south an individual receives throughout the day. Tabular and graphical presentations were used to describe life history and demographic patterns and trends.

Rainfall data were received from Texas Municipal Power Agency (TMPA) approximately 6.4 km northeast for correlation of annual counts of *S. parksii* at Twin Oaks, while rainfall data from Easterwood Airport (approximately 12.9 km southwest) were used for correlation with annual counts of *S. parksii* at Indian Lakes.

Results

Flowering Individuals

Annual counts of *S. parksii* individuals were 18 in 2006 and 118 in 2007; one from 2006 re-flowered and is included in the 2007 count. Data from previous surveys obtained from HDR Inc. yielded 32 and 774 *S. parksii* individuals in 2000 and 2001,

respectively (Fig. 4.1) (USFWS 2006). The fifteen *S. parksii* found in 2006 that were relocated occurred in close proximity to drainages, whereas many found in 2007 were located away from drainages (Fig. 4.2). Most *S. parksii* found in 2006 and 2007 occurred on two main soil types: Burlewash fine sandy loam, 1 to 5 percent slopes and 5 to 12 percent slopes, as mapped by the National Cooperative Soil Survey (NCSS) (Table 4.1). Although only a few data points are available, rainfall in August and rainfall during rosette growth and the beginning of influorescence production (previous year's December through the respective year's June, August and September) showed high correlations with number of *S. parksii* individuals found in 2000, 2001, 2006 and 2007 at Twin Oaks. Total annual rainfall did not show as high of a correlation (Fig. 4.3).

Plant trait measurements and phenological development were similar for *S*. *parksii* and *S. cernua* in fall of 2006, except that more flowering individuals of *S. cernua* formed a rosette on 21 November 2006 (40%) than individuals of *S. parksii* (13.3%) (Table 4.2). Mature height, stem diameter at 5 cm from the soil surface, and number of flowers per influorescence were not significantly different.

Rosettes

Rosettes of *S. parksii* and *S. cernua* cannot reliably be distinguished based on leaf traits. Surveys for these rosettes were mainly concentrated around previously found *S. parksii* or *S. cernua* flowering individuals, while other areas of known *S. parksii* occurrences were surveyed, therefore, the numbers given do not represent all of Twin

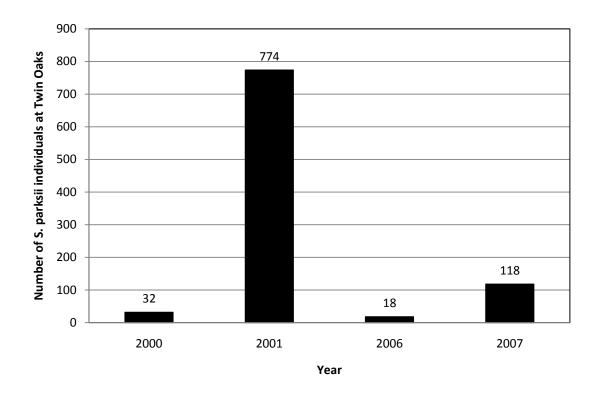


Figure 4.1. Annual counts of S. parksii individuals at Twin Oaks landfill.

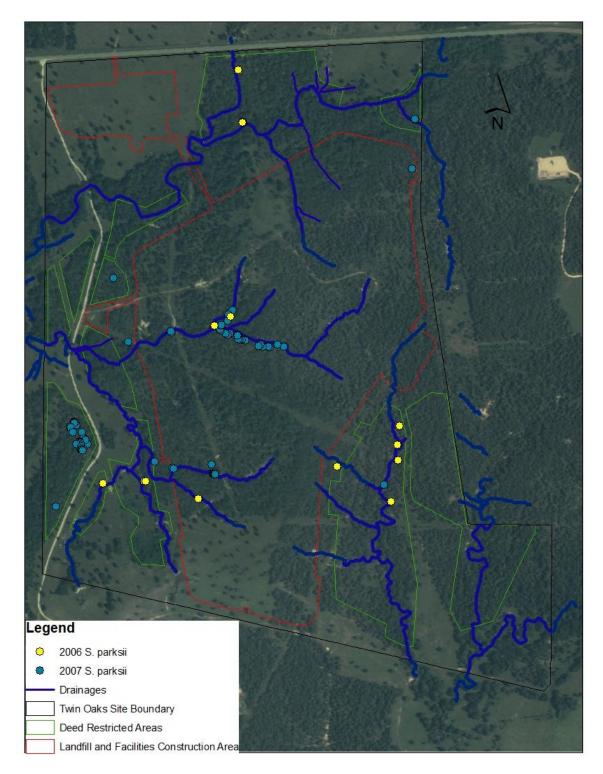


Figure 4.2. Distribution of flowering S. parksii *found in 2006 and 2007 at Twin Oaks.* 15 S. parksii *from 2006 mapped. 96 of 118 mapped from 2007. Some dots represent more than one individual, due to them occurring in close proximity.*

Soil Type	2006 S. parksii	2007 S. parksii
Burlewash fine sandy loam, 1 to 5 percent slopes		
Number	3	33
Number per ha	0.03	0.32
Burlewash fine sandy loam, 5 to 12 percent slopes		
Number	11	48
Number per ha	0.22	0.98
Burlewash-Gullied land complex, 5 to 15 percent slopes		
Number		4
Number per ha		1.16
Hatliff fine sandy loam, frequently flooded*		
Number	1	5
Number per ha	0.05	0.23
Robco loamy fine sand, 1 to 5 percent slopes		
Number		5
Number per ha		0.24
Shiro loamy fine sand, 1 to 5 percent slopes		
Number		1
Number per ha		0.07

Table 4.1. Number of *S. parksii* individuals found in 2006 and 2007 with the respective soil type at Twin Oaks.

* Soil was not seen as frequently flooded; due to an error on soil map. Most likely occurred on the adjacent soil: Burlewash fine sandy loam, 1 to 5 percent slopes.

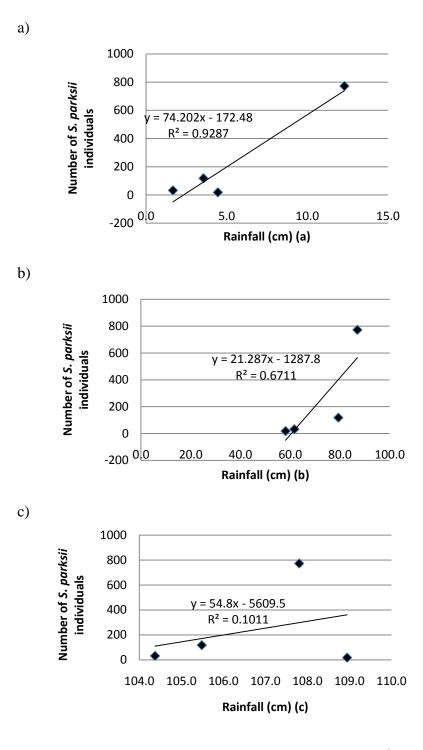


Figure 4.3. Simple linear regression with coefficient of determination (\mathbb{R}^2) values of S. parksii individuals found at Twin Oaks in 2000, 2001, 2006 and 2007 with a) August rainfall (p=0.036), b) previous year's December through June and August and September (p=0.18), and c) annual rainfall (p=0.68).

Table 4.2. Flowering stalk comparisons, plant trait measurements and phenological development data collected in fall of 2006 and subsequent seasons for *S. parksii* and *S. cernua* individuals. T-test p-values for plant height, stem diameter and number of flowers per plant are 0.25, 0.77 and 0.35, respectively.

Observations/Measurements	2006 S. parksii	2006 S. cernua
Found in full flower	25 Oct 2006 - 30 Oct 2006	25 Oct 2006 - 4 Nov 2006
Fruits dehiscing	21 Nov 2006	21 Nov 2006
Eaten by 11/22/06	2 of 15 (13.3%)	4 of 25 (16%)
Rosette present on 11/21/06	2 of 15 (13.3%)	10 of 25 (40%)
Plant height (cm)	21.26 (SE 1.06)	22.92 (SE 1.25)
Stem diameter (mm)	1.62 (SE 0.09)	1.65 (SE 0.05)
Number of flowers/plant	11.58 (SE 1.48)	12.52 (SE 1.06)
Rosette present in 2007	10 of 15 (66.7%)	(not monitored)
Flowering in Fall 2007	1 of 15 (7.6%)	3 of 29 (10.3%)*
Stake Flags displaced	3 of 15 (20%)	5 of 34 (14.7%)*
Rosette present in Spring 2008	6 of 10 (60%)**	15 of 29 (51.7%)*

* Additional individuals were re-located

** While the general vicinity (w/in .5m) is known for 5 of 15, can only be sure on location of 10 due to flag displacement by large mammals

Oaks. Five hundred forty-one and 276 rosettes of unknown *S. parksii* or *S. cernua* species were located in 2007 and 2008, respectively (Fig. 4.4). Of these individuals, 450 from 2007 and 156 from 2008 were logged into a GPS and most occurred on Burlewash fine sandy loam, 1 to 5 and 5 to 12 percent slopes, similar to *S. parksii* found in 2006 and 2007 (Table 4.3). Two hundred four of the 541 rosettes in 2007 were selected across the site to be continually monitored throughout the rosette stage in 2007 (Fig. 4.4). Seventy-seven percent had disappeared by 25 June 2007, while some of the remainder persisted until mid-September (Fig. 4.5). During the fall of 2007, twenty-six (13%) of the 204 rosettes produced an influorescence; 15 were *S. parksii*, 9 were *S. cernua*, and 2 were a closed flower form of *S. cernua* (Table 4.4). Rosettes that flowered as *S. parksii* showed a similar disappearance as the other rosettes monitored in 2007. On average, those rosettes that flowered as *S. cernua* persisted longer and 3 of them had a rosette present at the emergence of a flowering stalk on 11 September (Fig. 4.6).

Average total leaf lengths of *S. parksii* rosettes measured on 3 April 2007 that flowered the fall of 2007 were twice the length of rosettes that did not flower the following fall. Due to a low sample size, a high standard error, and uncertainty of a peak rosette growth, no definite conclusions can be made regarding the size of a rosette indicating flowering the following fall for an individual (Table 4.5).

Due to a loss of some stake flag markers, 193 (94.6%) of the original 204 were re-located to census for a rosette in 2008. Seventy-seven percent (149) produced a rosette. None of these rosettes persisted beyond 5 July 2008. In addition to the 193 of

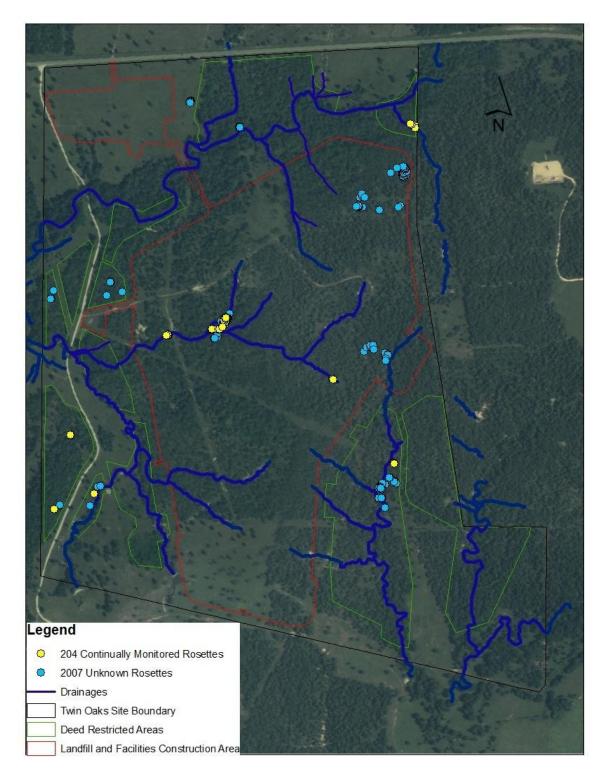


Figure 4.4. Distribution of 450 of 541 unknown species' rosettes located and marked in 2007 and 204 that were chosen to be continually monitored. Each dot may represent several individuals.

Soil Type	2007 Rosettes	2008 Rosettes
Burlewash fine sandy loam, 1 to 5 percent slopes		
Number	91	20
Number per ha	0.87	0.19
Burlewash fine sandy loam, 5 to 12 percent slopes		
Number	269	107
Number per ha	5.47	2.18
Hatliff fine sandy loam, frequently flooded*		
Number	8	12
Number per ha	0.37	0.56
Robco loamy fine sand, 1 to 5 percent slopes		
Number	15	2
Number per ha	0.73	0.56
Shiro loamy fine sand, 1 to 5 percent slopes		
Number	67	15
Number per ha	4.44	0.99

Table 4.3. Number of rosettes found in 2007, including ones that flowered as *S. parksii* and *S. cernua* the following fall, and unknown species' rosettes in 2008 with the respective soil type at Twin Oaks.

* Soil was not seen as frequently flooded; an error on soil map. Most likely occurred on the adjacent soil: Burlewash fine sandy loam, 1 to 5 percent slopes.

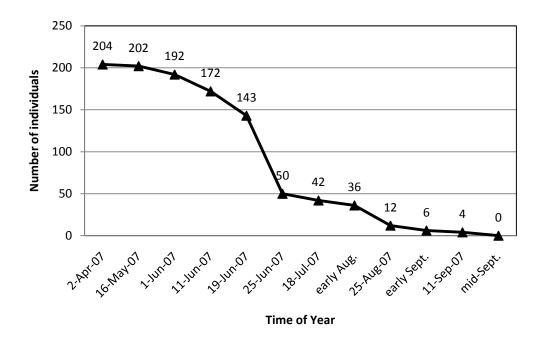


Figure 4.5. Number of unknown S. parksii or S. cernua rosette individuals (n=204) by date, 2007.

Table 4.4. Numbers and percentages of 204 unknown *S. parksii* and *S. cernua* rosettes that flowered the following flowering season per species.

