THE EFFECTS OF WOODY PLANT MANAGEMENT ON HABITAT CONDITIONS, PLANT DEMOGRAPHY, AND TRANSPLANTATION SUCCESS OF THE ENDANGERED ORCHID *Spiranthes parksii* CORRELL

A Thesis

by

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ABSTRACT

Spiranthes parksii Correll is a federally endangered species endemic to 13 counties of the Post Oak Savanna in Central Texas. Approximately 700 *S. parksii* are located on the Brazos Valley Solid Waste Management Agency's (BVSWMA) Twin Oaks landfill property in Grimes County, Texas. The opportunity to study *S. parksii* was created through the mitigation requirements set forth by the United States Fish and Wildlife Service (USFWS) on the property.

Studies were designed to assess the effect of woody plant management on the target species with the overall goal of enhancing the establishment, growth, and reproductive success of *S. parksii.* Evergreen midstory shrub removal and woody patch clearing to produce varying sized open grassland and woody patches to increase edge effect were studied to determine their influence on habitat for *S. parksii*. *S. parksii* were transplanted from at risk areas to permanently protected areas to evaluate potential effective procedures for transplanting of the species.

Seasonal variability in *S. parksii* and *Spiranthes* spp. numbers were common across all studies, however, flower production in the fall was positively correlated with the summed January through March precipitation. In general, the removal of encroached woody plants resulted in a positive orchid response, though poor herbicide efficacy may limit this treatment effect in the long-term. Transplantation of *S. parksii* into areas which received woody plant management resulted in at least a 50% survival rate 3 years post-transplant.

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I would like to thank my committee chair, Dr. Fred E. Smeins; he was the keystone which held the process together. His unwavering patience and enthusiasm provided me the opportunity to consistently progress despite the hardships and pitfalls inherent with scientific exploration. I would like to thank Dr. William E. Rogers; he sparked my interest in scientific research by providing me with the opportunity to work on the orchid during my undergraduate years and has been a superb mentor throughout my academic career. Dr. Anthony M. Filippi enlightened me through his teachings and has always been there to provide prompt assistance and feedback.

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Mom and Dad, thank you for your unconditional love and support. Ubuntu: “I am, because of you.”

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CHAPTER I

INTRODUCTION AND OBJECTIVES

Spiranthes parksii (Correll) (Navasota Ladies'-Tresses) is a federally listed endangered orchid endemic to 13 counties of the Post Oak Savanna in Central Texas (USFWS 2009). As of 2009 the known species population was conservatively estimated to be 3141 surviving individuals, of which 93% occur in Brazos and Grimes counties (USFWS 2009; Wonkka et al. 2012). Those two counties are undergoing substantial urban and exurban development which impacts survival of the species. Regulatory deed-restricted areas (DRA's) established on the Brazos Valley Solid Waste Management Agency's (BVSWMA) Twin Oaks landfill site contain approximately 700 *S. parksii* plants which provide an opportunity to conduct research on the species.

The biology and ecology of the species is not well understood, however, research conducted by ecologists at Texas A&M University over the past decade has substantially increased our knowledge (Hammons 2008, Hammons et al. 2010; Wonkka 2010; Wonkka et al. 2012; Ariza 2013). A large number of individual plants have been documented and followed since 2000 to better describe population dynamics including plant survival, flowering frequency, rosette density and demography, seedling establishment, seed production, mycorrhizal associations, and herbivory impacts. Habitat data including soil characteristics, litter depth, associated vegetation, light availability, topography, and elevation have been collected for multiple populations of *S. parksii*.

Belowground studies are being conducted to determine species of mycorrhizal fungi upon which *S. parksii* depends for germination, seedling development, and survival (Ariza 2013). Additionally, small scale patch burns are being used to alter the litter and herbaceous layers of these communities to better understand the influence fire may have on *S. parksii* biology and ecology (Wonkka 2009).

Historically the Post Oak Savanna ecosystem was influenced by fire. A fire frequency of 5-10 years helped maintain open patchy savannas throughout the landscape except in areas where water courses or other physical barriers limited its effects (Smeins & Diamond 1986). Fire suppression along with other land use changes have occurred for over a century and have created a landscape which is increasingly dominated by shrubs and trees. A general hypothesis is that decreasing light availability due to increases in woody cover contributes to *S. parksii* decline and that the species will benefit from the reintroduction of woody plant management on the landscape. Specific hypothesis/objectives of this study are:

Hypothesis 1: Establishment, growth and reproductive ability of *S. parksii* is driven by direct light quantity.

Objective 1: In a horizontally stratified, three-layered herbaceous, shrub (deciduous and evergreen), and tree Post Oak woodland containing scattered *S. parksii*, the evergreen shrub/small tree layer will be mechanically removed and chemically stump-treated to alter the light regime in order to enhance the establishment, growth, and reproductive success of *S. parksii*.

Objective 2: Selected soil/topographic/drainage regime variables will be evaluated to assess their interaction in order to assess their ability to enhance the establishment, growth, and reproductive success of *S. parksii*.

Hypothesis 2: Woody patch size, pattern, and amount of edge within a grassland matrix and the corresponding variation in light regime are related to the distribution, abundance and reproduction of *S. parksii*.

Objective 1: In several locations across the Twin Oaks site a vertically stratified, dense three-layered Post Oak Woodland will be mechanically altered by removing woody cover to create varying sizes of woody patches in order to enhance the establishment, growth, and reproductive success of *S. parksii*.

Objective 2: Selected soil/topographic/drainage regime variables will be evaluated for their responses to the various treatments in order to assess their ability to enhance the establishment, growth, and reproductive success of *S. parksii*.

Hypothesis 3: *S. parksii* populations are not stable from year to year.

Objective 1: Permanently locate and monitor flowering individuals and individual rosettes within the two above mentioned studies in order to assess the degree of variation of flower and rosette production from year to year and their ability to enhance the establishment, growth, and reproductive success of *S. parksii*.

Objective 2: Assess biophysical site factors including; relationship to woody cover and litter depth to determine factors that may relate to variations in *S.*

parksii numbers in order to assess their ability to enhance the establishment, growth, and reproductive success of *S. parksii*.

Hypothesis 4: Biophysical site traits have significant influence on success of transplanting *S. parksii*.

Objective 1: Determine the effectiveness of transplanting *S. parksii* plants from at risk donor sites to protected recipient sites with predicted favorable environments for persistence of *S. parksii* in order to enhance the establishment, growth, and reproductive success of *S. parksii*.

Objective 2: Evaluate the biophysical features of the donor and recipient sites to assess features most favorable to transplanting success in order to enhance the establishment, growth, and reproductive success of *S. parksii*.

CHAPTER II

LITERATURE REVIEW

Savannas are defined as areas with a continuous herbaceous cover and a discontinuous layer of trees or shrubs with 25-50% cover (Belsky 1990; McPherson 1997; Taft 1997). Generally they are characterized by seasonal climates, with distinct wet and dry seasons (Khavhagali & Bond 2008). Savannas are matrix grasslands that have periodic establishment and mortality of fire tolerant shrubs and trees including oak species (Curtis 1959). In North America, post-settlement reductions in fire frequency caused by landscape fragmentation, changing land use, and fire suppression have allowed significant structural changes across many North American ecosystems. One such change has been woody encroachment and closing of canopies (Stout 1944; Cooper 1960; Abrams 1986; Davis & Faber-Langendoen 1995; Agee 1996), which leads to a shift towards dominance by shade tolerant and fire-sensitive species (Heyward 1939; Garren 1943; Dickman 1978; Wright & Bailey 1982; Agee 1996).

The Post Oak Savanna is no exception and has experienced significant encroachment over the last century (Dyksterhuis 1957). The Post Oak Savanna encompasses roughly 3.4 million ha in east-central Texas and stretches from the Red River in the north, south to San Antonio (Gould 1969). Historically, the pre-European fire regime kept shrub species and Eastern red-cedar *(Juniperus virginiana)*[1] from

[1] Taxonomic nomenclature follows Diggs et al. 1999.

spreading throughout the landscape which helped to maintain a grassland community mixed with various shrub and oak tree species. The post-European removal of fire has allowed for woody encroachment across much of the Post Oak Savanna landscape.

Multiple studies conducted within North America have suggested woody encroachment within savanna ecosystems can alter herbaceous productivity (Hennessy et al. 1983), biodiversity (Archer 1995), and understory species composition (Anderson et al. 2000; Nielsen et al. 2003), though little is known beyond the effects on dominant grasses (Scholes & Archer 1997). Understory species react to the woody understory light, moisture, and nutrient regimes which results in compositional, biodiversity, and production differences between the woody and grassland patches (Heyward 1939; Cooper 1960; Wright & Bailey 1982; Belsky et al. 1989, 1993; Ko & Reich 1993; Scholes & Archer 1997; Grundel et al. 1998; Weltzin & Coughenour 1990; Mordelet & Menaut 1995; Anderson et al. 2001; Ludwig et al. 2004).