Species	Number	Percentage
S. parksii	15	7
S. cernua	9	4
S. cernua - peloric form	2	1

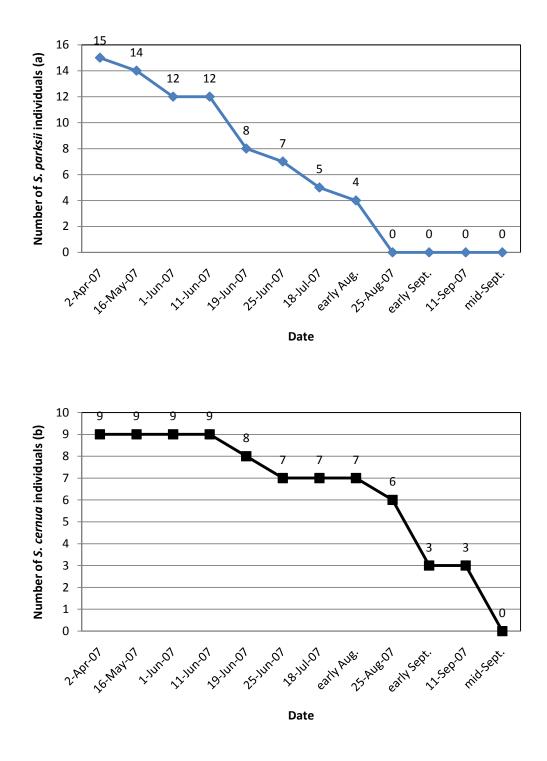


Figure 4.6. Number of a) S. parksii rosette individuals (n=15) and b) S. cernua rosette individuals (n=9) present by date prior to flowering in fall of 2007.

Table 4.5. Comparison of total leaf length (cm) for rosettes in spring of 2007 that flowered as *S. parksii* in fall of 2007 and those that did not flower in fall of 2007 that remain as unknown *S. parksii* or *S. cernua*.

Species	n	Min	Max	Mean	SD	SE
Unknown	44	2	30	12.8	5.8	0.9
S. parksii	7	7.1	39.1	22.5	10.5	4

the original 204, 183 more of the 541 unknown rosettes from 2007 were checked for presence or absence of rosette in the 2008 season, which yielded the presence of 237 (63%) presenting a rosette again in 2008.

Average total leaf length, measured on 14 March 2008, for *S. parksii* rosettes that previously flowered were longer by 2.7 cm than unknown *S. parksii* or *S. cernua* rosettes that did not flower (Table 4.6). Further conclusions cannot be made since it is not known if all rosettes first presented themselves aboveground at the same time or how much longer they persisted (i.e. if they were in peak growth).

Herbivory

Estimated percent herbivory was recorded for unknown and *S. parksii* rosettes in 2008 on 8 February and 8 March where individuals on each date were exclusive of each other. Individuals with herbivory increased from 8 February to 8 March in both populations, although the percent herbivory per individual only increased for the unknown *S. parksii* or *S. cernua* rosettes and not the *S. parksii* rosettes (Table 4.7).

Eight different types of herbivory were also recorded: 1) Edges – edges of the leaves consumed, 2) Middle – holes eaten through the leaves, without disturbing the edges, 3) Top half – a clean bite would remove the top half of the leaf, 4) Whole leaf – leaf bitten off at the base, 5) Whole plant – all leaves bitten off at the base, 6) Yellow leaf – the leaf would be yellow, 7) Brown leaf – the leaf would be brown, and 8) Leaf fungus – where yellow and brown holes formed throughout the leaf surface. The

Table 4.6. Total leaf length (cm) for unknown *S. parksii* or *S. cernua* rosettes that did not flower the previous flowering season and *S. parksii* rosettes that did flower the previous flowering season; measured on 14 March 2008.

Species	n	Min.	Max.	Mean	SD
Unknown	47	4	23	9.9	3.9
S. parksii	29	3	23	12.6	4.2

Table 4.7. Herbivory in 2008 for unknown *S. parksii* or *S. cernua* rosettes and known *S. parksii* rosettes on 8 Feb. and 8 Mar.

Species	n	Number with herbivory	Percent with herbivory	Mean % herbivory per individual
Unknown Rosettes 8 Feb 2008	115	18	15.7	1.7 (SD 6.9)
Unknown Rosettes 8 Mar 2008	172	105	61.0	17.3 (SD 28.9)
S. parksii Rosettes 8 Feb 2008	36	13	36.1	22.6 (SD 29.5)
S. parksii Rosettes 8 Mar 2008	27	16	59.3	22 (SD 34)

percentage of unknown *S. parksii* or *S. cernua* and *S. parksii* rosette individuals having each type of herbivory were recorded throughout the rosette season (Table 4.8). In both populations, "edges" occurred most frequently, while "top half" and "whole leaf" followed as being the next most frequent (Table 4.8).

Growth Cycle

Most flowering *S. cernua* and *S. parksii* stalks emerged by 11 September 2007. Flowering *S. parksii* were found in full flower from 17 October 2007 to 27 November 2007, although most were found 28 October to 1 November. The lower fruits began to dehisce from 27 November 2007 to 13 December 2007 with the later flowering individuals' fruits dehiscing later. Rosettes began appearing as early as 5 January 2008 from flowering *S. parksii* plants with either an absent or dead flowering stalk, although some didn't appear until mid-late January. Unknown *S. parksii* or *S. cernua* plants that did not flower in 2007 produced a rosette as early as 26 November 2008.

All rosettes from flowering *S. parksii* had senesced by 5 July 2008 (Fig. 4.7). All rosettes marked in 2007 that flowered as *S. parksii* in 2007 also presented a rosette in 2008 except for one that did not present a rosette in 2008 (n = 21). Six (7%) out of 83 *S. parksii* that were found in 2007 did not produce a rosette after flowering, whereas, the remaining 77 (93%) did.

Table 4.8. Data for the percentage of individuals with each type of herbivory for
unknown S. parksii or S. cernua rosettes and S. parksii rosettes in spring of 2008.

Species	n	edges	middle	-			brown leaf	·	leaf fungus
S. parksii	20	50	5	20	40	10	5	5	
Unknown	180	52.2	18.3	29.4	18.9	6.7	1.7	5.0	1.1

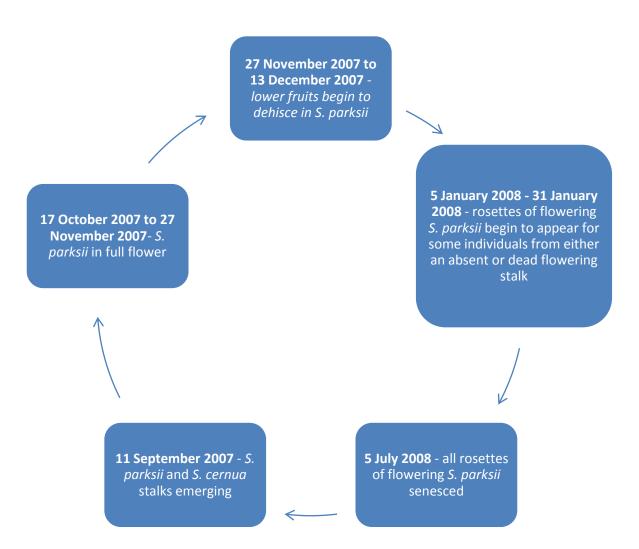


Figure 4.7. Growth cycle for the 2007 flowering season and 2008 rosette season of **S**. parksii.

No *S. parksii* or *S. cernua* flowering stalks, and few unknown *S. parksii* or *S. cernua* rosettes, were found to occur in 0 to 40 percent shade. Most individuals in all three population samples were found to occur in 41 to 60 percent shade throughout the day, while some occurred in 61 to 80 percent and 81 to 100 percent shade (Fig. 4.8).

Discussion

While *S. parksii* is thought to only occur in close proximity to drainages in the Post Oak Savannah, *S. parksii* individuals have been found up to 305 m from drainages (USFWS 2006). For this study, environmental conditions were more favorable for *S. parksii* in 2007 as 100 more individuals were found and occurred farther up in the landscape along the upper segments of drainages than individuals found in 2006 (Fig. 4.2). This is most likely due to more rainfall in the previous season's rosette stage and early flowering, as there was approximately 20 cm more rainfall in these periods for 2007 individuals than 2006 individuals (Fig. 4.3). Smaller drainages not mapped in Figure 4.3 are seen around populations of *S. parksii* from 2007, particularly the large population in the southwestern portion. The *S. parksii* individuals found in 2007 farthest from any drainage (including the smaller drainages not mapped in Figure 4.3) are the southwestern most individual and the individual just south of the northeastern

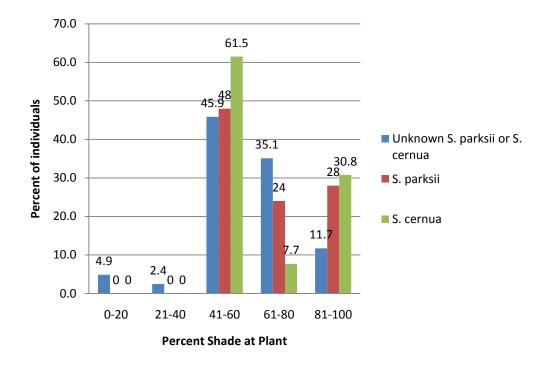


Figure 4.8. Percentage of unknown S. parksii or S. cernua (n=205), S. parksii (n=25), and S. cernua (n=13) individuals and their estimated percent shade received throughout the day with a slight southern tilt of the sun between 8 March 2008 to 8 April 2008.

most individual. The drainage closest to these individuals can be seen as one of the drainages mapped and are approximately 82 m from each individual (Fig. 4.2).

Additionally, the soils should be assessed in more detail at Twin Oaks, rather than relying on county soil surveys. By relying on the county soil surveys alone, an associated soil type for *S. parksii* may not be accurately conveyed. Upon observation of the *S. parksii* individuals occurring on the Hatliff fine sandy loam, frequently flooded soil, this area was not seen to be frequently flooded, rather it was a small elevated area around a frequently flooded area.

Rainfall eight to ten weeks before flowering has been suggested as critical for the flowering of *S. parksii* (Parker 2001). Lower and higher deviations from the average rainfall in spring and fall are also thought to be correlated with lower and higher numbers of *S. parksii*, respectively (Wilson 2006b). Rainfall just prior to the emergence of flowering stalks (August) showed the highest correlation with the annual counts of *S. parksii* at Twin Oaks. However, an analysis with annual counts of *S. parksii* from five years at a location approximately four miles southwest of Twin Oaks at Indian Lakes Conservation Easement showed annual counts of *S. parksii* individuals were not correlated with August rainfall (Fig. 4.9). Similar to the correlations at Twin Oaks, rainfall during rosette (previous year's December through June) and early flowering (August and September) showed a close correlation with number of *S. parksii* individuals at Indian Lakes (Fig. 4.9). Differences between the two sites could be due to patchy rainfall events within a given month while over several months they may have

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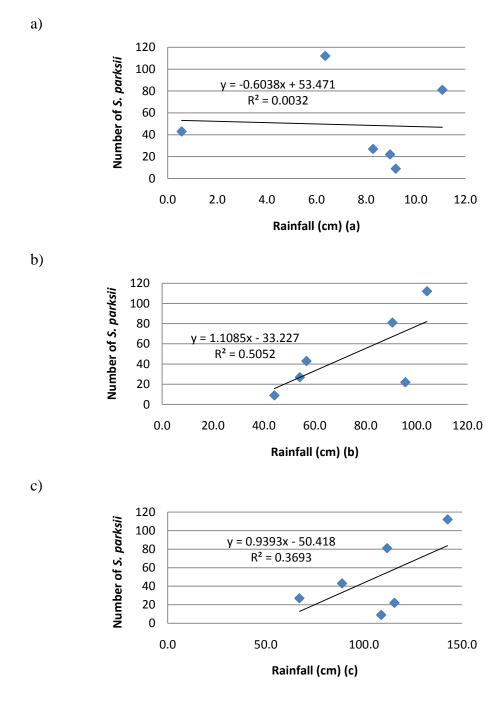


Figure 4.9. Simple linear regression with coefficient of determination (R^2) values of S. parksii individuals found at Indian Lakes in 2000 through 2005 with a) August rainfall (p=0.92), b) previous year's December through June and August and September (p=0.11), and c) annual rainfall (p=0.20).

similar precipitation. More accurate local weather data should be used to correlate rainfall with number of *S. parksii* individuals.

S. parksii typically appears in full flower from mid-October to early December (Parker 2001). While most *S. parksii* were found in late October, some individuals were found in full flower in late November. The area these individuals were found was surveyed in late October and not seen. Also, *S. parksii* individuals found on 25 October 2007 were seen to be forming fruit in the lower flowers, therefore, could have been in full flower as early as late September. While observations suggest that most *S. parksii* peak in late October, it is necessary to expand the survey season to obtain more accurate data on the number of *S. parksii* in a given area. Perhaps the variation in duration and peak of flowering is related to precipitation variation in the fall. Future investigations should obtain site specific rainfall data to document this relationship.

As many as three rosettes emerging from the same place in the soil was seen. This was a rare occurrence, but more commonly, individuals just 1 cm away from the originally marked plant were seen to emerge. Care should be taken when permanently marking individuals in the field, so that the original plant is not confused as being the newer individual just 1 cm away.

Individuals of *S. cernua* or *S. parksii* growing within 1 cm of each other has led many observers to believe these individuals have a rhizome, however, this was not seen upon close examination of individual rosettes growing within 0.5 cm of each other when conducting bare-root transplants. Rather, these individuals were seen to exhibit characteristics of vegetative reproduction. This type of reproduction has been noted in *S*. *spiralis* when the lateral buds on the leafy stem develop separate root tubers and eventually become detached from the parent plant when the connecting stem dies away (Summerhayes 1951; Wells 1967).

Although rosettes of known *S. parksii* and *S. cernua* individuals were examined for morphological differences, none were found. Identifying differences in leaf morphology or other characteristics between the two species' rosette leaves would be useful and should be studied further. Dr. Robert Ferry from Victoria, TX, a retired orchid enthusiast, is currently looking at intercellular characteristics to possibly determine a difference between the two species' leaves.

The different types of herbivory seen across rosette individuals raises the question of what herbivores are attributed to each type. Herbivory from snails was thought to have devastating effects on the survivability of other terrestrial orchids, although the occurrence of a snail was seen only once at a *S. parksii* or *S. cernua* rosette (Tamm 1972). Top Half herbivory was very consistent across rosette individuals and was almost always seen as a clean bite taking the top half of the rosette leaf. Some individuals that were caged in an attempt to perform a greater study on herbivory were seen to have this type of herbivory, while the cage was 46 cm tall with a diameter of 10 cm with 1 cm x 1 cm openings made from 1 mm galvanized wire; therefore, the type of individual to take this type of bite would have to be able access the rosette through the 1 cm x 1 cm openings. Edges was the most frequent and was seen as very small bites.

Tree and shrub encroachment in a temperate savannah system has been documented as having negative effects on other terrestrial orchids, including *S. spiralis*, therefore canopy relationships were evaluated (Farrell 1991; Tamm 1972, 1991). While most individuals occurred from 41 to 100 percent shade throughout the day, it is an estimate and does not take into account leaf area index. More accurate measurements (i.e. light meter) should be conducted to give a more accurate representation of the incoming light around *S. parksii*.

CHAPTER V

LOCAL HABITAT

Introduction

Objective 2 aims to assess the local habitat of *S. parksii* and compare it to areas where *S. parksii* is not found to occur. Within the local habitat, indicator species or a combination of herbaceous species are sought to be identified. Additionally, the percent woody cover above a *S. parksii* and some abiotic factors surrounding are thought to be critical and will be evaluated. This analysis might also indicate that the occurrence of *S. parksii* is not driven by any of these factors and might not occur in some areas due to other reasons, such as the absence of a specific mycorrhiza or a limitation in seed dispersal. The results from this study will also help in identifying suitable habitat for the placement of transplants in the deed-restricted areas on Twin Oaks.

Since the identified and permanently marked individuals of *S. parksii* was limited (18) on the Twin Oaks site, and some were in close proximity to one another, an additional area with identified and permanently marked plants was used to complement the Twin Oaks samples and increase the sample size. This area is a 38 acre conservation easement established by the Texas Department of Transportation (TxDOT) to mitigate for loss of plants due to highway construction in the area. The site is located on the north side of William D. Fitch Parkway (SH 40) approximately 0.5 miles southwest of

the intersection with SH 6 south of College Station, TX. Ongoing research of this site has permanently marked *S. parksii* (15) that can be added to those at Twin Oaks.

Vegetation of the TxDOT site is dominated by a semi-open post oak (*Quercus stellata*) – yaupon (*Ilex vomitoria*) woodland. Soils are similar to Twin Oaks with Burlewash fine sandy loams, 1 to 5 percent slopes, Derly – Rader Complex loams, 0 to 1 percent slopes, Sandow loams and Singleton fine sandy loams. These soil series have a fine sandy loam texture, are poorly to well-drained, have low to high available water capacity, and moderate to severe hazard of erosion, similar to Twin Oaks (USDA 2002).

Methods

Between a 1 m x 1 m quadrat was centered around a known *S. parksii* plant, which was located during the 2006 survey, by aligning two 1-m dowels north-south and east-west with *S. parksii* at the midpoint of each dowel, forming a cross. After this, the midpoint of each four 1 m wooden dowels was placed at the 0.5 m mark from *S. parksii* made by the original two wooden dowels aligned north-south and east-west to make a 1 m x 1 m quadrat (Fig. 5.1). An accompanying quadrat was randomly chosen at a twenty degree compass direction (i.e. 20, 40, 60....360) and between two to fifteen meters from the original quadrat to represent a random location on a small landscape scale. If the random quadrat landed in a creek bed, man-made disturbance, a known *S. parksii* flowering plant or known *S. parksii* or *S. cernua* rosette, another location was randomly drawn. Percent foliar cover (to the nearest percent) was estimated for herbaceous, tree

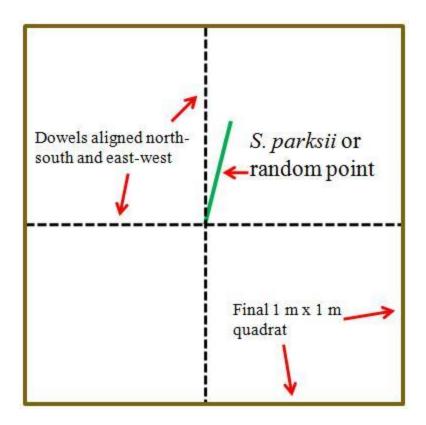


Figure 5.1. Diagram showing the methodology for placement of 1 m x 1 m quadrat around S. parksii or randomly chosen point.

and shrub species for the following height categories: 0-0.5, 0.5-1.0, 1.0-2.0, and greater than 2.0 m (values may exceed 100 percent since foliar cover is estimated per species at each height categories). A Non-metric Multidimensional Scaling (NMS) ordination (PC-ORD ver. 4 MjM Software Design) was used to identify combinations of species that may be associated with *S. parksii* and to characterize the herbaceous and woody canopy cover on quadrats occupied and unoccupied by *S. parksii*. NMS is generally the most effective ordination method for ecological community data since it avoids the assumption of linear relationships (McCune & Grace 2002). This analysis was conducted using 48 species, including *S. parksii*, which occurred in 4 or more quadrats.

Abiotic factors were also recorded at each quadrat. Percent leaf litter was estimated by the percentage of bare soil covered with leaf litter. Leaf litter depth was estimated by the number of stacked leaves above the soil surface in the majority of area covered by leaf litter. The categories were as follows:

> Light – 1 leaf Light-Medium – 1 to 3 leaves Light-Heavy – 1 to 5 leaves Medium – 3 leaves Medium-Heavy – 3 to 5 leaves Heavy – 5 or more leaves

For example, if stacked leaves were approximately evenly distributed throughout the leaf litter from 1 leaf deep to 2 and 3 and some with even 5 leaves deep, then it would be considered light-heavy, whereas, if there were 3 leaves stacked consistently throughout

the majority of the leaf litter, then it would be determined medium. Percent moss cover was estimated to the nearest percent. Presence of a drip line above and a game/cattle trail within 2 m of the quadrat were recorded. Slope aspect was determined with a compass. The location of the quadrat on its given slope was determined by evaluating whether the quadrat occurred on the low, mid or high position of the slope. Distance to a nearest water feature was determined by the distance to the bank of the nearest water feature. Canopy cover viewed to the south of the plant was estimated by percentage of time throughout the day the plant was shaded. Depth to the hardpan or claypan was determined by pushing a metal dowel (dia. 6.3 mm) into the soil and measuring the depth to significant resistance. Three soil cores were collected and pooled from the 0 to 15 cm depth within the quadrat and analyzed at Texas AgriLife Extension Soil, Water and Forage Testing Laboratory for pH, conductivity, nitrogen, phosphorus, potassium, calcium, magnesium, sulfur, and sodium. All data for this study was collected between 11 July 2007 and 27 July 2007. Independent samples t-test and graphical presentation were used to compare biotic and abiotic factors between quadrats with and without S. parksii.

Results

Following data collection, an unknown *S. parksii* or *S. cernua* rosette emerged in a quadrat that originally did not have *S. parksii* in 2006, therefore this quadrat was removed from further analyses. Another quadrat that originally did not have a *S. parksii*

45

in 2006, had a *S. parksii* emerge in 2007, and therefore this quadrat was put into the category with *S. parksii* for all analyses.

Associated Herbaceous Species

A total of 25 quadrats with *S. parksii* and 22 without *S. parksii* were used in the following analyses except for when the number of species per quadrat at Twin Oaks and the number of species per quadrat at TxDOT were compared (Table 5.1).

Among all quadrats, a total of one hundred nine plant species were found (Appendices A & B). No difference (p < 0.05) was found in the number of species between quadrats or between sites (Table 5.1). Quadrats at Twin Oaks with *S. parksii* had an average of 4 more species than quadrats with *S. parksii* at TxDOT (p = 0.11). Also, all quadrats with *S. parksii* had 10 percent more total foliar cover from herbaceous species than quadrats without *S. parksii* (p = 0.19).

The ordination analysis did not show a cluster of quadrats with and without *S*. *parksii* or quadrats from Twin Oaks and TxDOT (Fig. 5.2). Three distinct clusters shown in the ordination were driven by the presence of three perennial bunch grasses: *Schizachyrium scoparium, Chasmanthium laxum* var. *sessiliflorum*, and *Andropogon ternarius* (Fig. 5.3). Only 2 out of 25 quadrats with *S. parksii* and 4 out of 22 quadrats without *S. parksii* had no occurrence of one of these bunch grasses. No individual or group of species was found to be highly associated with *S. parksii* (Table 5.2). Some species sampled in quadrats only occurred with one type of perennial grass, which

Quadrat	Ν	Mean	SE	t	df	p-value	
Number of Species							
With S. parksii	25	13.3	1.2	1.02	45.0	0.32	
Without S. parksii	22	11.3	1.6			0.02	
All at Twin Oaks	18*	13.8	1.1	1.33	46.0	0.24	
All at TxDOT	30	11.5	1.4	1.00		0.2	
At Twin Oaks with S. parksii	9	15.8	0.9	2.04	21.7	0.11*	
At TxDOT with S. parksii	16	11.9	1.7				
At Twin Oaks without S. parksii	8	11.6	1.9	0.14	20.0	0.87	
At TxDOT without S. parksii	14	11.1	2.3				
Percent foliar cover							
All herbaceous with S. parksii	25	51	4.7	1.34	45.0	0.19	
All herbaceous without S. parksii	22	41 6.2		1.34	43.0	0.19	

Table 5.1. Independent samples t-test statistics for number and total percent foliar cover of herbaceous species in quadrats with and without *S. parksii* at Twin Oaks and TxDOT.

* Quadrat containing an unknown *S. parksii* or *S. cernua* rosette the following spring is included in analysis

** Variances not equal (p < 0.05 for Levene's Test for Equality of Variances)

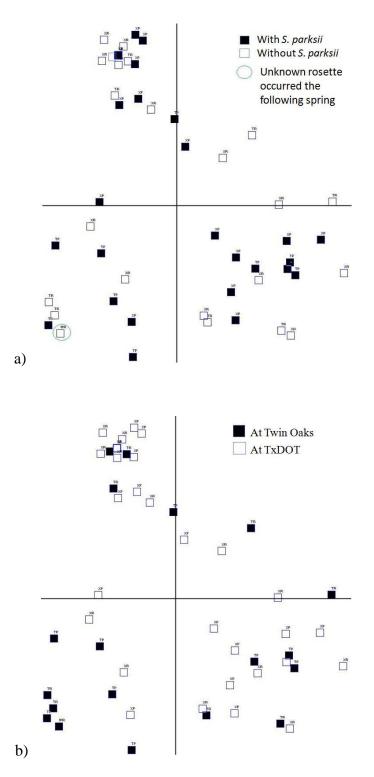


Figure 5.2. Non-metric Multidimensional Scaling (NMS) ordination showing no clustering of quadrats a) with (P) and without (R) S. parksii or b) from Twin Oaks (T) and TxDOT (X), based on percent foliar cover of herbaceous species within the quadrat.

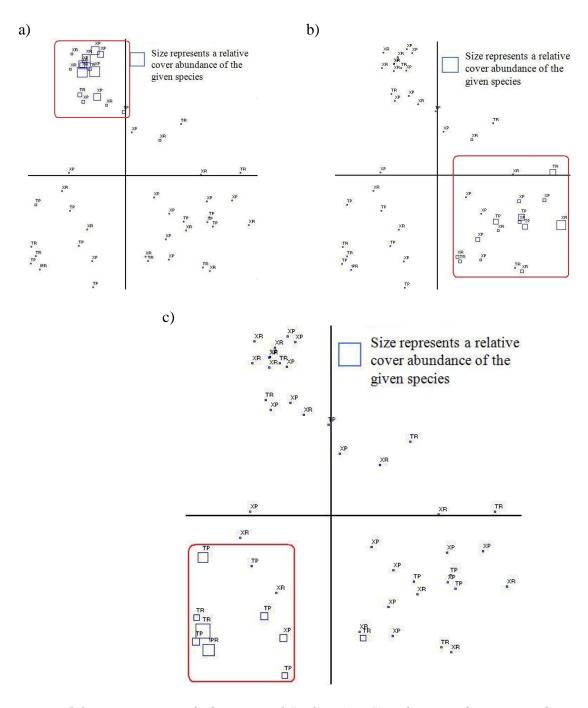


Figure 5.3. Non-metric Multidimensional Scaling (NMS) ordination showing quadrats with a relative abundance of a) Chasmanthium laxum var. sessiliflorum, b) Schizachyrium scoparium, and c) Andropogon ternarius. T represents quadrats from Twin Oaks, X are quadrats from TxDOT, P is a quadrat with S. parksii, R is a quadrat without S. parksii, and 1PR is the quadrat that had an unknown rosette emerge the following spring.