Woody canopy increase is generally agreed to be a major factor in the decrease of many orchid species within savannas, and possibly for *S. parksii* within the Post Oak Savanna Ecoregion of Central Texas (Farrell 1991; Tamm 1972, 1991; Hammons 2008). In a long-term study of orchids within eastern Sweden, Tamm (1972) stated that a decrease in orchid numbers within a majority of their sampling areas could be attributed to increased light competition from encroaching woody species. They further suggest the woody encroachment seen within their study resulted from environmental and land use changes which include discontinued cattle grazing, haying, and mowing.

S. parksii, first discovered in Texas in 1945, is one of 15 native terrestrial orchids in Texas (Dueck & Cameron 2008). The orchid was described by Correll (1947) and was listed as a Federally Endangered Species in 1982 (MacRoberts & MacRoberts 1997). Currently there are 24 reserves established across central and east Texas to protect *S. parksii* through habitat conservation, many of which are located within Brazos and Grimes counties. These two counties contain 93% of the known *S. parksii* populations, though this percentage is probably inflated due in large part to the substantial number of surveys and research conducted in these counties (Wonkka et al. 2012). One reserve, created as part of the mitigation process of the Twin Oaks BVSWMA landfill located in Grimes County, is the site for this research.

General observations and limited research characterize the habitat for the *S. parksii* as upland Post Oak Woodland with grassland patches, often along stream banks of upland stream tributaries (Correll 1978; Luer 1975; Catling & McIntosh 1979; USFWS 1980, 1982, 1984, 1993, 2006, 2009; Bridges & Orzell 1989; Parker 2001; Wilson 2006; Hammons 2008; Wonkka et al. 2012). The species is rarely found in areas severely disturbed by anthropogenic impacts, such as road sides, power line easements, or open fields (Wilson 2006). *S. parksii* occur on acid sandy loams and loamy sands (Correll & Johnston 1970).

Morphologically, *S. parksii* are clearly distinct from all other orchids except *S. cernua*. During the rosette stage, *S. parksii* and *S. cernua* appear identical, which poses difficulties for distinguishing the endangered orchid from the more abundant *S. cernua* when only rosettes are present. However, when in full flower the two species can be distinguished (Hammons 2008; Wonkka 2010; Wonkka et al. 2012; Ariza 2013). Therefore the identification of *S. parksii* can only be accomplished during the fall flowering period.

The seeds of orchids including *S. parksii* are among the smallest known in the plant kingdom (Rasmussen 1995). They lack an endosperm and require a symbiotic relationship with mycorrhizae for protocorm development (Wells 1981). Their seeds are well adapted to dispersal by wind, water, and animals as they have a thin seed coat and impermeable testae which contain air bubbles (Arditti & Ghani 2000). Little is known regarding their dispersal mechanisms, but it has been suggested that their patchy distribution is potentially representative of a limited dispersal shadow (Wonkka et al. 2012).

After germination there are three main stages of orchid life cycle development; protocorm, mycorrhizome, and adult plant. Rasmussen (1995) states that "The protocorm stage may be defined in practice as the stage from germination until the seedling has a shoot tip with primordial leaves but no roots, the mycorrhizome stage being initiated when the apical meristem elongates and the first roots develop." The initiation of the adult stage is characterized by the formation of the basal rosette. The time spent by *S. parksii* in each stage is still unknown (Hammons 2008), though for orchids in general the mycorrhizome stage lasts between 2-4 years and about 2 years for *S. cernua* (Rasmussen 1995).

Both rosette and flower production are highly variable from year to year among orchids (e.g., Tamm 1972; Wells 1967). *S. parksii* generally produce rosettes beginning in late December with peak rosette production occurring from late February into early March. While they may produce flowers as early as September, generally peak flowering occurs in late October to early November followed by seed dispersal occurring in December (USFWS 1984; Parker 2001; Hammons 2008; Wonkka et al. 2012; Ariza 2013). *S. parksii* survive underground as dormant rhizomes via root stores from the time of rosette senescence in the late spring/early summer until they reemerge as a flowering stalk and/or rosette during the fall (Hammons 2008; Wonkka et al. 2012; Ariza 2013).

CHAPTER III

STUDY AREA

The study area is located on the 246 ha Twin Oaks BVSWMA landfill site approximately 6.4 km west of Carlos, TX and 20 km east of College Station, TX. (96˚8'51.86"W, 30˚35'47. 25"N) (Figure 1).The total parcel consists of an 86 ha landfill footprint, 56 ha in thirteen *S. parksii* deed-restricted areas (DRA's) and a few remaining undesignated areas. Of the total deed-restricted area, 45 ha are presumed to be potentially suitable for *S. parksii* (USFWS 2006). The site is located within the Navasota River watershed and on site there are 10 intermittent and 17 upland ephemeral streams that total approximately 10.3 km (HDR 2002).

The climate of the Post Oak Savanna is characterized by a bimodal precipitation pattern with maxima occurring in late spring/early summer and late summer/early fall (Figure 2). The thirty year mean annual precipitation is 101.8 cm and mean annual temperature is 20.5^{o}C although considerable variability exists in both measures. Annual rainfall totals for the period of these studies varied from 50.6 to 105.6 cm (Table 1). The average freeze-free period is approximately 285 days. The site is located on the Wellborn Geologic Formation (Eocene) and Alluvium (Holocene) (BEG 2007). The Wellborn Formation is primarily sandstone and clay with an average thickness of 45 m (BEG 2007). Soils are primarily Alfisols and mapped primarily as Burlewash fine sandy loam at 1-5% and 5-12% slopes (NCSS 2007). Other soil series on the site are Boonville, Hatliff, Koether-Rock, Padina, Robco, Shiro, and Tabor (NCSS 2007). These series are mainly loamy fine sands to fine sandy loams over a claypan subsoil, are well drained, and have low to moderate water holding capacity (USDA 1996).

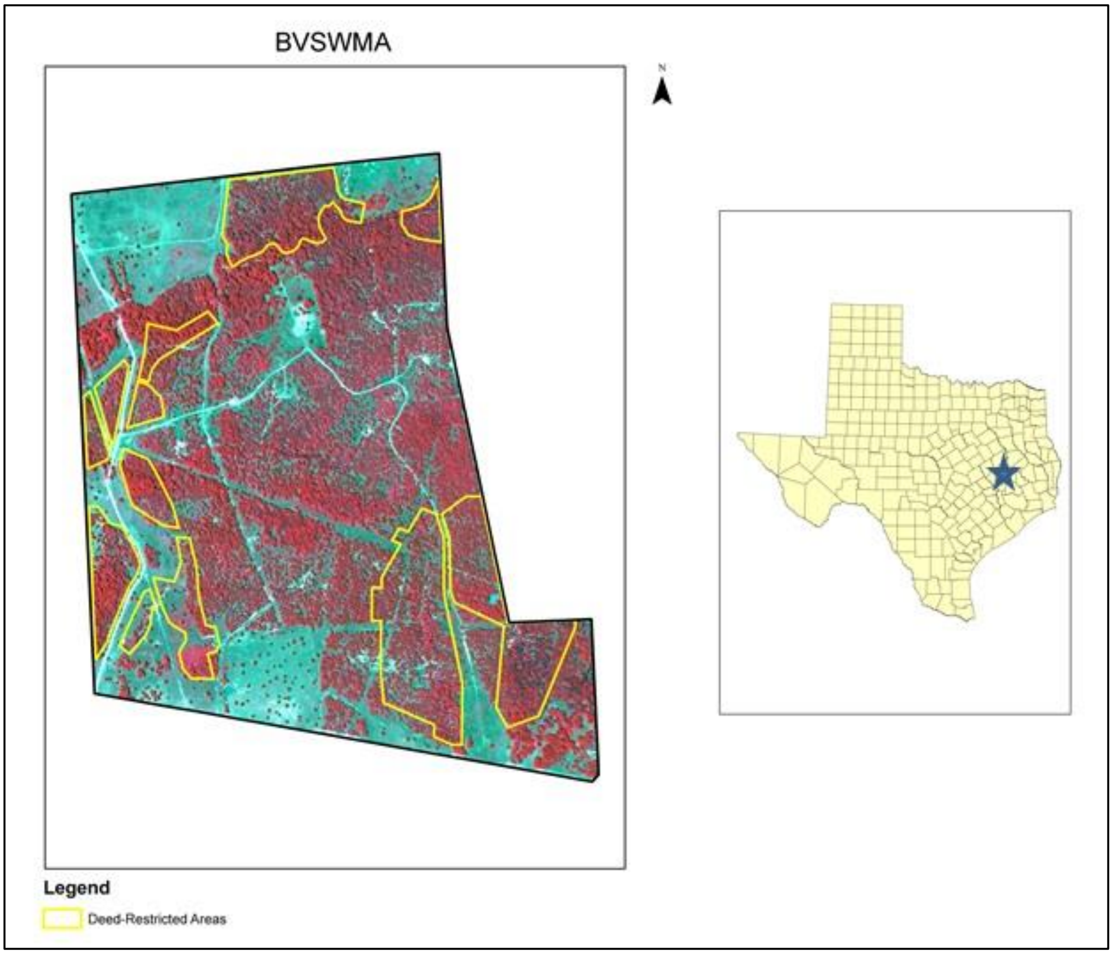


Figure 1. Map of the thirteen DRA's locations within the BVSWMA *Twin Oaks landfill in Grimes County, TX.*