Table 5.2. Percent frequency (F) of 47 species in quadrats with and without *S. parksii* for each perennial grass cluster in the NMS ordination and in all quadrats sampled.

		hium laxum siliflorum		chyrium arium		opogon 1arius	All q	uadrats
	With S.	Without S.	With S.	Without S.	With S.	Without S.	With S.	Without S.
	parksii	parksii	parksii	parksii	parksii	parksii	parksii	parksii
	(n=12)	(n=10)	(n=10)	(n=8)	(n=6)	(n=4)	(n=25)	(n=22)
Species in Quadrat	F	F	F	F	F	F	F	F
Woody								
Hypericum hypericoides	17	0	40	13	0	0	20	5
Ilex vomitoria	42	60	20	25	50	75	32	41
Juniperus virginiana	8	20	10	25	17	0	8	14
Quercus nigra	17	10	0	0	17	25	8	9
Quercus stellata	25	40	30	25	17	25	24	27
Smilax bona-nox	0	40	0	0	0	0	0	23
Ulmus alata	83	60	50	38	50	0	64	45
Vaccinium arboreum	8	10	10	13	17	25	12	14
Herbaceous								
Acalypha gracilens	17	10	20	38	50	25	20	18
Alophia drummondii	0	0	10	13	0	0	12	9
Andropogon ternarius	25	0	10	13	100	100	24	14
Aristida sp.	17	0	30	25	67	25	28	14
Aster pratensis	8	0	0	13	17	50	4	9
Carex sp.	8	10	10	13	17	25	8	5
Chasmanthium laxum var.		-					-	
sessiliflorum	100	100	10	25	50	0	48	45
Chrysopsis pilosa	8	0	30	25	17	25	24	14
Crotalaria sagittalis	0	10	30	13	33	25	16	5
Croton capitatus	0	10	20	50	17	0	8	18
Croton monanthogynus	33	0	80	50	100	75	60	32
Cyperus sp.	0	10	30	25	17	0	24	18
Desmodium sp.	33	30	50	50	33	50	40	41
Dichondra carolinensis	8	20	10	13	0	0	8	9
Diodia teres	8	10	70	63	50	75	44	32
Erigeron strigosus	17	0	20	13	17	25	12	5
Evax sp.	25	10	30	13	33	50	24	14
Fabaceae sp.	0	0	30	38	17	0	16	14
Gamochaeta sp.	25	10	70	50	83	25	48	23
Juncus marginatus	0	0	10	38	0	0	8	14
Juncus sp.	8	10	10	25	17	25	8	9
Lechea mucronata	0	0	30	25	0	0	16	9
Liatris squarrosa	58	10	40	13	83	75	48	23
Linum medium	33	10	60	63	67	50	48	32
Mushroom sp.	17	50	10	13	17	0	16	27
Nasella leucotricha	8	20	10	0	0	0	8	9
Oldenlandia boscii	0	10	20	25	0	0	12	9
Oxalis corniculata	8	10	20	38	17	0	12	14
Panicum aciculare	42	0	70	75	67	50	60	36
Panicum brachyanthum	25	0	40	38	67	100	32	23
Panicum sphaerocarpon	33	40	90	100	50	50	56	50
Paspalum plicatulum	0	20	30	50	17	0	16	23
Paspalum setaceum	0	0	30	13	0	0	12	5
Plantago virginica	0	0	30	13	17	0	12	5
Rudbeckia hirta	8	0	10	13	17	0	12	5
Schizachyrium scoparium	8	20	100	100	17	25	40	36
Scleria sp.	42	50	70	63	50	100	56	55
Sorghastrum nutans	8	0	20	25	17	0	12	9
Sporobolus compositus var. clandestinus	0	0	10	13	0	50	4	14

contributed to the clusters in the ordination, while other species were seen to occur with all perennial grasses (Table 5.2). For example, *Scleria* sp. occurred in all three clusters, *Lechea mucronata* only occurred with *Schizacyrium scoparium*, and *Smilax bona-nox* only occurred with *Chasmanthium laxum* var. *sessiliflorum* (Table 5.2).

Woody Canopy Cover

No difference was found between quadrats with and without S. parksii for tree and shrub species at all heights, except for total canopy cover > 2 m (p = 0.026; Table 5.3 & Fig. 5.4). All quadrats containing S. parksii had an average of 55.2 percent woody canopy above 2m, while all quadrats without S. parksii averaged 96.8 percent. Total woody canopy cover was 44 percent less at quadrats with S. parksii than quadrats without S. parksii (p = 0.09). The total woody canopy from 1 - 2 m was 40 percent less at quadrats with S. parksii than quadrats without S. parksii (p = 0.08). Total woody canopy cover plus the total herbaceous cover was 33 percent less at quadrats with S. *parksii* than quadrats without *S. parksii* (p = 0.17). No difference was found for total woody canopy cover between quadrats with and without S. parksii at TxDOT, whereas there was a difference between quadrats at Twin Oaks (p = 0.029; Table 5.4). NMS ordinations were run on woody species using the summation of cover across all height strata for each woody species, and for percent woody cover at each height interval for each species. No distinct clusters or patterns emerged relative to quadrats with and without S. parksii or between sites (Figs. 5.5 & 5.6). A NMS ordination does not show

Canopy Cover	Mean	SE	t	df	p-value
Total woody canopy					
With S. parksii	106	16.4	-1.71	45.0	0.09
Without S. parksii	150	19.6	-1./1	43.0	0.09
Total shrub canopy cover					
With S. parksii	52	13	0.77	45.0	0.45
Without S. parksii	67	15.2	-0.77	45.0	0.45
Total tree canopy cover					
With S. parksii	54	10.8	1 5 1	45.0	0.14
Without S. parksii	82	15.5	-1.51	45.0	0.14
Total woody canopy $> 2 \text{ m}$					
With S. parksii	55.2	11.2	0.01	15.0	0.000
Without S. parksii	96.8	14.5	-2.31	45.0	0.026
Total woody canopy 1 - 2 m					
With S. parksii	25.7	6.3	0.05	45.0	
Without S. parksii	23.5	5.3	0.27	45.0	0.79
Total woody canopy $1 - 2m$					
With S. parksii	80.9	14.8	1	45.0	0.00
Without S. parksii	120.3	16.7	-1.77	45.0	0.08
Total woody canopy 0.5 - 2 m					
With S. parksii	37.1	8.2	0.04	45.0	0.01
Without S. parksii	39.7	7	-0.24	45.0	0.81
Total woody canopy 0.5 - 1 m					
With S. parksii	11.37	2.7		45.0	0.07
Without S. parksii	16.18	3.5	-1.11	45.0	0.27
Total woody canopy 0 - 1 m					
With S. parksii	25.3	5.6	0.46	45.0	0.6
Without S. parksii	29.3	5.8	-0.49	45.0	0.6
Total woody canopy + total herbaceous					
With S. parksii	158	16.3	1.00	45 0	0.15
Without S. parksii	191	17.3	-1.39	45.0	0.17

Table 5.3. Woody and herbaceous canopy cover (%) over quadrats with and without *S. parksii*.

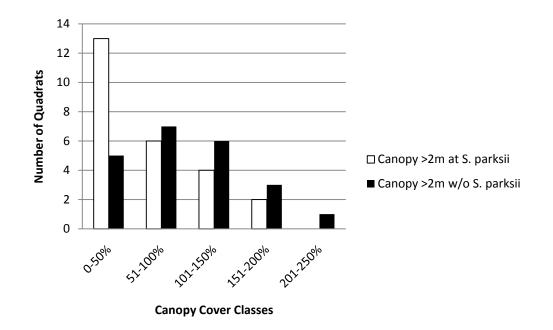


Figure 5.4. Total canopy cover at > 2 m for quadrats with (open bars) and without (filled bars) S. parksii (p = 0.026).

Quadrat	n	Mean	SE	t	df	p-value	
Twin Oaks							
With S. parksii	9	51.4	16.1	0.22	10.0	0.020*	
Without S. parksii	8	140.0	34.6	-2.33	10.0	0.029*	
TxDOT							
With S. parksii	16	130.9	22.5	7.2	20	0.47	
Without S. parksii	14	155.0	24.5	-7.3	28	0.47	

Table 5.4. Total woody canopy cover for all levels summed (0-0.5m, 0.5-1m, 1-2m and >2m) separated by quadrats at each site with and without *S. parksii*.

* Variances not equal (p < 0.05 for Levene's Test for Equality of Variances)

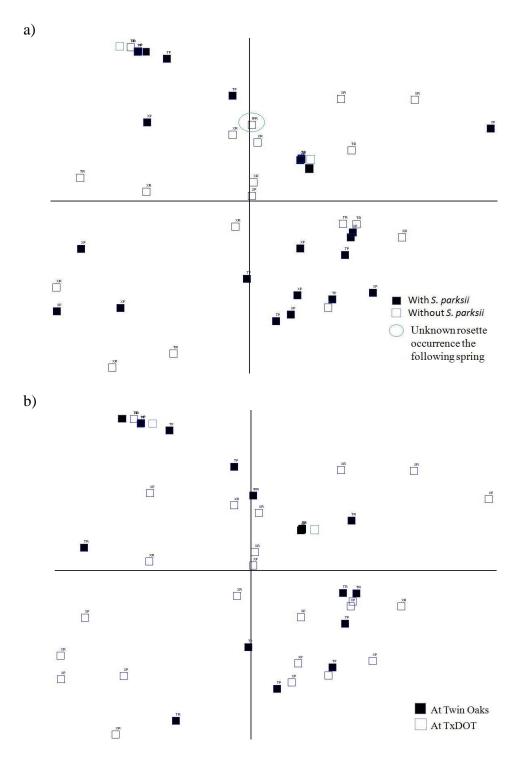


Figure 5.5. Non-metric Multidimensional Scaling (NMS) ordination of 42 out of 48 quadrats (the other 6 quadrats had no canopy values) a) with (P) and without (R) S. parksii and b) at Twin Oaks (T) and TxDOT (X) with total canopy cover for each species at each quadrat.

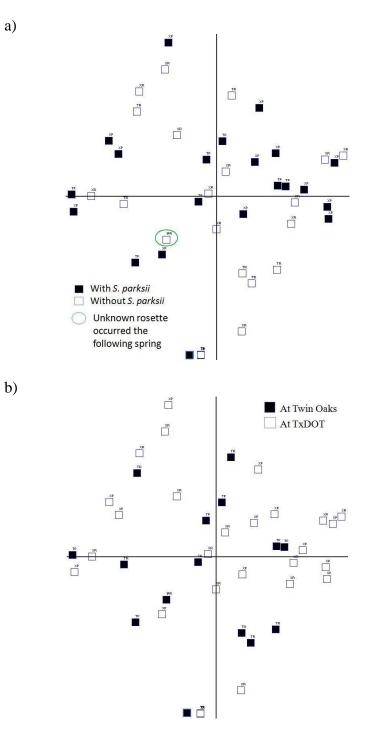


Figure 5.6. Non-metric Multidimensional Scaling (NMS) ordination of 42 out of 48 quadrats (the other 6 quadrats had no canopy values) a) with (P) and without (R) S. parksii and b) at Twin Oaks (T) and TxDOT (X) with canopy cover per species for each height category.

any separation of quadrats with and without *S. parksii* or between the two sites with all 48 herbaceous species and canopy cover per species at each strata (Fig. 5.7).

Abiotic Factors

No significant (p < 0.05) difference was found between quadrats with and without *S. parksii* for all factors measured (Table 5.5). Additionally, when all quantitative abiotic factors (leaf litter depth, percent moss, bare soil, percent slash < 2.5, 2.5-10, and > 10 cm, and slope grade) are added to the 48 herbaceous species and canopy cover per species at each strata, no cluster of quadrats with and without *S. parksii* or between sites is seen (Fig 5.8). While all abiotic factors showed no significant difference between quadrats, graphical representation can summarize a likely local habitat for *S. parksii* (Figs. 5.9 – 5.14).

S. parksii is likely to occur with 41-100% of leaf litter at a light to light-heavy depth. It is likely that consistent heavy leaf litter would drastically decrease the light availability for the emergence of *S. parksii*, however, 4 out of 25 quadrats with *S. parksii* occurred with medium-heavy to heavy leaf litter. Moss of 0.1-10 percent and bare soil of 0-30 percent occurred within most quadrats with *S. parksii*. Small amounts (< 5%) of slash < 2.5 cm and 2.5 – 10 cm occurred, as well as larger pieces of slash > 10 cm were found in quadrats with *S. parksii*. Many of the slopes *S. parksii* occurred on were 0 – 4 degrees at a northerly aspect and located from the middle to high portion. As would be predicted from many canopy cover values above quadrats, *S. parksii* was seen to likely

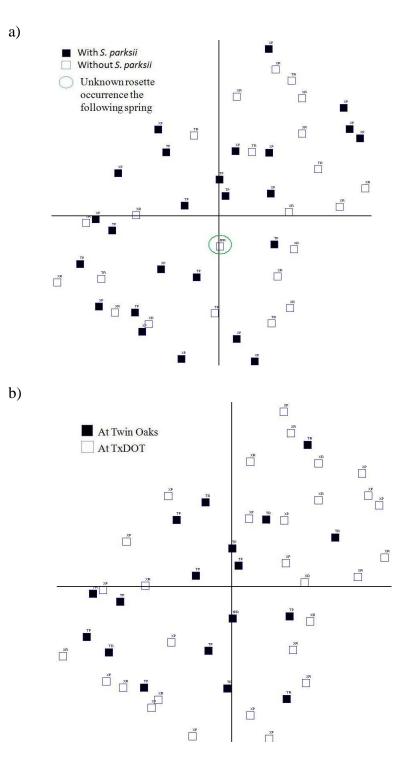


Figure 5.7. Non-metric Multidimensional Scaling (NMS) ordination of all quadrats a) with (P) and without (R) S. parksii and b) at Twin Oaks (T) and TxDOT (X) based on 48 herbaceous species and canopy cover per species at each strata.

Factor	n	Mean	SE	t	df	p-value
Leaf litter cover (%)						
With S. parksii	21*	68.8	4.1	-0.83	29.6	0.47****
Without S. parksii	19*	75.5	7	-0.85	29.0	0.4/*****
Leaf litter depth (***)						
With S. parksii	25	2.5	0.3	-1.83	45	0.08
Without S. parksii	22	3.5	0.4	-1.85	45	0.08
Moss (%)						
With S. parksii	25	4.1	1.8	0.00	15	0.22
Without S. parksii	22	2.1	0.9	0.96	45	0.33
Bare soil (%)						
With S. parksii	25	15.5	2.4	1.04	45	0.21
Without S. parksii	22	11.5	3.2	1.04	45	0.31
Slash < 2.5 cm (%)						
With S. parksii	25	2.6	0.8	0.05	45	0.20
Without S. parksii	22	1.8	0.3	0.85	45	0.38
Slash 2.5-10 cm (%)						
With S. parksii	25	1.7	0.7	0.01	15	0.02
Without S. parksii	22	2	0.9	-0.21	45	0.83
Slash > 10 cm(%)						
With S. parksii	25	0.8	0.4	1.00	04****	0.07**
Without S. parksii	22	0	0	1.98	24****	0.07**
Slope grade (°)						
With S. parksii	25	3.1	0.6	1 10	24.6	
Without S. parksii	22	5.9	2.3	-1.19	24.6	0.22****
Distance to drainage bank (m)						
With S. parksii	22*	3.5	1	0.47	4.1	0.64
Without S. parksii	21*	4.1	1	-0.47	41	0.64
Shade throughout day (%)						
With S. parksii	24*	72.5	3.9	0.10	10	0.00
Without S. parksii	21*	73.3	5	-0.13	43	0.90
Depth to hard/clay pan (cm)						
With S. parksii	23*	70.4	5.8	0.24	4.4	0.01
Without S. parksii	20*	68.4	6.1	0.24	41	0.81

Table 5.5. Abiotic factors and moss cover at quadrats with and without S. parksii.