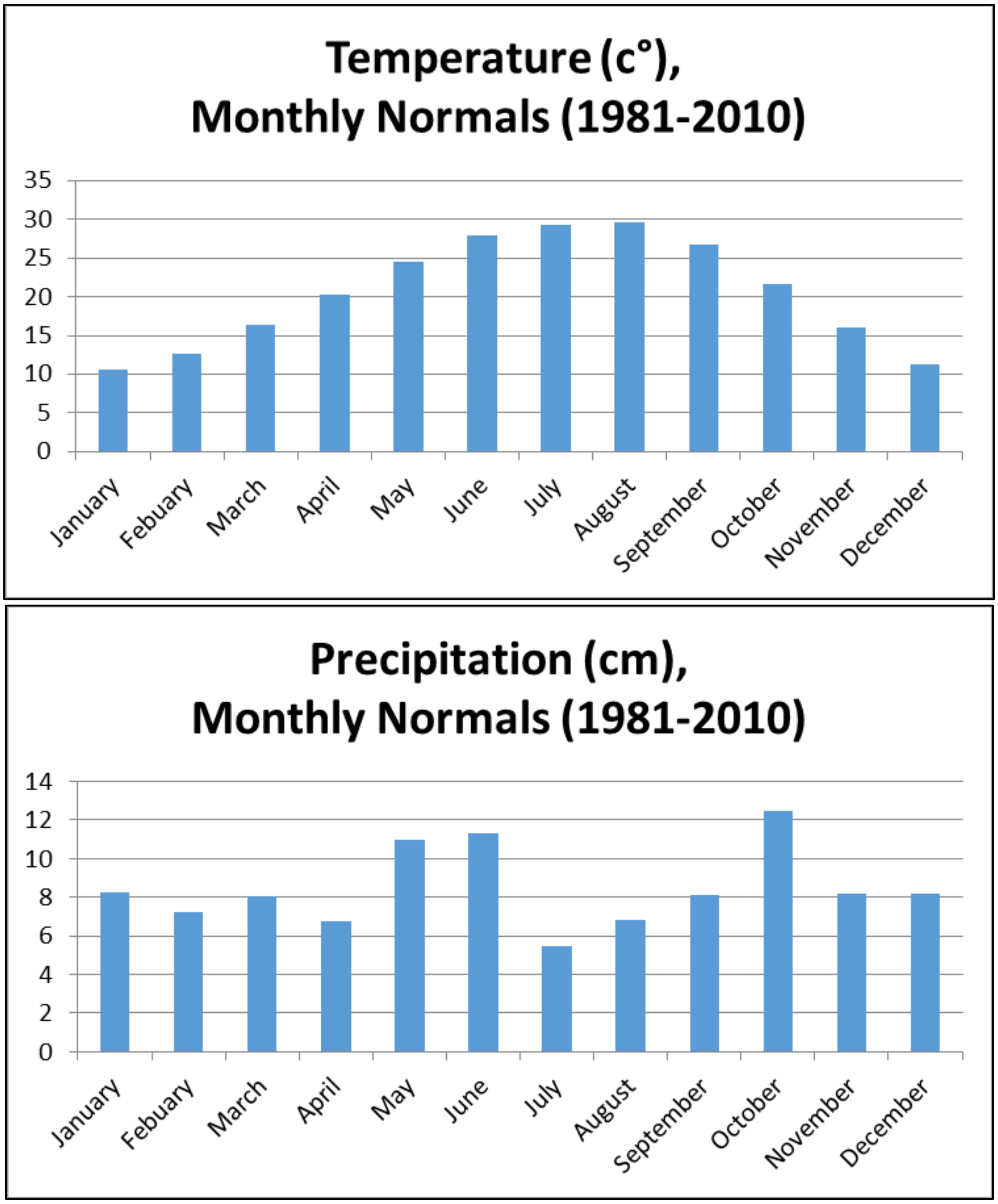


Figure 2. Monthly normal temperature (C °) and precipitation (cm) (1981-2010) for Easterwood Airport, College Station, TX.

Table 1. Monthly rainfall totals (cm) 2007-2012 for Easterwood Airport, College Station, TX.

	Annual Rainfall (cm)					
	2007	2008	2009	2010	2011	2012
January	12.7	5.2	1.8	7.4	7.6	7.1
February	0.2	8.4	1.7	7.1	1.6	23.7
March	17.1	9.7	12.9	6.7	1.8	22.0
April	7.3	7.0	15.5	2.8	0.00	1.5
May	10.2	10.9	3.6	5.1	8.6	4.6
June	12.7	0.7	0.00	18.5	7.3	5.4
July	11.7	1.0	6.2	3.1	0.1	11.6
August	3.6	16.5	1.8	0.8	0.7	4.3
September	3.7	8.8	18.8	14.8	5.7	8.2
October	6.8	4.4	21.0	0.00	2.4	5.5
November	9.9	3.4	8.7	2.3	6.1	1.4
December	9.8	2.0	7.1	2.1	8.7	9.5
Total	105.6	78.1	99.1	70.6	50.6	104.7

The study area ranges from relatively open grassland to varying numbers and sizes of individual or woody patches of trees and shrubs within a grassland matrix to a closed canopy woodland often with a dense shrub understory. The understory often consists of post oak saplings (*Quercus stellata*), Eastern red-cedar, yaupon (*Ilex vomitoria*), American beauty-berry (*Callicarpa americana*), farkle-berry (*Vaccinium arboretum*), and winged elm (*Ulmus alata*). The herbaceous layer of the savanna and the patches of mixed open native grassland and tame pasture are dominated by grasses including; little bluestem (*Schizachyrium scoparium)*, hairy-awn muhly *(Muhlenbergia capillaris),* and bahia grass *(Paspalum notatum)* in open grasslands and more shade tolerant species such as various panic grasses *(Panicum* spp.*)* and narrow-leaf wood-oats (*Chasmanthium laxum* var. *sessiliflorum)* that occur under woody canopies.

The following information was gathered from Hammons (2008) regarding previous land use over the site. Over the past several decades the property had been moderately stocked with cattle at an estimated yearlong stocking rate of 1 animal unit per 3 ha. Wild game harvesting took place over the last 50-60 years, with an estimated 1-2 white-tail deer (*Odocoieus virginianus*) and 20 feral hogs (*Sus scrofa*) taken yearly from the site. It is presumed that cotton farming occurred over small portions of the site adjacent an old homestead due to the close proximity of a local cotton gin during that time period. Prescribed burns were conducted over select areas on three year intervals over the last 50-60 years. Bull dozing occurred over portions of the site and these areas were often seeded to improved livestock pastures using bahia grass.

CHAPTER IV

WOODY PLANT MANIPULATIONS

Evergreen Shrub and Sapling Removal Analysis

Introduction

Savannas are matrix grasslands that have periodic establishment and mortality of fire tolerant shrubs and trees including oak species (Curtis 1959). Reductions in fire frequency caused by landscape fragmentation, changing land use, and fire suppression have allowed significant structural changes across many North American ecosystems. Multiple studies conducted within North America have suggested woody encroachment within savanna ecosystems can alter herbaceous productivity (Hennessy et al. 1983), biodiversity (Archer 1995), and understory species composition (Anderson et al. 2000; Nielsen et al. 2003). General agreement exists supporting woody encroachment as a major factor in the decrease of many orchid species within savannas, and *S. parksii* within the Post Oak Savanna Ecoregion of Central Texas (Farrell 1991; Tamm 1972, 1991; Hammons 2008).

The goal of this study was to assess the long-term response of naturally occurring *S. parksii* and *S. cernua* individuals to the removal of the evergreen woody shrub layer of a Post Oak Woodland. It has been hypothesized that woody encroachment and the subsequent increased competition for light and other limited resources such as water or nutrients may be a key factor in the decline of *S. parksii* (Hammons 2008).

This investigation was conducted in the 10.1 ha, deed-restricted area (DRA) 2 of the Twin Oaks landfill (Figure 3). This area was selected since initial surveys by HDR Engineering, Inc. in 2001(HDR) located several populations of *S. parksii* in the area (HDR 2002; Hammons 2008).

The area selected has undergone little management over the last decade and combined with the removal of cattle grazing pressure and periodic fires has become severely encroached within the understory shrub canopy. Therefore, an attempt was made to remove a substantial portion of this encroaching shrub layer. All *S. parksii* as well as the entire *Spiranthes* spp. population within the study plots were documented from the spring of 2007 through the spring of 2013.