* Some plots were not measured, due to an error in data collection

** Only 5 occurrences of slash > 10cm in plots with *S. parksii* and no occurrences in plots without *S. parksii*

*** Number of overlapping leaves: Light (1), Light-Med (2), Light-Heavy (3), Med (4), Med-Heavy (5), and Heavy (6)

**** Variances not equal (p < 0.05 for Levene's Test for Equality of Variances)

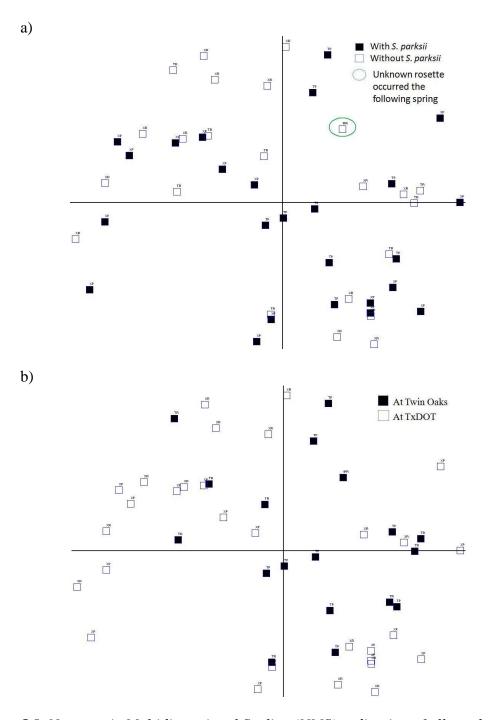


Figure 5.8. Non-metric Multidimensional Scaling (NMS) ordination of all quadrats a) with (P) and without (R) S. parksii and b) at Twin Oaks (T) and TxDOT (X) based on biotic (48 herbaceous species and canopy cover per species at each strata) and all quantitative abiotic factors sampled at all quadrats (leaf litter depth, percent moss, bare soil, slash < 2.5, 2.5-10, and > 10 cm, and slope grade).

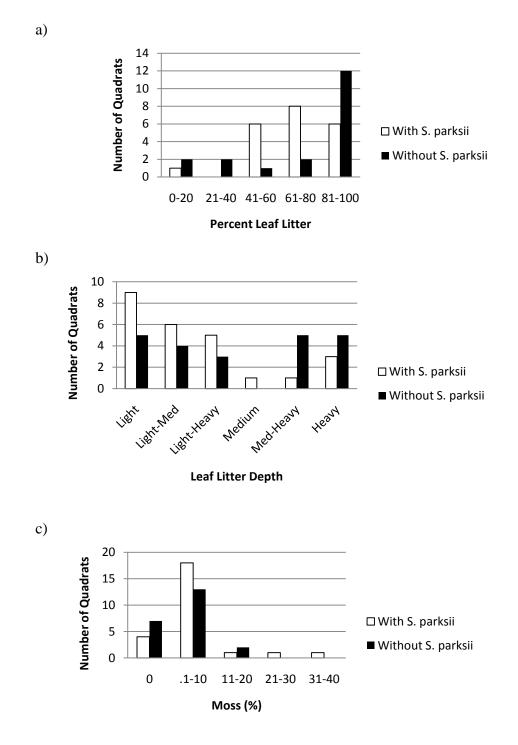


Figure 5.9. Graphical representations of a) percent leaf litter in quadrats with (n=21) and without (n=19) S. parksii, b) leaf litter depth at quadrats with (n=25) and without (n=22) S. parksii, and c) percent moss cover in quadrats with (n=25) and without (n=22) S. parksii.

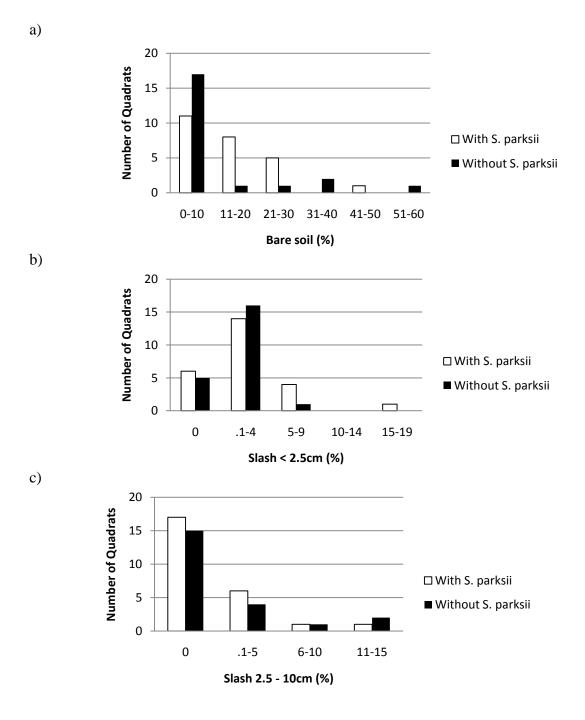


Figure 5.10. Graphical representations of a) percent bare soil in quadrats with (n=25) and without (n=22) S. parksii, b) percent slash < 2.5 cm in quadrats with (n=25) and without (n=22) S. parksii, c) percent slash 2.5-10 cm in quadrats with (n=25) and without (n=22) S. parksii.

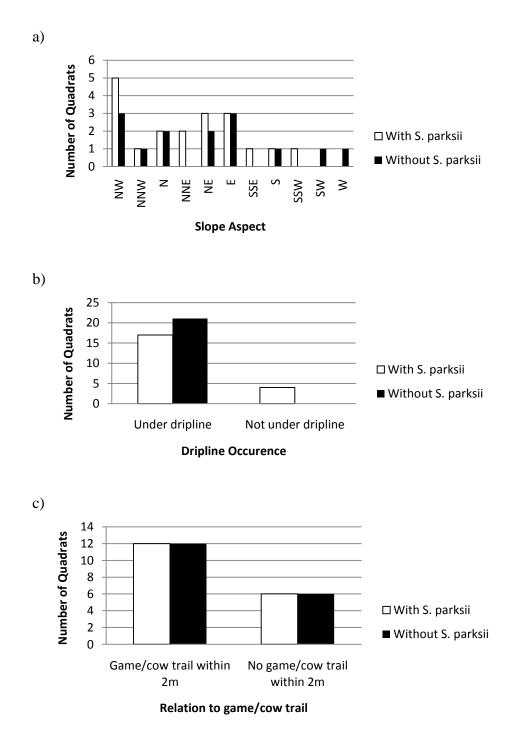


Figure 5.11. Graphical representations of a) slope aspect of quadrats with (n=19) and without (n=14) S. parksii, b) dripline occurrence above quadrats with (n=21) and without (n=21) S. parksii, and c) occurrence of a game/cattle trail within 2 m of quadrats with (n=18) and without (n=18) S. parksii.

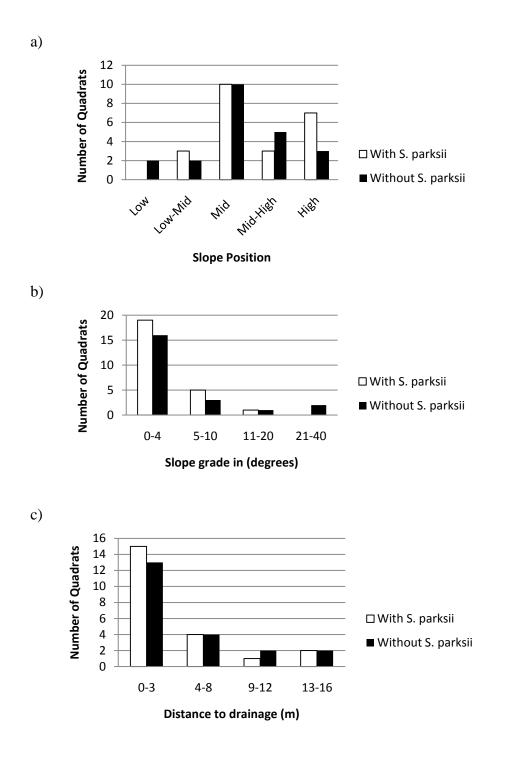


Figure 5.12. Graphical representations of a) slope position of quadrats with (n=23) and without (n=22) S. parksii, b) slope grade at quadrats with (n=25) and without (n=22) S. parksii, and c) distances to a drainage bank from quadrats with (n=22) and without (n=21) S. parksii.

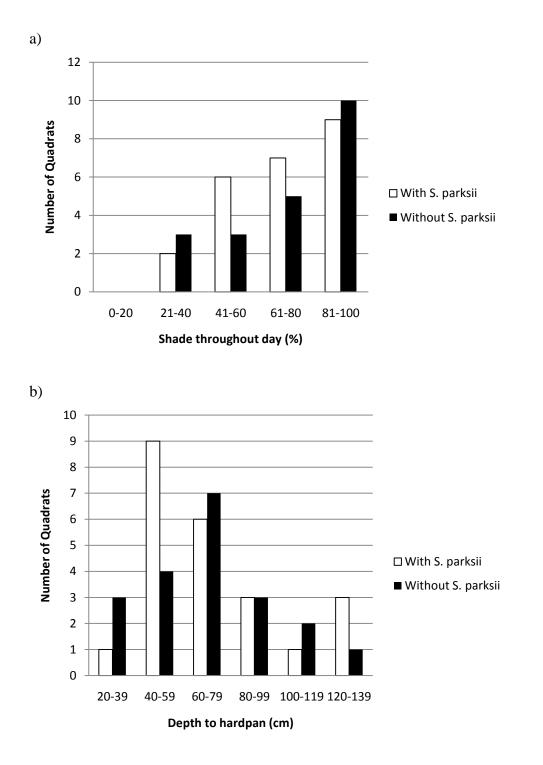
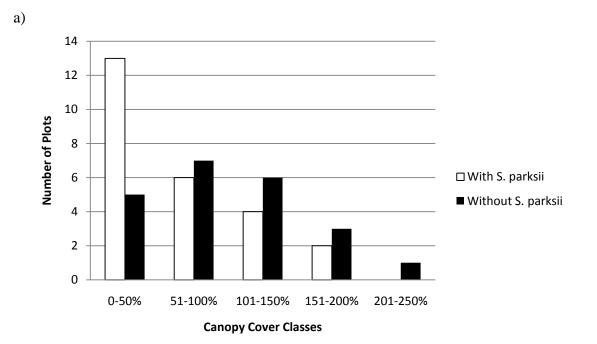
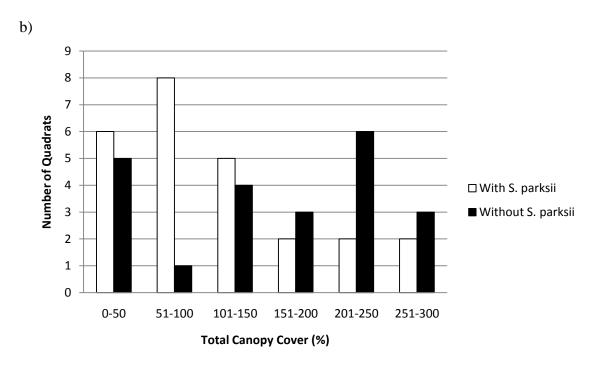
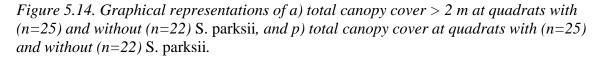


Figure 5.13. Graphical representations of a) percent shade throughout the day with a slight southern tilt of the sun at quadrats with (n=24) and without (n=21) S. parksii and b) depth to hard/clay pan at quadrats with (n=23) and without (n=20) S. parksii.







occur under a dripline. *S. parksii* was also seen occur within 2m of a game/cattle trail and within 8 m of a drainage bank. *S. parksii* was seen to occur in areas with 41-100 percent shade throughout the day to the south. The hard/claypan was mostly found at 40 – 99 cm in quadrats with *S. parksii*.

There were no significant differences between soil properties of quadrats with and without *S. parksii*, except for sodium (p = 0.02). Quadrats with *S. parksii* had a lower mean value of sodium (166.8 ppm) than quadrats without *S. parksii* (192.6 ppm) (Table 5.6).

Discussion

S. parksii occurred frequently with *Chasmanthium laxum* var. *sessiliflorum*, which was not expected since this perennial bunch grass occurs in more closed canopy environments. It is possible that *S. parksii* is occurring frequently with *Chasmanthium laxum* var. *sessiliflorum* because of woody encroachment. *S. parksii* is perennial, therefore might have been present in an area prior to woody encroachment, but is still able to persist under more closed canopy conditions.

S. parksii is mostly found within the patchy openings of the Post Oak Savannah (Parker 2001; USFWS 1993). Results from this study quantify this, as the woody canopy cover from 0.5 - 2 m was far from significant (p = 0.81) while the canopy cover at > 2 m was significant (p = 0.026) between quadrats with and without *S. parksii*. Consequently, *S. parksii* was more likely to occur under low growing shrubs/trees, but

Soil Property	Mean	SE	t	df	p-value	
рН					-	
With S. parksii	4.40	0.25	0.10	0.0	0.04	
Without S. parksii	4.50	0.20	-0.18	8.0	0.86	
Conductivity (µmho/cm)						
With S. parksii	32.60	2.68	1.02	0.0	0.11	
Without S. parksii	51.00	9.76	-1.82	8.0	0.11	
Nitrate-N (ppm)						
With S. parksii	1.40	0.24	0.00	0.0	1.00	
Without S. parksii	1.40	0.24	0.00	8.0	1.00	
Phosphorus (ppm)						
With S. parksii	8.80	1.71	-1.52	0.0	0.17	
Without S. parksii	12.00	1.22	-1.52	8.0	0.17	
Potassium (ppm)						
With S. parksii	63.40	9.77	1.00	0.0	0.00	
Without S. parksii	111.20	22.36	-1.96	8.0	0.09	
Calcium (ppm)						
With S. parksii	349.60	113.71	1 17	0.0	0.29	
Without S. parksii	611.80	192.55	-1.17	8.0	0.28	
Magnesium (ppm)						
With S. parksii	90.60	12.63	1.07	8.0	0.32	
Without S. parksii	114.00	17.93	-1.07			
Sulfur (ppm)						
With S. parksii	10.00	0.32	1 5 4	A A	0.10*	
Without S. parksii	12.20	1.40	-1.54	4.4	0.19*	
Sodium (ppm)						
With S. parksii	166.80	6.55	0.07	7.0	0.02*	
Without S. parksii	192.60	5.69	-2.97	7.8	0.02*	

Table 5.6. Independent samples t-test and descriptive statistics for soil properties (0-15 cm) in quadrats with (n=5) and without (n=5) *S. parksii*.