Methods

Study site

Research plots are located within DRA 2 in the northern portion of the Twin Oaks property. The area was selected based on previous verified sightings of *S. parksii* during 2001 surveys conducted by HDR (HDR 2002; Hammons 2008). The site is representative of a typical Post Oak Savanna/Woodland with post oak dominating the overstory and yaupon, farkle-berry, and American beauty-berry composing the majority of the midstory. The understory is composed of little bluestem and narrow-leaf wood-oats along with a great diversity of less abundant grasses and forbs. The plots are located on an upland portion of the DRA on two sides of an intermittent stream which runs through the center of the DRA. Plots were distributed in a grid pattern with slight adjustments to capture known locations of orchids. The soils are Burlewash fine sandy loams.

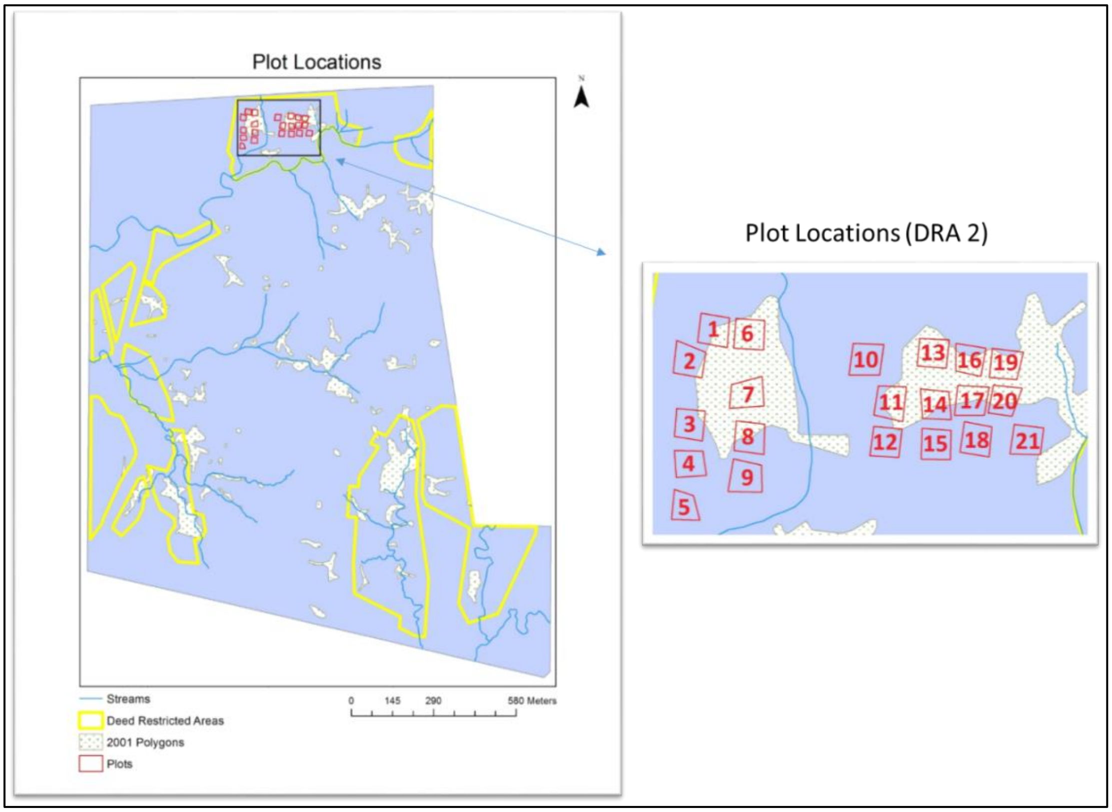


Figure 3. Location of evergreen shrub and sapling removal plots (1-21) in deed-restricted area 2, Twins Oaks site.

Treatments

Twenty-one 20 x 20 m plots were randomly assigned to one of three treatments; unmanipulated control, cut-stump, or cut-stump with a post-cut herbicide application. This provided a total of seven replicates for each whole plot treatment. All yaupon and Eastern red-cedar with a height of greater than 1 m but a trunk base diameter of less than 3 cm were cut at approximately 10-15 cm above the ground and removed from the plots by hand. This treatment took place in January 2008, prior to the peak rosette period. The herbicide application occurred in August 2008, by re-clipping the re-sprouting shrubs within the cut-herbicide plots and applying a herbicide mixture. The mixture (25% Remedy (3,5,6-trichloro-2-pyridinyloxyacetic acid) in 75% diesel) was applied immediately after re-clipping using a 5 gallon backpack sprayer and applying it to the exposed cambium of the stump and bark to the point of saturation. No manipulations occurred in the control plots.

Data collection

Intensive surveys were conducted seasonally (fall and spring) both pre- and post-treatment starting in the spring of 2007 and continued through the spring of 2013. Pre-treatment measurements included *Spiranthes* spp. rosette and flowering individuals. All *Spiranthes* spp. which occurred above ground in 2007 were GPS located and permanently marked using a metal flag with an accompanying identification tag at 30 cm north of the plant location. The number of rosette leaves, leaf length and width, and a visual estimation of the percentage of herbivory damage during periods of peak rosette growth were recorded. Flowering individuals were identified to species, stalk and inflorescence lengths were measured, the numbers of flowers per stalk were counted, flower color was noted (white versus cream) and the percentage of herbivory damage was visually assessed. Habitat data were collected including measurements of litter depth, estimates of canopy cover type (shade; under the canopy of the tree/shrub layer, dripline; transition between the tree/shrub canopy and the open savanna, or open; in the open savanna), and assessments of whether the orchid was situated along a riparian drainage.

Rainfall data was obtained for Easterwood Airport, College Station, TX which is located approximately 13 mi west of the Twin Oaks site.

Data were collected prior to this study which included measurements to evaluate the woody vegetation manipulation effect on orchids within the plots. These data included pre-(fall 2007) and post-treatment (fall 2008) woody cover estimates as well as instantaneous light measurements. Unfortunately, these data were not available specifically the pre-treatment data, which made the characterization of the pre-treatment vegetation condition and post-treatment vegetation response to treatment impossible. Therefore, this analysis is mainly a study of the demographic characteristics of a population of orchids and any suggestions of the orchid responses to treatments should be considered speculative.

Statistical analysis

The relationship between several summed rainfall totals and orchid production was assessed using a simple linear regression. Assessments of; total annual rainfall, rainfall during the month of August, and the separate assessments of the combined total rainfall during the months of July and August and January through March were all conducted.

A MANOVA Repeated Measures test was conducted to assess year to year differences in both flower and rosette production, by plot treatment. Data were ranked prior to running the analysis. The Sphericity Test result was reported along with the

Univariate unadjusted Epsilon values. Year to year mean differences were assessed by conducting a Student's t test with results reported as mean ± standard deviation.

A statistical analysis was conducted to assess the plot treatment effect on the production of fall and spring rosettes and the number of times the recorded individuals within the plots remained belowground. Test for normality was conducted using a Shapiro-Wilk W test with transformations attempted where data did not meet parametric assumptions. In all instances the data was not normally distributed and in no instances were transformations successful. Test of homogeneity of variances was conducted using four tests (O'Brien, Brown-Forsythe, Levene and Bartlett). All analyses were conducted using nonparametric methods. Across group differences were analyzed using a Kruskal-Wallis test and a Wilcoxon Each Pair test was used to assess differences in the means.

A statistical comparison was conducted to assess the difference in flowering plant height and spring rosette leaf area between *S. parksii* and *S. cernua.* The within species variability in both flowering plant height and spring rosette leaf area was also assessed. Test for normality was conducted using a Shapiro-Wilk W test with transformations attempted where data did not meet parametric assumptions. Test of homogeneity of variances was conducted using four tests (O'Brien, Brown-Forsythe, Levene and Bartlett).

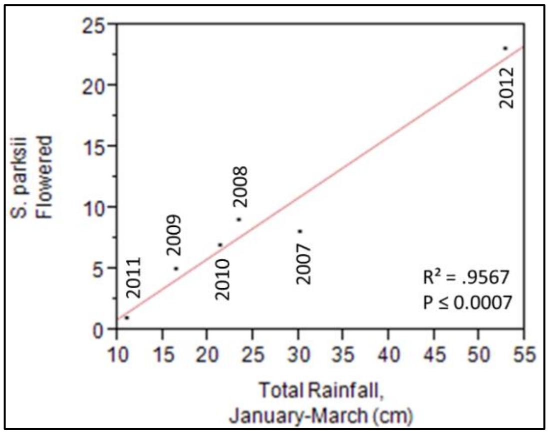
When data met the assumptions of parametric statistics, across group differences were analyzed using a Student's T-test and are represented as mean ± standard error. Among group differences were assessed using an Analysis of Variance (ANOVA) test with significant differences represented with different lowercase letters.

In instances where data did not meet parametric assumptions, across group differences were analyzed using a Wilcoxon test and are represented as mean ± standard deviation. Among group differences were assessed using a Kruskal-Wallis test with differences in means tested using a Wilcoxon Each Pair test with significance represented as different lowercase letters.

In all instances statistical analyses were conducted using JMP 10 (SAS 2010).

Results

August rainfall showed no significant ($p = 0.69$) correlation with *S. parksii* flowering numbers while rainfall for July and August produced an improved but not significant relationship for *S. parksii* ($p = 0.16$) and *S. cernua* ($p = 0.06$). Total rainfall, January through March and the production of flowers later that year were highly correlated for both *S. parksii* ($p < 0.01$) and *S. cernua* ($p < 0.01$) (Figure 4). However, January through March total rainfall was not significantly correlated with spring rosette numbers ($p = 0.44$). Annual rainfall was assessed and no relationship was found with rosette density or with either *S. parksii* or *S. cernua* flower production.



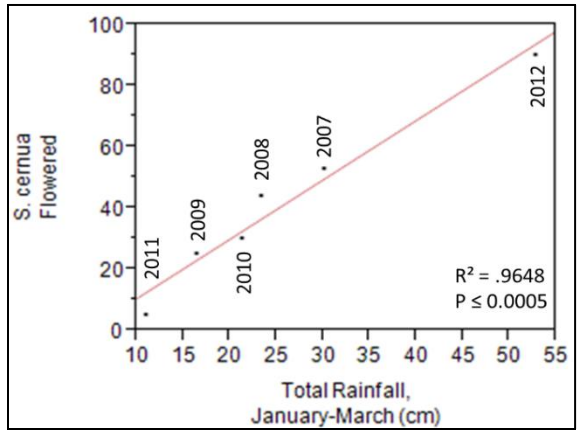


Figure 4. Simple linear regression of number of flowering individuals 2007-2012 and total rainfall for January through March (cm) each year for Easterwood Airport, College Station, TX.

A MANOVA Repeated Measures test was conducted on the ranked yearly flower production (*S. cernua* plus *S. parksii*) values for the period of fall 2007 through fall 2012 (Figure 5). Statistical analysis on individual species was not conducted as *S. parksii* flowering numbers were insufficient. Pre-treatment there was no statistical difference in the number of *Spiranthes* spp. flowers produced by plot treatment (Table 2). The first year post-treatment (fall 2008) and again in the fall of both 2011 and 2012, there was significantly greater mean flower production within the cut versus control plots.

Neither understory canopy manipulation treatment had a statistically significant effect on fall flowering plants ($p = 0.68$) or spring rosette production ($p = 0.13$). Likewise, there were no statistical differences in the number of times previously identified plants remained belowground during either a fall or spring sampling period.

The distribution of both flowering *S. parksii* and *S. cernua* across plots was variable. Three plots produced no flowering individuals and few rosettes and thus were among the lowest orchid producing plots in the study (Figure 6). While two of these plots were undisturbed controls the other was located in a cut-herbicide treatment. Only eight plots contained flowering *S. parksii*, with two of those plots (plots 3 and 12) containing 63% of the total (Table 3). *S. cernua* were more widely distributed, but the majority were contained in only five plots (3 cut stump and 2 control). The production of *S. parksii* and *S. cernua* flowers was similar across treatments with control plots containing 32% and 31%, cut plots containing 50% and 53%, and the cut-herbicide plots containing 18% and 16%, respectively (Table 3). These data suggest a positive cut treatment response as the treatment had larger increases in numbers of flowering *S. parksii* (0 to17) and *S. cernua* (28 to71) as well as in total individuals (65to 253) when compared to the other treatments (Table 3).

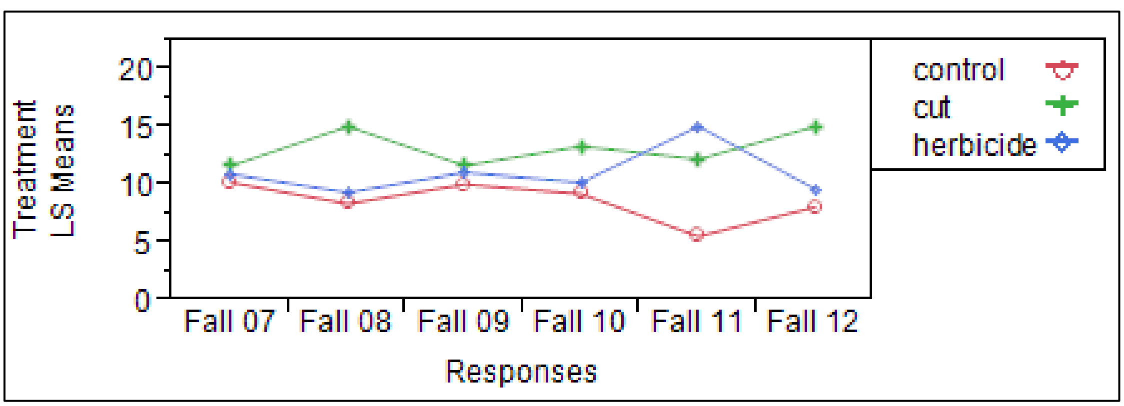


Figure 5. Repeated Measures analysis of flowering Spiranthes spp. by plot treatment, 2007-2012. Sphericity Test value (.2504), Univariate unadjusted Epsilon; within interactions (.0044), time (< .0001), time by treatment (.0044).

Table 2. Treatment comparison of mean flowering *Spiranthes* spp., 2007-2012. Student's t results (mean ± SD) with different uppercase letters representing significant differences between means within a year.

	Fall 07	Fall 08	Fall 09	Fall 10	Fall 11	Fall 12
Control	10.2 ± 8.2 **A**	8.4 ± 7.2 **B**	10.1 ± 7.8 **A**	9.2 ± 8.4 **A**	5.6 ± 5.7 **B**	8.1 ± 6.3 **B**
Cut	11.7 ± 6.8 **A**	15.1 ± 5.4 **A**	11.7 ± 7.6 **A**	13.4 ± 5.5 **A**	12.3 ± 5.8 **A**	15.1 ± 6.3 **A**
Herbicide	11.0 ± 3.7 **A**	9.4 ± 4.0 **AB**	11.1 ± 2.9 **A**	10.3 ± 4.2 **A**	15.1 ± 2.4 **A**	9.7 ± 4.3 **AB**

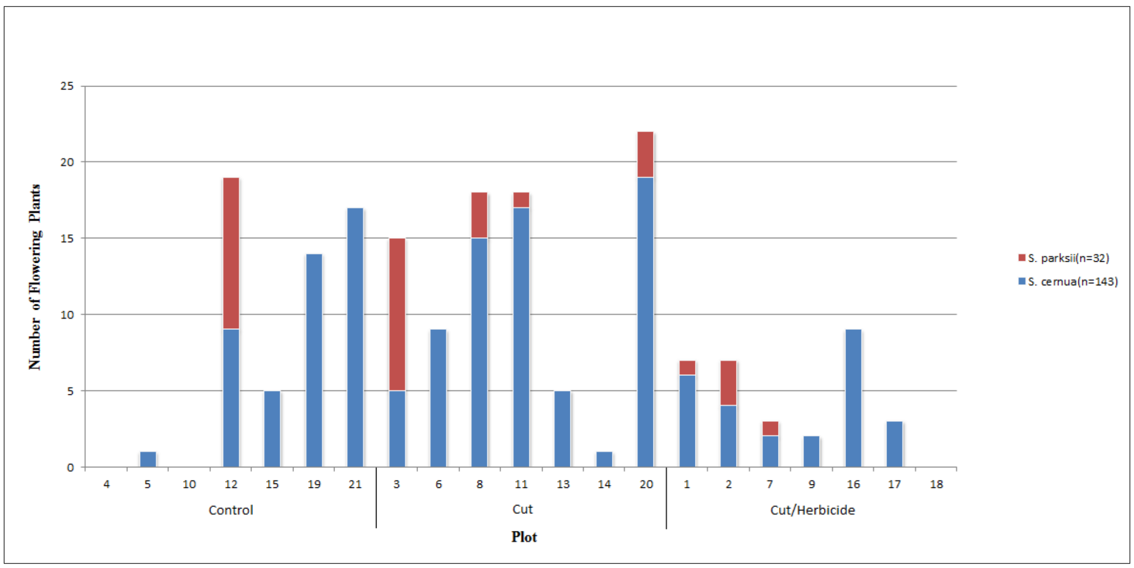


Figure 6. Number of flowering S. parksii and S. cernua by treatment, summed across 2007-2012.

Table 3. Total number pre- (2007) to post-treatment (2012) of flowering *S. parksii*, *S. cernua* and unknown individuals (rosettes and flowers) across plots by treatment. Percent of totals in parenthesis.