* Variances not equal (p < 0.05 for Levene's Test for Equality of Variances)

when the higher canopy closed in from mature trees and possibly shrubs, it was not likely to occur. While this was much the case for Twin Oaks, *S. parksii* at TxDOT appears to be able to persist in more closed canopy conditions as the mean percent woody cover > 2 m was 54 percent greater than at Twin Oaks (p = 0.016) (Table 5.7).

The higher occurrence of lighter leaf litter at quadrats with *S. parksii* could have been maintained with prescribed burning, as Twin Oaks has been burned every 3 years in the past 50 to 60 years (R. Trant, personal communication). Caution should be taken as the deed-restricted areas at Twin Oaks will be fenced off and land use will be drastically changed. Grazing, prescribed burning and the hunting of feral hogs appear to be important to *S. parksii*, as these management practices have been in place for the last 50 to 60 years at Twin Oaks. Grazing and prescribed burning likely maintained a more open canopy on Twin Oaks as both are known to lessen the degree of woody encroachment. If feral hogs increase in number, many *S. parksii* individuals will be in danger of being dug up as feral hogs are well known for grubbing.

The small amounts of slash at quadrats with *S. parksii* likely offer protection from herbivores as they could prevent game/cattle from stepping on the plant or eating it. *S. parksii* was likely to be seen within 2 m of a game/cattle trail since many of the areas a game/cattle trail is seen to occur in are more open areas along drainages where water is available.

S. parksii is thought to occur along drainages where natural soil erosion has occurred. This natural soil erosion has formulated a hypothesis that by nearly exposing the clay subsoil provides shallow subsurface flow of water that is available to *S. parksii*

Canopy Cover > 2 m	n	Mean	SE	t	df	p-value
Twin Oaks						
With S. parksii	9	21	9.3	-2.49	8.4	0.019*
Without S. parksii	8	98	29.7			
TxDOT						
With S. parksii	16	75	14.6	-0.98	28	0.336
Without S. parksii	14	96	16			
With S. parksii						
At Twin Oaks	9	21	9.3	-3.12	22.7	0.016*
At TxDOT	16	75	14.6	-3.12	22.1	0.016*

Table 5.7. Woody canopy cover > 2 m for quadrats with and without *S. parksii* at Twin Oaks and TxDOT.

* Variances not equal (p < 0.05 for Levene's Test for Equality of Variances)

(USFWS 2007). An attempt to measure this was done, however, resulted in being very deep and should be assessed more accurately with other methods to determine more definite conclusions. When collecting the soil samples, a claypan was found at 5 - 10 cm, however, the metal dowel seemed to push right through it. While the percent shade estimate showed that most *S. parksii* occurred at greater than 41 percent, it did not take into account the degree of shade within this range (i.e. leaf area index). Continuous light measurements should be taken at areas with and without *S. parksii* to better quantify this measurement.

While all values for macro nutrients in the soil samples were higher for quadrats without *S. parksii*, no conclusions can be made due to a low sample size (Table 5.6).

CHAPTER VI

LANDSCAPE LEVEL HABITAT

Introduction

It is hypothesized that *S. parksii* is light sensitive. It is infrequently found in open grassland habitats and is typically absent from densely shaded, wooded habitat, although it occasionally occurs in both. Its presence in dense woody environments might be as a remnant. Wilson (1993) hypothesizes that *S. parksii* is not able to establish in dense woody environments, however, it occurs in them because the area was once more open, but has recently become more wooded. To address Objective 3, historical (1958) and current (2004) aerial imagery were used to assess changes in land cover on the Twin Oaks site to determine the pattern and relative degree of change in herbaceous and woody dominated cover. This may provide explanations for some of the current distribution patterns of *S. parksii* and may suggest approaches for vegetation management. *S. parksii* is most often found in small natural openings within the open woodland along or adjacent to upland watercourses between the flood plain and open grassland of the Post Oak Savannah Ecosystem of Central and East Texas (Parker 2001; USFWS 2006, 2007; Wilson 2007).

Methods

Aerial imagery from 7 March 1958 and 15 October 2004 were acquired from the Texas Natural Resources Information System (TNRIS). The 1958 image was received in two portions, east and west, both of which were panchromatic in 1 m pixels. These two portions had to be geometrically corrected using image to image registration with a 1996 image of the same area that was received from TNRIS in UTM projection Zone 14 and North America 1983 Datum. After both 1958 images had been geometrically corrected, they were mosaicked together using a georeferenced based procedure. The 2004 image was a three band (infrared, red and green) image received in UTM projection Zone 14, North America 1983 Datum and also in 1 m size pixels. With both images in UTM projection Zone 14 and North America 1983 Datum, the study site could be cropped out of each (Figs. 6.1 & 6.2).

The study site was cropped out by setting a vector file of the Twin Oaks site boundary as an ROI in ENVI ver. 4.4 (ITT Visual Information Solutions). This Region of Interest (ROI) was then overlaid onto the image and exported as a subset data into a separate file to only include image information inside the Twin Oaks site boundary. After isolating the study site of interest, both images were classified.

Since the 1958 image is panchromatic, a texture had to be run to better differentiate the woody vegetation pixels from the herbaceous vegetation pixels. After this texture was run, the image was classified with an ISODATA unsupervised

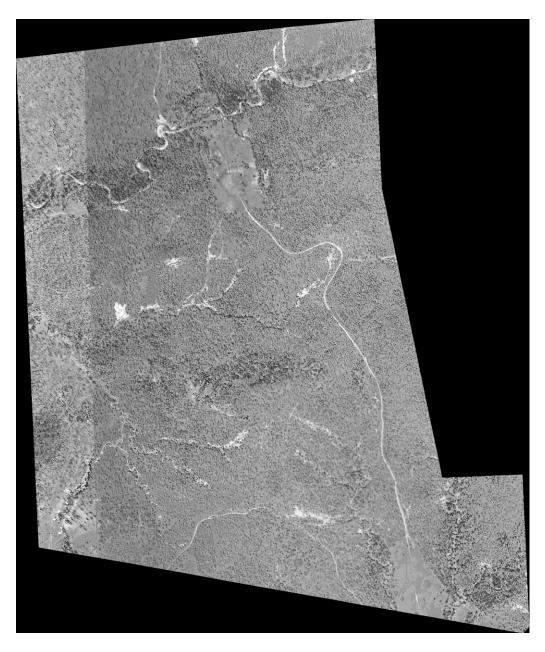


Figure 6.1. Image of the Twin Oaks landfill on 3 March 1958.

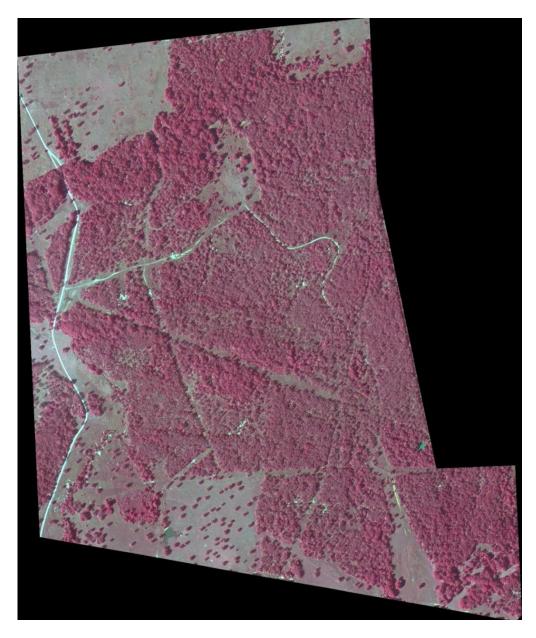


Figure 6.2. Image of the Twin Oaks landfill site on 15 October 2004.

classification set at 5 classes and 10 iterations. ISODATA is an effective classification method due to its large number of passes (Jensen 2005). To better smooth out the image and remove isolated pixels, a majority analysis was run. Four of the five classes were then combined to form the herbaceous class and the left over class was defined as woody vegetation (Fig. 6.3). The 2004 image was classified into herbaceouss, woody, and road/rock outcrop class by selecting a ROI of 44,773 pixels for herbaceous, 6,466 pixels for woody vegetation and 1616 pixels for road/rock outcrop. A supervised classification using the Mahalanobis distance measure was then run to give the best results for the three classes (Fig. 6.3). Following this, polygons of high *S. parksii* occurrences from the initial 2001 survey conducted by HDR Inc. were overlaid on the 2004 classified image to give a representation of the composition of different classes within each polygon (Fig. 6.4).

Results

Geometric Correction

An ideal RMS error for the 1958 image would have been 0.5 or less (since it has 1m pixels), but this accuracy could not be achieved due to a lack of good ground control points (GCP). The RMS error for the 1958 western image was 7.7 with 36 GCP and a RMS of 6.4 with 23 GCP in the 1958 eastern image. Change detection between 1958

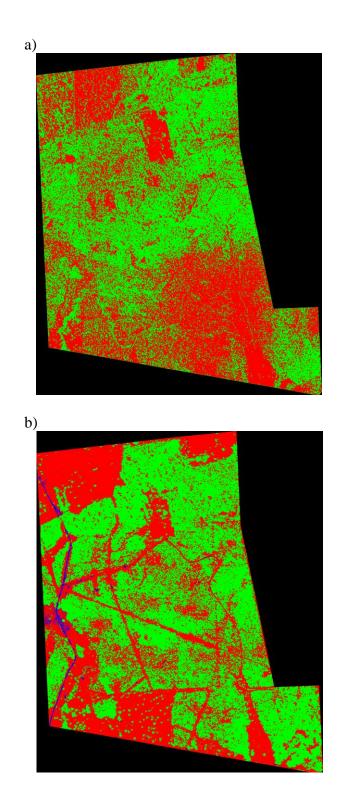


Figure 6.3. Image of the Twin Oaks landfill from a) 3 March 1958 and b) 15 October 2004, classified into herbaceous (red) and woody (green) vegetation.

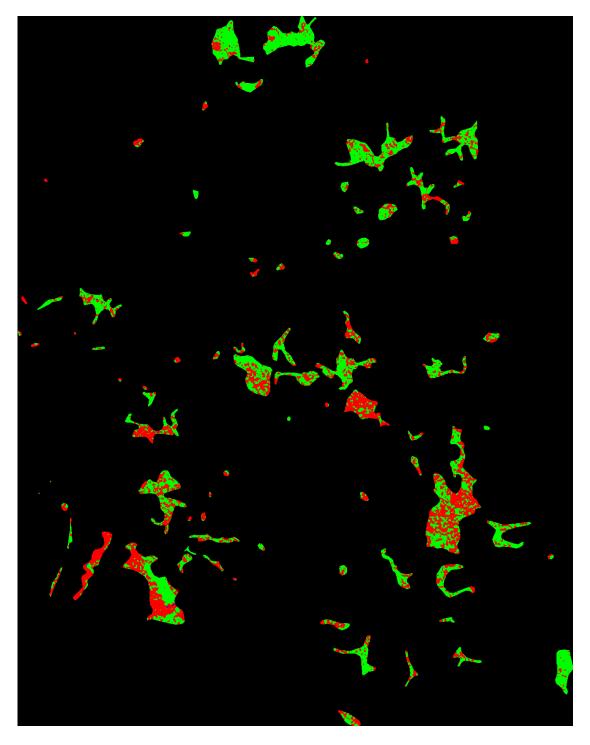


Figure 6.4. Polygons with high S. parksii occurrences from a 2001 survey overlaid onto the 2004 classified image of herbaceous (red) and woody (green) vegetation classes.

and 2004 images would have been applied to give a much more quantitative change per pixel, but the RMS could not be met, so visual interpretation was used.

Feature Classification

An accuracy of 91.8% was achieved in the unsupervised classification of the 1958 photo by using 322 herbaceous and 277 woody vegetation points. The supervised classification in the 2004 image yielded a 99.9% accuracy with 374 herbaceous and 277 woody vegetation reference points. The ROI Separability Report yielded nothing below 1.98 between any two classes. Overall class statistics for the 1958 photo were 48.7% herbaceous and 51.3% woody, while for the 2004 classified image they were 44.8% herbaceous, 54.4% woody and 0.8% road/rock outcrop. The percentage of herbaceous and woody vegetation did not change significantly between 1958 and 2004, however, the spatial distribution of each class changed significantly. In 2004, the herbaceous vegetation was concentrated in areas where bulldozing and mechanical clearing occurred and the woody vegetation increased in the non-bulldozed areas (Figs. 6.1 & 6.2).

Visual Interpretation

Areas that have been mechanically cleared, encroached by woody cover, and some that have remained unchanged are seen in Figure 6.5. Upon extracting the herbaceous and woody vegetation within polygons of high *S. parksii* occurrences from a

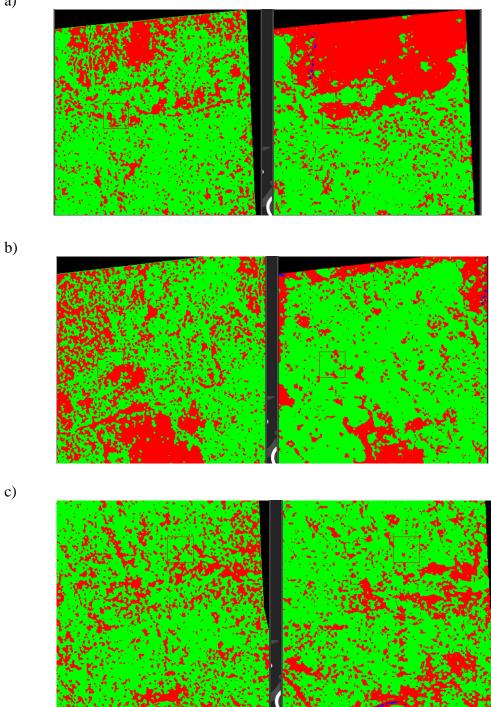


Figure 6.5. Areas of Twin Oaks on the same geographic location from 1958 (left) and 2004 (right) showing a) mechanical clearing, b) woody encroachment, and c) little change. Herbaceous is represented by red and woody by green.

a)

2001 survey from the 1958 and 2004 classified images, little change in herbaceous/woody cover is seen within the polygons (Fig. 6.6). The vegetation composition within most of the polygons from both years is almost a 50:50 woody and herbaceous mix with slightly more woody vegetation in 2004.