	Plot	*S. cernua*		*S. parksii*		Unknown		Total	
		Pre-	Post-	Pre-	Post-	Pre-	Post-	Pre-	Post-
Control	**4**	0	0	0	0	2	5	2	5
Control	**5**	1	1	0	0	0	2	1	3
Control	**10**	0	0	0	0	2	7	2	7
Control	**12**	7	9	6	10	18	59	31	78
Control	**15**	4	5	0	0	2	4	6	9
Control	**19**	7	14	0	0	14	44	21	58
Control	**21**	7	17	0	0	3	27	10	44
Sub-total		26 (38%)	46 (32%)	6 (67%)	10 (31%)	41 (44%)	148 (28%)	73 (42%)	204 (29%)
Cut	**3**	4	5	0	10	5	38	9	53
Cut	**6**	4	9	0	0	0	20	4	29
Cut	**8**	5	15	0	3	19	98	24	116
Cut	**11**	6	17	0	1	7	42	13	60
Cut	**13**	0	5	0	0	0	9	0	14
Cut	**14**	1	1	0	0	3	3	4	4
Cut	**20**	8	19	0	3	3	43	11	65
Sub-total		28 (41%)	71 (50%)	0 (0%)	17 (53%)	37 (39%)	253 (48%)	65 (38%)	341 (49%)
Cut-herbicide	**1**	4	6	1	1	3	34	8	41
Cut-herbicide	**2**	1	4	2	3	2	26	5	33
Cut-herbicide	**7**	2	2	0	1	3	15	5	18
Cut-herbicide	**9**	2	2	0	0	1	1	3	3
Cut-herbicide	**16**	3	9	0	0	2	22	5	31
Cut-herbicide	**17**	3	3	0	0	4	19	7	22
Cut-herbicide	**18**	0	0	0	0	1	7	1	7
Sub-total		15 (22%)	26 (18%)	3 (33%)	5 (16%)	16 (17%)	124 (24%)	34 (20%)	155 (22%)
Total		69	143	9	32	94	525	172	700

Mean flowering plant height (stalk plus inflorescence length) of *S. parksii* was significantly shorter than *S. cernua* in three of the four years assessed; 2007, 2008, and 2012 (Table 4). Two seasons (2009 and 2011) were removed from the analysis due to insufficient numbers of flowers produced to satisfy statistical requirements. *S. parksii* produced a total plant height which was not statistically different regardless of the year it was produced, while *S. cernua* varied in height from year to year (14.2 to 27.7 cm) ($p < 0.01$).

There was a significant difference in mean total rosette leaf area between *S. parksii* and *S. cernua* with *S. cernua* producing a larger leaf during the spring of 2008 ($p = .02$), though no differences occurred in any other season (Table 4). Both *S. parksii* and *S. cernua* produced rosettes which was significantly smaller in the spring of 2012 compared to the other years.

There were no significant differences in the number of flowers produced per inflorescence or in the flower color between the two species. The amount of herbivory recorded was not different for the rosette or flowering individuals of the two species or between treatments. There was no difference in the canopy cover type or the depth of litter in which *S. parksii* and *S. cernua* were found.

Table 4. Mean flowering plant height of *S. parksii* and *S. cernua* and mean spring rosette leaf area by year from 2007 through spring 2013.

Flowering Plant Height (cm)				
	S. parksii[1]	***S. cernua***[2]	**Chi-square/T-ratio#**	**P-value**
2007	15.9 ± 3.3 **a** (6)	23.5 ± 1.3 **b** (38)	-2.11#	0.0407
2008	19.8 ± 1.7 **a** (7)	27.3 ± 0.8 **a** (30)	-0.31#	0.0003
2009	23.5 ± 0.00 (1)	27.7 ± 5.4 (16)	-	*
2010	19.2 ± 13.7 **a** (7)	24.1 ± 5.8 **ab** (23)	3.38	0.0659
2011	-	14.2 ± 7.1 (5)	-	*
2012	13.6 ± 2.2 **a** (19)	19.6 ± 1.2 **c** (59)	0.07#	0.0176
2013	-	-	-	-

Leaf Area (cm^2)				
	S. parksii[2] **(n = 32)**	***S. cernua***[2] **(n = 143)**	**Chi-square**	**P-value**
2007	-	-	-	-
2008	3.1 ± 4.0 **a**	5.8 ± 7.4 **a**	5.50	0.0195
2009	3.5 ± 3.5 **a**	6.7 ± 9.9 **a**	0.10	0.7508
2010	-	-	-	-
2011	-	-	-	-
2012	2.5 ± 6.9 **b**	4.9 ± 13.9 **b**	0.09	0.7588
2013	7.4 ± 8.9 **a**	9.1 ± 12.7 **a**	0.00	0.9623

* The number of individuals (N-value) was too low for statistical analysis. N-values in parenthesis. - No data recorded. [1]ANOVA or [2]Kruskal-Wallis test used for within group comparisons, significant differences ($p < .05$) represented by different lowercase letters. Wilcoxon test (mean ± SD) or #T-test (mean ± SE) used for across group comparisons.

Discussion

The relationship between August rainfall and flower production for *S. parksii* and *S. cernua* found by Hammons (2008) was not seen within this study. However, correlation was found between total rainfall for January through March and the production of both *S. parksii* and *S. cernua* flowers for 2007 through 2012. This suggests that rainfall prior to and during the spring rosette period may be as important, if not more important, than late summer rains for the development of flowering individuals in a given year.

The specifics driving this relationship are unknown, but one could speculate that higher rainfall just prior to and during the spring rosette period allows for the development of larger root stores which subsequently increases flower production. Rainfall has been determined to contribute to orchid flowering patterns in many (Wells et al. 1998; Inghe & Tamm 1988; Light & MacConaill 2006), but not all studies (Falb & Leopold 1993; Coates et al. 2006).Therefore, further study regarding the effects of seasonal and/or annual rainfall fluctuations on *S. parksii* should be conducted, especially when long-term datasets are available.

A positive cut treatment response existed for both *Spiranthes* spp.. This is especially encouraging for the endangered target species, *S. parksii*. A statistical difference existed between the control and treated plots in total *Spiranthes* spp. flower production during several years, with the cut plots producing more flowering orchids in 3 of the 6 yrs. The cut treatment also showed the greatest increase in *Spiranthes* spp. numbers over the duration of the study. Additionally, within the cut treatment both *S.*

parksii and *S. cernua* realized their greatest gains (28 to 71 and 0 to 17 individuals, respectively).

Few studies exist which have tested the effect of woody plant removal on encroached orchids. Brumback et al. (2011) found a positive response by the federally threatened woodland orchid, *Isotria medeoloides,* to the removal of shrubs during a canopy-reduction management study and suggested that canopy thinning may help to promote long-term conservation for the orchid. In another study, the orchid *Orchis mascula,* was found to respond positively to the increased light through the reintroduction of coppicing, with increased flowering rate and fruit set (Jacquemyn et al. 2008). The results of these studies help to strengthen the positive treatment response exhibited by *Spiranthes* spp. and *S. parksii* to the cut treatments.

Comparing the year to year variation in total flower production between the cut and cut-herbicide plots suggests a difference between treatments. Both treatments received similar removal of the shrub layer, however, the cut-herbicide treatment responded similarly to the control in all but one year (2011). Additionally, post-treatment numbers were higher within the cut versus cut-herbicide treatment, with more *S. parksii* (17 vs. 5), *S. cernua* (71 vs. 26) as well as total orchids (341 vs. 155) produced. Though, it should be noted that pre-treatment values were higher within the cut plots.

S. parksii produced a smaller inflorescence than *S. cernua* during every flowering season except in 2010, a difference which is suggested by others (Walters 2007). Interestingly, the data suggests that unlike *S. cernua*, *S. parksii* had a total flowering plant height which was not different regardless of the year it was produced (Table 4). While the exact cause of the lack in variation in flowering plant height across years for *S. parksii* is unknown, it does suggest between species differences and should further be investigated.

The majority of plots and all plot treatments showed increases in orchid numbers over the duration of the study. This was most likely driven by two factors: one, the difficulty in locating these orchids due to their small size and likelihood of being hidden amongst the litter and herbaceous vegetation, which resulted in additional individuals being located across years which were missed during previous sampling periods, especially in rosette form, and two, their natural seasonal variation in population density (e.g., Hammons 2008) a trend expressed by many orchids (Tamm 1972; Hutchings 1987; Gregg 1991; Light & MacConaill 1991; Falb & Leopold 1993; Kindlmann & Balounova 1999; Brzosko 2002).

A few additional factors were present within this study which caused issues with analysis and interpretation of the data. The locations chosen for the plots, unbeknownst at the time, did not uniformly capture the native orchid population. Subsequently, high variability existed pre-treatment in the number of orchids per plot, which created issues during data analysis. *S. parksii* numbers were not sufficient to allow independent analysis of plot treatment response; however, the above assessment of the raw data suggests that a post-treatment effect existed. Additionally, as of fall 2013 visual assessment suggests significant variation exists in the amount of woody cover by plot, both in the understory and overstory. In general, plots which received woody removal are beginning to reestablish, with much of the shrub development exceeding 1 m in height, though the density of reestablishment is highly variable. Again, this variation in regrowth is not consistent across plots, thus the impact of the reestablishing shrub layer is highly variable both within and across treatments and lack of quantitative data for this change in shrub layer cover prevented analysis of this factor.