Discussion

The open patchy savannah landscape that is found in 2004 within each 2001 *S. parksii* polygon (Fig. 6.3) is also seen across the entire site in the 1958 image; therefore, it is possible that Twin Oaks historically contained individuals of *S. parksii* in more areas than are currently found (USFWS 2007). These results also suggest that since the landscape has not changed much around occurrences of *S. parksii* from 1958 to 2004, *S. parksii* was likely present in the same locations in 1958.

Flight dates for the 1958 and 2004 images were 7 March 1958 and 15 October 2004. While classification of the 2004 image is thought to be highly accurate, leaves on the trees were arguably not in great numbers on 7 March 1958, therefore the woody class may not be well differentiated from the herbaceous. Woody encroachment is seen from 1958 to 2004 in the southeastern portion of Twin Oaks (Fig. 6.3). This woody encroachment is so pronounced that the results had to be questioned. Upon further investigation of the original 1958 image (Fig. 6.1), the herbaceous class might be overrepresented in 1958, but does appear to have been much more open than in 2004, as more rock outcrops and drainages can be distinguished in 1958 than 2004 (Figs. 6.1 &

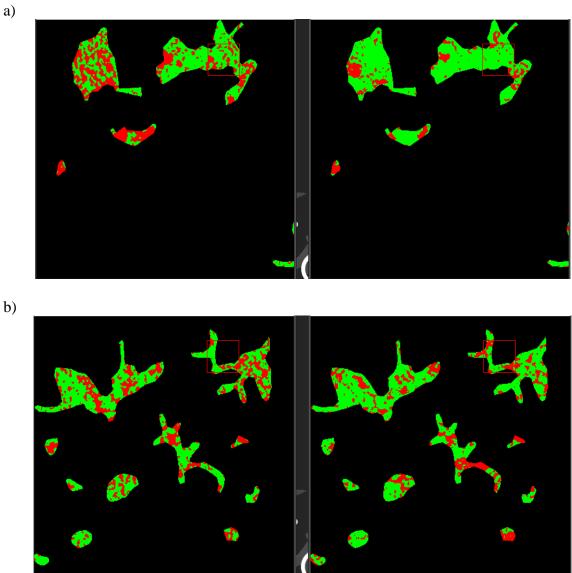


Figure 6.6. Polygons of high occurrences of S. parksii from a 2001 survey overlaid onto the classified 1958 (left) and 2004 (right) images showing a) woody encroachment and *b) little change. Herbaceous is represented by red and woody by green.*

6.2). The spectral responses in this area are not as dark as other pixels, and therefore were not as heavily classified as woody. However, they appear to be darker than surrounding pixels that are thought to herbaceous, so are thought to be small shrubs rather than mature trees.

CHAPTER VII

TRANSPLANTATION

Introduction

Little is known of the ability of *S. parksii* to establish in nature, or degree of success of transplanting extant individuals from threatened sites to areas where they may be protected. Methods for successful transplanting of *S. parksii* is necessary to meet the mitigation requirements outlined in the Biological Opinion for Twin Oaks, as *S. parksii* individuals will be destroyed in the construction of the landfill (USFWS 2006).

Transplanting *S. parksii* individuals on site with the immediate soil intact was noted as being successful, although no long term data was recorded (Parker 2001). This method is thought to be the most effective due to the mycorrhizal association terrestrial orchids, particularly *S. cernua*, are known to have (Rasmussen 1995; Pileri 1998). However, transplanting *S. parksii* individuals grown *in vitro* were not successful upon transplantation to sites of known *S. parksii* occurrences (Parker 2001). The failure of these transplants is likely due to the individual's inability to adapt to native field conditions as they were grown in optimal greenhouse conditions. It is the purpose of Objective 4 to assess the success of *in situ* soil-intact and bare-root methods for transplantation of *S. parksii*.

Methods

Two methods were implemented for transplantation of *S. parksii* (soil-intact and bare-root). Both methods transferred plants out of the footprint (Twin Oaks landfill area) and into deed-restricted areas where *S. parksii* was known to occur (Fig. 7.1). Rosettes of unknown *S. parksii* or *S. cernua* were searched for, chosen, and transferred to areas where high numbers of *S. parksii* had been located from the 2001 survey conducted by HDR Inc. (Fig. 7.2). Within these areas of high *S. parksii* occurrences, the transplants were placed with associated vegetation and light availability similar to areas from which individuals were taken. Transplants were moved when some rosettes at Twin Oaks were seen to be senescing, therefore at or near maximum growth, and when the soil was near field capacity, except for some soil-intact transplants that were transplanted when the soil was saturated and even inundated in parts of the deed-restricted areas.

Relative to other individuals seen at Twin Oaks, ten rosettes of smaller (approx. 5 -10 cm in total leaf length) and larger (20 -35 cm in total leaf length) sizes were selected on 18 April 2007, excavated, and soil removed to measure maximum lateral tuber length and greatest depth of tuber (Fig. 7.3). A shovel was submerged well below the plant then the soil lifted. After this, the soil surrounding the root tubers would break up so that the root tubers could be safely separated from the soil without any destruction to them. The tubers were then wrapped in a saturated paper towel until they were transplanted to the deed-restricted areas the same date approximately 2 to 4 hours after

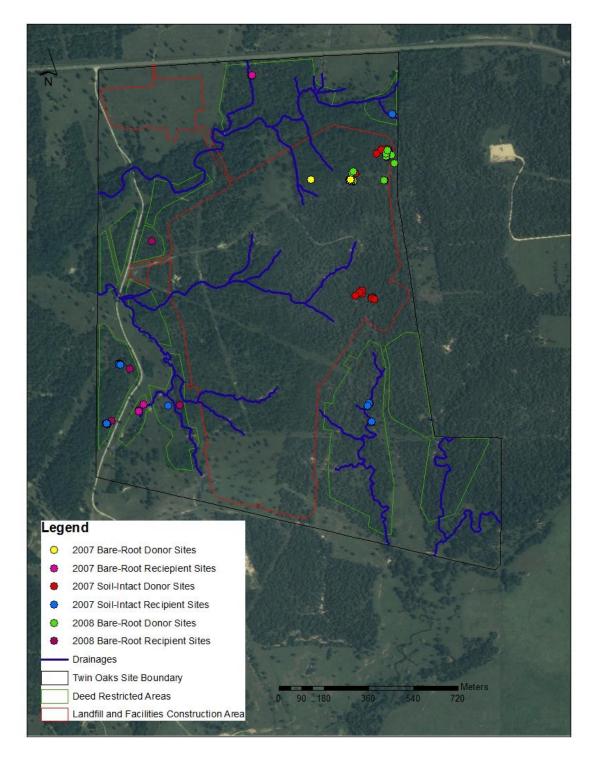


Figure 7.1. Location of all donor and recipient sites for transplants from 2007 and 2008.

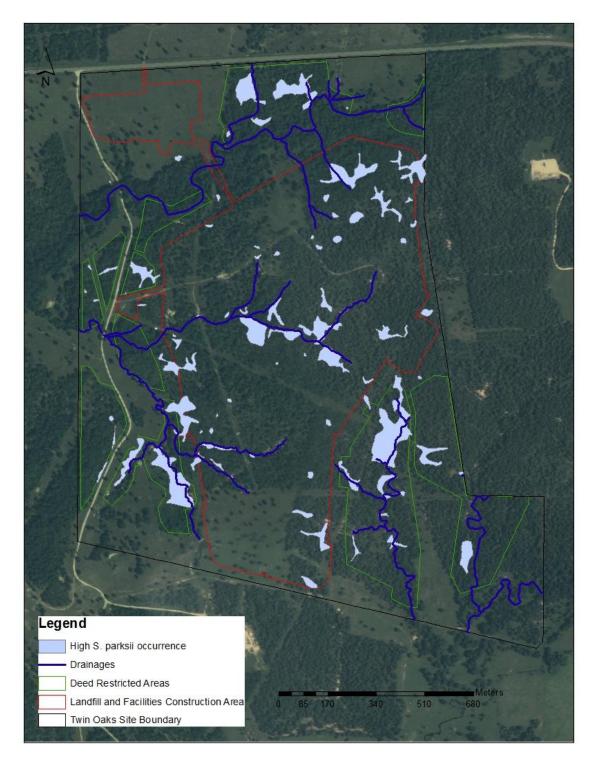


Figure 7.2. Location of areas of high S. parksii occurrences from the 2001 survey by HDR, Inc.

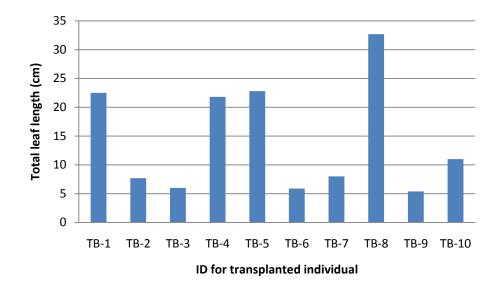


Figure 7.3. Total leaf length of individuals prior to transplanting on 18 April 2007 which were selected as smaller (approx. 5 - 10 cm) and larger (approx. 20 - 35 cm) rosettes.

excavation. Since the soil was at or near field capacity, some soil remained on the root tubers while the root tuber's length could still be measured.

Sixty-two unknown *S. parksii* or *S. cernua* rosettes were selected between 27 April 2007 and 12 May 2007 to be included in 59 soil-intact transplants (two transplants contained 2 and 3 rosettes each). Based on the root tuber measurements from the bareroot transplants prior and to keep the soil intact, a 20.32 cm PVC pipe 15.24 cm deep was centered around an individual, hammered into the soil until the top of the pipe section was level with the soil surface. A small trench was excavated to one side of the PVC pipe just below 15 cm so that a flat blade shovel could be pushed underneath the submerged PVC pipe. The PVC pipe remained around the plug of soil until the transplant recipient site was reached and a hole was made for the transplant to be placed in. After placing the transplant in the hole, the PVC pipe was removed and soil that was dug up to make the recipient hole was used to fill in the air space where the PVC pipe had been placed. These transplants were also placed at their recipient site on the same day of excavation within 2 to 4 hours.

For both bare-root and soil-intact transplants, a 38 cm aluminum stake flag was placed 7 cm north of the plant location to record presence or absence in following seasons and years and total leaf length of rosettes on approximately the same day before and after transplantation. A paired samples t-test was used to detect any differences in the total leaf length of individuals before and after the soil-intact transplantation.

Preliminary results from the 2007 bare-root transplants were encouraging. As a result, an additional study using a larger sample size (n = 59) for the bare-root method

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was conducted between 30 April 2008 to 13 May 2008 to later compare with the soilintact transplants from 2007.

Results

Preliminary Bare-Root Transplants

Maximum root tuber depth was 9 cm with a maximum lateral length of 8 cm (Table 7.1). Number of root tubers ranged from 2 to 8 and were strongly correlated with the sum of root tuber lengths data. The sum of root tuber lengths was also strongly correlated with the sum of total rosette leaf lengths (Fig.7.4). Persistence of rosettes in 2007 were similar to undisturbed rosettes (Chapter IV, Fig. 4.5), but seemed to persist longer with five individuals still present at the end of August (Fig. 7.5). Two individuals flowered as *S. cernua* and one individual flowered as the peloric form of *S. cernua* (Table 7.2a). One individual that flowered as *S. cernua* still had a rosette present at the time of stalk emergence on 11 September 2007.

Six individuals (60%) re-appeared as a rosette in spring of 2008, also similar to undisturbed rosettes found in 2007 (Table 7.2b). The three individuals that flowered the following fall, were all from the large rosette category, while those that appeared as a rosette in spring of 2008 were a 50:50 mix of small and large individuals (Table 7.2c). Total leaf length increased for four of five individuals that re-appeared in spring of 2008 and were able to be re-measured on 18 April 2008 (Fig. 7.6). The remaining sixth

	Maximum root	Maximum overall root
ID	tuber depth (cm)	tuber width (cm)
1	8	7
2	5.7	5
3	4	4
4	9	12
5	8	14.5*
6	1.7	2.6
7	3	2.5
8	7.5	12.8
9	3	3.8
10	5.5	3.5

Table 7.1. Maximum depth and overall width of root tubers for 10 bare-root transplants of unknown species.

* maximum lateral root tuber distance from plant was 8 cm

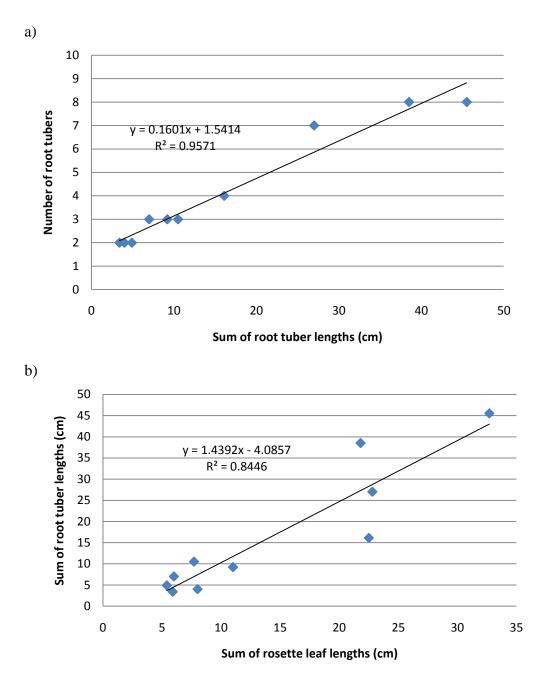


Figure 7.4. Correlation of a) number of root tubers and the sum of all root tuber lengths (cm) (p=.000) and b) sum of all root tuber and rosette leaf lengths (cm) (p=.000) for 10 bare-root transplants transferred on 18 April 2007.

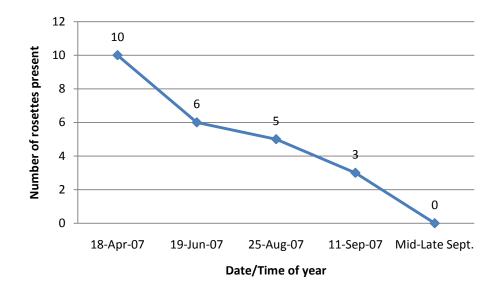


Figure 7.5. Persistence of 10 bare-root transplanted unknown S. parksii or S. cernua rosettes through spring of 2007.

Table 7.2. Percentage of undisturbed and transplanted rosettes that a) flowered as *S. parksii, S. cernua*, and *S. cernua* peloric form in fall of 2007, b) re-appeared as a rosette in spring of 2008, and c) 10 bare-root transplanted individuals that re-appeared as flowering in 2007 and rosette in 2008 based on its total leaf length size class, small (approx. 5 - 10 cm) and large (approx. 20 - 35 cm).

	Undisturbed Rosettes (n = 204)	Transplants - Soil- Intact (n = 59)	Transplants - Bare- Root (n = 10)
a) Species	Percentage	Percentage	Percentage
S. parksii	7	3	0
S. cernua	4	2	20
S. cernua - peloric form	1	2	10

b)	Cohort	Re-appearance of rosette in 2008 (%)	
	2007 soil-intact transplants (n=62)	63.8	
	2007 bare-root transplants (n=10)	60	
	2007 Undisturbed rosettes (n=376)	63	

c)			Flowering occurrence in fall 2007	Occurrence of rosette in spring 2008
	Small			
	TB-9	5.4	no	yes
	TB-6	5.9	no	no
	TB-3	6.0	no	no
	TB-2	7.7	no	yes
	TB-7	8.0	no	yes
	TB-10	11.0	no	no
	Large			
	TB-4	21.8	yes	yes
	TB-1	22.5	no	yes
	TB-5	22.8	yes	no
	TB-8	32.7	yes	yes

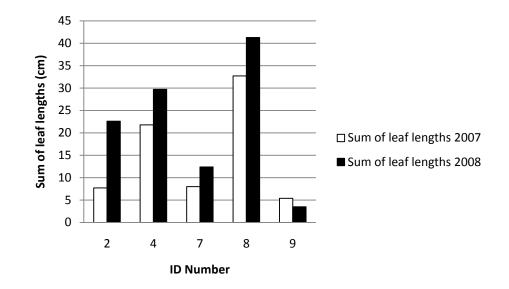


Figure 7.6. Sum of all rosette leaf lengths that were able to be re-measured for bare-root transplanted rosettes on 18 April 2007 (prior to transplanting) and 18 April 2008 (after transplanting).

individual that re-appeared in spring of 2008 disappeared before leaf measurements were taken on 18 April 2008.

Transplant Soil-Intact

Persistence for all transplanted rosettes was not as long as undisturbed rosettes (Chapter IV, Fig. 4.5). Most transplants were gone by late May and none persisted past the month of July (Fig. 7.7). Two of the 62 flowered as *S. parksii*, one flowered as *S. cernua*, and one flowered as *S. cernua* peloric form (Table 7.2). Five other transplants had a flowering stalk emerge in them, but never reached full flower so the species could be identified. Sixty-four percent developed a rosette again in spring of 2008, similar to undisturbed and bare-root transplanted individuals (Table 7.2). Total leaf length was measured within 10 days prior or 20 days after the previous year's total leaf length. Some individuals had a longer total leaf length in 2008 and some a shorter. A paired samples t-test revealed no significant difference (p = 0.445) in the total leaf length between rosette size in 2007 and 2008 (Table 7.3).

Discussion

Results found from the soil-intact method were similar to Parker (2001), as she found *S. parksii* can be successfully transplanted by a soil-intact method. She also stated that a bare-root method for *S. parksii* individuals grown *in vitro* was unsuccessful

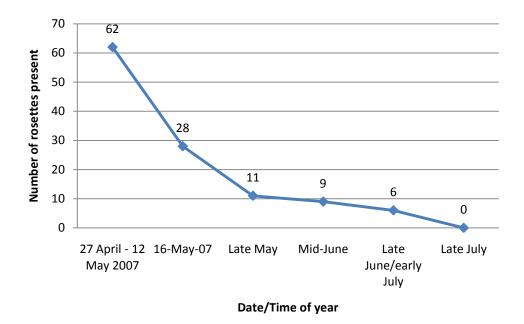


Figure 7.7. Persistence of 62 rosettes in 59 soil-intact transplants in spring of 2007.

Table 7.3. Paired samples t-test descriptive for the total leaf length measurements for individuals before (2007) and after (2008) soil-intact transplantation. No significant difference was found among all individuals (p = .445).

Year	Mean	SE	t	df	p-value
2007	15.97	1.53	-0.77	34	0.45
2008	17.22	1.27	-0.77	54	0.45

(Parker 2001). The results reported show success of bare-root transplantation of individuals *in situ*. This could be due to associated mycorrhizae within the root tubers for *in situ* individuals.

When both cohorts of bare-root transplants were conducted, the soil was near field capacity, therefore much of the immediate soil on the root tubers remained while their length could still be measured on the preliminary cohort. This could be an important part of the success of the preliminary bare-root transplants as mycorrhizae is thought to be facultative for a mature individual (Pileri 1998; Rasmussen 1995; Wells 1967).

Care should be taken in making these successful conclusions as other terrestrial orchids are known to build up carbohydrate reserves in the root tubers for subsequent years of growth (Wells & Cox 1991). It is unknown how long an individual could persist on these reserves.

CHAPTER VIII

SUMMARY AND CONCLUSIONS

Little published research exists on *S. parksii* and most information on the species has been derived from observations (Correll 1950; Luer 1975; Catling & McIntosh 1979; USFWS 1980, 1982, 1984, 1993, 2006, 2007; Bridges & Orzell 1989; Parker 2001; Wilson 2006b). These studies were conducted to better identify life cycle and demographics of *S. parksii*, local and landscape habitat, and provide results for effective transplantation of *S. parksii* individuals. Additionally, much of the results will aid in future research of *S. parksii*.

Number of *S. parksii* individuals is highly variable between years at Twin Oaks and may be attributed to seasonal rainfall during rosette and early flowering growth, although site specific rainfall data should be used for future correlations. Distance to drainages increases with more favorable years of flowering of *S. parksii* and is probably due to more available soil moisture, but should be measured to be better quantified. *S. parksii* likely occurs predominantly on Burlewash soil series on Twin Oaks, but should be further investigated as they occurred on differing soil types according to the county soil survey.

Size of rosettes seems to be correlated to the likelihood of flowering in *S. parksii*, but due to low sample sizes, no conclusions could be made in this study. Additionally, selected individuals for this type of study should be continually monitored on a weekly to bi-weekly basis to document a maximum growth and persistence as all rosettes do not

appear and disappear at the same time. Like rosettes, flowering individuals of *S. parksii* did not all appear aboveground at the same time, therefore, surveys should be conducted from mid-September to late November at Twin Oaks. Permanently marked individuals should be continually monitored for more detailed information of the longevity of *S. parksii*.

A likely local habitat of *S. parksii* occurred with *Schizachyrium scoparium*, *Chasmanthium laxum* var. *sessiliflorum*, and *Andropogon ternarius*, 41-100% leaf litter at a light to light-heavy depth, 0-30% bare soil, small amounts of slash and on mid to high position of a 0-4 degree slope at some type of northerly aspect. Additionally, *S. parksii* is likely to occur with a lighter woody canopy > 2 m of approximately 20-50%. While some of these variables were not consistent for all *S. parksii*, they should be used when assessing the habitat for *S. parksii*, and more importantly for transplantation of *S. parksii* individuals to deed-restricted areas.

Historical photos (1958) of Twin Oaks suggest that much of the site has been encroached by woody vegetation and areas that do not show woody encroachment are more open due to mechanical clearing. Additionally, by using current aerial imagery, open patches within woody cover are seen in proximity to current populations of *S*. *parksii*, therefore *S. parksii* likely occurred in 1958 in current locations and was probably more widespread across Twin Oaks.

Transplantation of *S. parksii* was documented successful by the soil-intact method, and while the bare-root method showed success with *S. cernua*, no conclusions can be made of its success for *S. parksii*. This was likely due to a small sample size in

the preliminary bare-root transplants (n = 10). A larger sample of bare-root transplants was conducted in spring of 2008 and will be continually monitored to determine success.

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APPENDIX A

FORTY-EIGHT SPECIES INCLUDED IN THE NMS ORDINATION AND OTHER

ANALYSES

	GRAMINOIDS
Family	Species
	Annual
Poaceae	Panicum brachyanthum
	Annual or Perrenial
Cyperaceae	Cyperus sp.
Cyperaceae	Scleria sp.
Juncaceae	Juncus sp.
Poaceae	Aristida sp.
	Perennial
Cyperaceae	Carex sp.
Juncaceae	Juncus marginatus
Poaceae	Andropogon ternarius
Poaceae	Chasmanthium laxum var. sessiliflorum
Poaceae	Nasella leucotricha
Poaceae	Panicum aciculare
Poaceae	Panicum sphaerocarpon
Poaceae	Paspalum plicatulum
Poaceae	Paspalum setaceum
Poaceae	Schizachyrium scoparium
Poaceae	Sorghastrum nutans
Poaceae	Sporobolus compositus var. clandestinus

FORBS

Family	Species
	Annual
Asteraceae	Chrysopsis pilosa
Asteraceae	Evax sp.
Asteraceae	Gamochaeta sp.
Euphorbiaceae	Acalypha gracilens
Euphorbiaceae	Croton capitatus
Euphorbiaceae	Croton monanthogynus
Plantaginaceae	Plantago virginica
Rubiaceae	Diodia teres
	Annual or Perennial
Asteraceae	Erigeron strigosus
Asteraceae	Rudbeckia hirta
Fabaceae	common name: wild mercury
Fabaceae	Crotalaria sagittalis
Oxalidaceae	Oxalis corniculata
	Perennial
Asteraceae	Aster pratensis
Asteraceae	Liatris squarrosa
Cistaceae	Lechea mucronata
Convolvulaceae	Dichondra carolinensis
Fabaceae	Desmodium sp.
Iridaceae	Alophia drummondii
Linaceae	Linum medium
Orchidaceae	Spiranthes parksii
Rubiaceae	Oldenlandia boscii

	WOODY VEGETATION		
Family	Species		
	Perennial		
Aquifoliaceae	Ilex vomitoria		
Clusiaceae	Hypericum hypericoides		
Cupressaceae	Juniperus virginiana		
Ericaceae	Vaccinium arboreum		
Fagaceae	Quercus nigra		
Fagaceae	Quercus stellata		
Smilacaceae	Smilax bona-nox		
Ulmaceae	Ulmus alata		
	MUSHROOMS		
	any type of mushroom		

APPENDIX B

ALL OTHER SPECIES NOT INCLUDED IN THE NMS ORDINATION AND

OTHER ANALYSES

GRAMINOIDS			
Family	Species		
	Annual or Perennial		
Poaceae	Aristida sp.		
Cyperaceae	Eleocharis sp.		
Juncaceae	Juncus diffusissimus		
Poaceae	Panicum acuminatum var. acuminatum		
	Perennial		
Cyperaceae	Fimbristylis puberula		
Juncaceae	Juncus validus		
Poaceae	Chasmanthium sp.		
Poaceae	Panicum rigidulum		
Poaceae	Paspalum notatum		
Poaceaa	Paspalum setaceum		
Poaceae	Setaria parviflora		

FORB	S

Family	Species
	Annual
Apiaceae	Bifora americana
Asteraceae	Chrysopsis pilosa
Asteraceae	Conyza canadensis var. canadensis
Asteraceae	Gutierrezia (Amphiachyris) dracunculoides
Asteraceae	Pyrrhopappus pauciflorus
Droseraceae	Drosera brevifolia
Fabaceae	Chamaecrista fasciculata??
	Annual or Perennial
Asteraceae	Coreopsis sp.
Asteraceae	Gutierrezia dracunculoides
Asteraceae	Thelesperma filifolium
Fabaceae	Lotus sp.
	Perennial
Acanthaceae	Ruellia sp.
Apiaceae	Eryngium yuccifolium var. synchaetum
Asclepiadaceae	Asclepias sp.
Asteraceae	Ambrosia psilostachya
Asteraceae	Bigelowia nuttallii
Asteraceae	Boltonia asteroides
Asteraceae	Ratibida columnifera
Asteraceae	Solidago sp.
Asteraceae	Vernonia sp.
Cactaceae	Opuntia sp.
Commelinaceae	Tradescantia sp.
Euphorbiaceae	Tragia sp.
Fabaceae	Desmanthus sp.
Fabaceae	Mimosa nuttallii
Scrophulariaceae	Linaria vulgaris

WOODY		
Family	Species	
	Perennial	
Anacardiaceae	Toxicodendron radicans	
Rhamnaceae	Berchemia scandens	
Rosaceae	Crataegus marshallii	
Salicaceae	Salix nigra	
Verbenaceae	Callicarpa americana	
Vitaceaa	Parthenocissus quinquefolia	
	FERNS	
Family	Species	
	Perennial	
Delemedicese	Dlagastic native disides	

Polypodiaceae Pleopeltis polypodioides

MOSS

Family	Species
Polytrichaceae	Atrichium angustatum
Scapaniaceae	Scapania nemorea

Notes on Species Not Identified

(broadleaf forb) (hairy grama look a like) (lamb's ear forb) (id 1, 012P) (id 1, 013PR) (id 1, 014P-016P) (id 3, 013P) (id 6 forb, 7/11/07) (ID 13 7/27/07, dillweed?) (ID 14, 7/27/07, whorled leather, Liatris?) (ID 16 - 7/27/07, little round fruit, flax?) (id 18 7/27/07 Liatris?-prob. Not) (id 2 7/25/07, plant 2P) (ID 5, 7/26/07, purple top? (ID 7, 7/26/07, red base) (ID 6, 7/27/07, fuzzy grass leaf, fringe leaf) (ID 7, sparce spatulate leather, 7/27/07)

VITA

Name:	Jonathan Ryan Hammons
Permanent Address:	Department of Rangeland Ecology and Management c/o Dr. Fred Smeins TAMU College Station, TX 77843-2126
Email Address:	ryanhammons2000@yahoo.com
Education:	B.S., Renewable Natural Resources, Texas A&M University at College Station, 2006M.S., Rangeland Ecology and Management, Texas A&M University at College Station, 2008