In summary, rainfall summed January through March was highly correlated with flower production later that fall for both *S. parksii* and *S. cernua*. This result is not surprising as rainfall has been correlated with production in other orchid studies. There was a positive response by *Spiranthes* spp. to the cut treatment, an encouraging sign especially for the endangered orchid, *S. parksii*. Though, issues related to sampling of the orchid population and the characterization of the general vegetation response caused concerns related to data analysis and interpretation.

Woody Patch Analysis

Introduction

Savannas are matrix grasslands that have periodic establishment and mortality of fire tolerant shrubs and trees including oak species (Curtis 1959). Reductions in fire frequency caused by landscape fragmentation, changing land use, and fire suppression have allowed significant structural changes across many North American ecosystems. Multiple studies conducted within North America have suggested woody encroachment within savanna ecosystems can alter herbaceous productivity (Hennessy et al. 1983), biodiversity (Archer 1995), and understory species composition (Anderson et al. 2000; Nielsen et al. 2003). Woody canopy increase is generally agreed to be a major factor in the decrease of many orchid species within savannas, and possibly for *S. parksii* within the Post Oak Savanna Ecoregion of Central Texas (Farrell 1991; Tamm 1972, 1991; Hammons 2008).

During surveys conducted on the BVWSMA site in 2001 by HDR Engineering, Inc., (HDR) populations of *S. parksii* were located in various areas across the property (HDR 2002; Hammons 2008). Subsequently, these areas were the focus of seasonal surveys from 2007 through 2010, at which time no *S .parksii* were observed. Many of these areas have been encroached by woody vegetation since the 2001 HDR surveys. This factor combined with the absence of *S. parksii* created an opportunity to study the effects of woody encroachment on the species.

The study was designed to test the hypothesis that woody plant encroachment and closing of the woody canopy led to decline of the *S. parksii* populations present within locations across the property in 2001. The hypothesis was tested by selecting areas across the site which both contained and were void of *S. parksii* in 2001 and then conducting woody plant removal treatments to randomly assigned plots across these two groups.

The concept of this study was to create varying sized woody patches within areas of dense woodland (~60-100% canopy closure) in order to evaluate their effect on *S. parksii*. There were two questions regarding the patches; one, would the removal of woody vegetation cover increase *S. parksii* numbers and two, would the size and dispersion of patches and the amount of edge created have varying effects on *S. parksii*.

Methods

Study site

The research plots are situated across three areas of the BVSWMA property and are located within, or directly adjacent to, DRA's. The plot locations were selected based on previous verified sightings of *S. parksii* during 2001 surveys conducted by HDR (2001 polygons) (Hammons 2008) (Figure 7). The site is representative of a typical Post Oak Savanna/Woodland with post oak dominating the overstory and yaupon, farkle-berry, and American beauty-berry composing the majority of the midstory. The understory is composed of little bluestem and narrow-leaf wood-oats along with a great diversity of less abundant grasses and forbs.

Vegetation data collection

A 30 x 30 m plot was determined to be of sufficient size for this study, small enough to allow ample replication while representing a landscape scale alteration. The treatment design was created to resemble natural Post Oak Savanna growth patterns while allowing for a measurable and repeatable application. A total of 24 plots were situated on Burlewash soils within three areas across the Twin Oaks landfill (Figure 7). Twelve of the plot locations were selected based on previous known presence of *S. parksii* (2001 polygons) with their subsequent absence since that initial sampling period. The other 12 plots were located in areas that had no recorded *S. parksii* sightings, but were within 20-100 m of known *S. parksii* occurrences and in areas of predicted favorable habitat based on prior site characterizations (Hammons 2008; Ariza 2013).

Each plot was randomly assigned one of four treatments; 5 meter, 10 meter, or 15 meter diameter cleared patches, or control/no treatment (Figure 8). Multiple patch sizes were utilized because of the inherent variation in length of edge created given the same amount of volume of woody plant removal. The 5, 10, and 15 m plots consisted of a total of 36, 9, or 4 identical circles, respectively. Of the total number of identical circles within each plot, a subset were randomly assigned to be receive woody plant treatment creating ratios of treated to untreated circles (28/36, 7/9, or 3/4) within each plot. The various ratios of cleared to uncleared circles were selected to create a uniform treatment of approximately 60% remaining woody cover within each plot. This percentage was chosen in attempt to mimic the patchiness of the Post Oak Savanna as described by Hammons (2008) and other savanna ecosystems (e.g., Dyksterhuis 1957; Ko & Reich 1993; McPherson 1997; Khavhagali & Bond 2008).

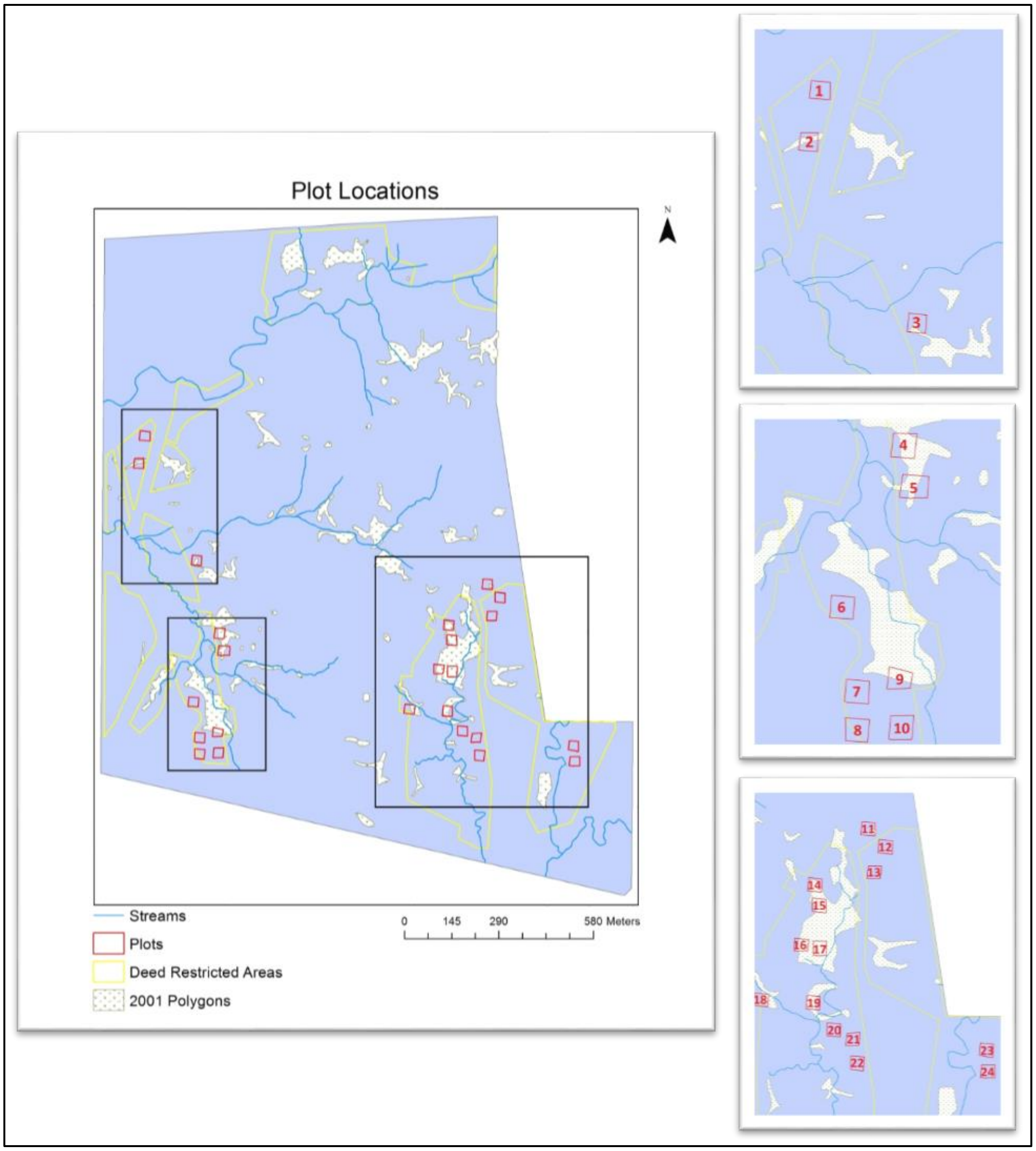


Figure 7. Location of created woody patch plots (1-24) within three areas of the Twin Oaks site.

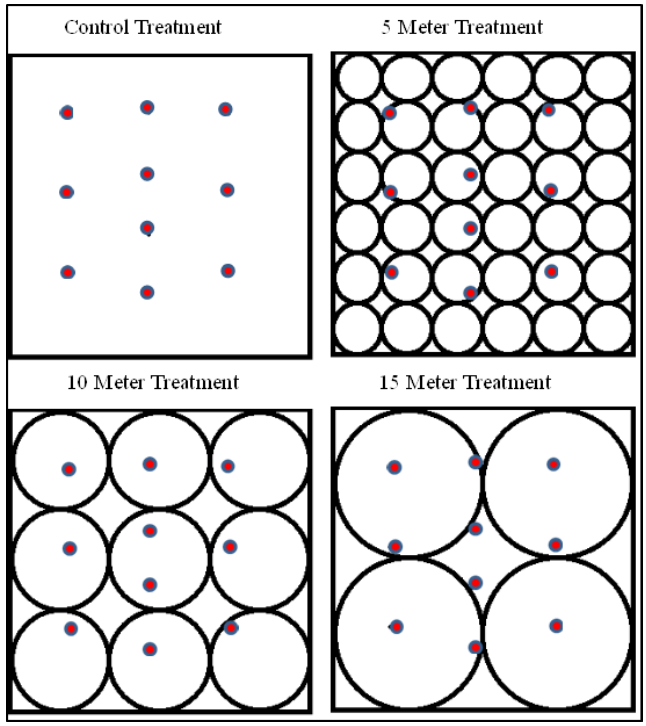


Figure 8. Woody patch treatments to create 5, 10, or 15 meter diameter patches within 30 x 30 m plots. Red dots represent the relationship of the 10 fixed sampling points to the varying sized cleared patches across treatments.

Removal of woody cover was conducted in late July through early August of 2010 by cutting and removing by hand all woody individuals within the cleared circles which had a diameter at breast height (dbh) of less than 13.2 cm. This was done by cutting the stems 7.6 cm from the soil surface and immediately applying a herbicide mixture of 5% Remedy (3,5,6-trichloro-2-pyridinyloxyacetic acid) in 95% diesel to the entire stump. Woody individuals with a (dbh) greater than 13.2cm were deemed too large to remove from the plot and therefore were girdled and treated with the same herbicide mixture.

Ten permanent sampling points were GPS located along three transects within each plot (Figure 9). Woody canopy cover was measured using an upright metered pole situated directly above the sampling point. A woody species was recorded as cover if the pole passed through the circular plane created by its' outer most branches. This interception was evaluated for three different strata; 0-2, 2-4, and > 4 m. A 1 x 1 m quadrat centered north-south on the sampling point was evaluated for total herbaceous cover to the nearest percent. Graminoid species composition was recorded for the single most abundant or two equally abundant individuals to either genus or species. Graminoid species percent cover was not determined. Woody cover was estimated below 1 m to the nearest percent. Litter depth was recorded within the four quadrants of each sampling quadrat and averaged. Sampling was conducted pre-treatment in July 2010 and post-treatment in July 2012.

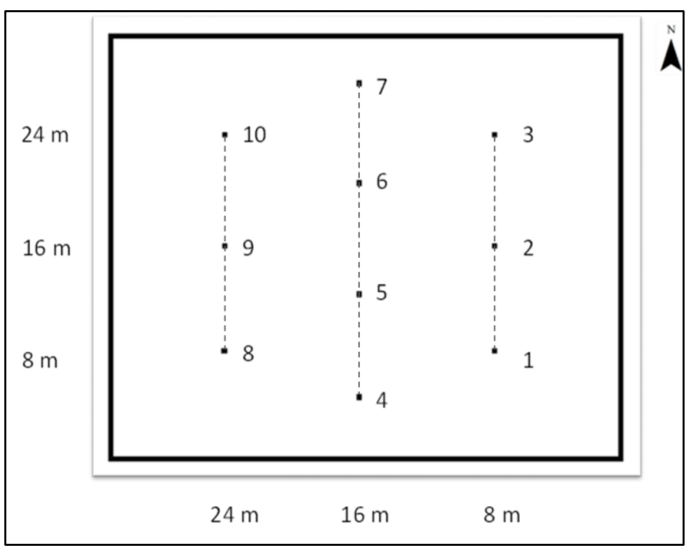


Figure 9. Location of fixed sampling points in each plot along three north-south transects.

Spiranthes data collection

Orchid demographics and habitat data were collected during the fall flowering and spring rosette periods beginning in the fall of 2011 through the spring of 2013. All *Spiranthes* spp. which occurred above ground were GPS located and permanently marked using two metal flags, one placed 15 cm north and the other 15 cm south of the plant location and were accompanied by an identification tag. The number of rosette leaves, leaf length and width, and a visual estimation of the percentage of herbivory damage during periods of peak rosette growth were recorded. Flowering individuals were identified to species, stalk and inflorescence lengths were measured, the numbers of flowers per stalk were counted, flower color was noted (white versus cream) and the percentage of herbivory damage was visually assessed. Habitat data were collected including measurements of litter depth, estimates of canopy cover type (shade; under the canopy of the tree/shrub layer, dripline; between the tree/shrub canopy and the open savanna, or open; in the open savanna), and assessments of whether the orchid was situated along a riparian drainage.

Statistical analysis

A statistical analysis was conducted to assess the change in; woody canopy cover, woody cover (below 1 m), and herbaceous cover between treatments. Test for normality was conducted using a Shapiro-Wilk W test with transformations attempted where data did not meet parametric assumptions. In all instances the data was not normally distributed and in no instances were transformations successful. Test of homogeneity of variances was conducted using four tests (O'Brien, Brown-Forsythe, Levene and Bartlett). All analyses were conducted using nonparametric methods. Across group differences were analyzed using a Kruskal-Wallis test and a Wilcoxon Each Pair test was used to assess differences in the means.

Statistical analyses were conducted using JMP 10 (SAS 2010).

Vegetation analysis

Woody canopy cover change was determined by examining each of the three height ranges stratified by the sampling pole. For each height range where a woody species was recorded, a value of 0.33 (33%) was assigned. No woody hits resulted in 0% woody cover and a hit in all three height ranges resulted in 100% woody cover. This resulted in woody canopy cover categories of 0, 33, 66 or 100%. Categories were created for both the pre- and post-treatment data. This analysis was designed to interpret change in woody canopy cover from a vertically stratified three-layered viewpoint.

Additionally, graminoid response was assessed both pre- and post-treatment. The six most recorded (abundant) species/species groups were used and a seventh category was created, which lumped the other species (Table 5). All six categories were used to assess the variation pre- to post-treatment while only little bluestem and narrow-leaf wood-oats were analyzed for shifts in specific species dominance.

Table 5. List of 6 graminoid categories used to assess the mean pre- to post-treatment variation.

Common Name	Scientific Name	Abbreviation Used
little bluestem*	*Schizachyrium scoparium*	LBS
narrow-leaf wood-oats	*Chasmanthium laxum* var. *sessiliflorum*	NWO
purpletop	*Tridens flavus*	PTP
brown-seed	*Paspalum plicatulum*	BSD
panic grass spp.	*Panicum* spp.	PNG
sedge spp.	*Cyperus/Carex* spp.	SDG
other		

* This category includes low quantities of splitbeard bluestem (*Andropogon ternarius*).

Herbicide efficacy analysis

The herbicide efficacy was assessed by surveying the sampling points in May of 2013 for the presence of resprouting woody vegetation. Every sampling point across the study was visited, regardless of treatment. The sampling points were referenced to their position within either a treated, untreated or control circle and the presence or absence of resprouting woody vegetation was recorded. The presence of woody resprouting vegetation was recorded for instances where green live woody growth was seen protruding directly from a cut-stump within the sampling point quadrat.

Results

Vegetation response

Pre-treatment showed no statistical difference in the mean woody canopy cover across treatments (Table 6). Post-treatment the 5 meter, 10 meter, and 15 meter treatments had significantly less woody canopy cover (37.8% to 42.1%) compared to the control plots (64.1%). However, the relative change in woody canopy cover was not significantly different across treatments (p = 0.08).

The change in mean woody cover (below 1 m) was assessed by treatment (Table 7). No significant differences were found either pre-treatment, post-treatment or when the relative change in cover was assessed.

Table 6. Mean pre- to post-treatment percent woody canopy cover change by plot treatment 2010-2012.

	Plot Treatment					
	Control (n = 28)	**5 Meter (n = 63)**	**10 Meter (n = 64)**	**15 Meter (n = 60)**	**Chi-square**	**P-value**
Pre-treatment	62.9 ± 35.6	56.7 ± 24.9	53.2 ± 24.8	57.4 ± 30.6	2.53	0.4690
Post-treatment	64.1 ± 38.4 A	38.9 ± 34.6 B	37.8 ± 36.9 B	42.1 ± 41.1 B	9.45	0.0239
Change	2.4 ± 26.8	-17.9 ± 37.5	-17.6 ± 40.4	-15.4 ± 49.3	6.85	0.0769

Across group analysis conducted with a Kruskal-Wallis test (mean ± SD). Differences in the means tested with a Wilcoxon Each Pair test with different uppercase letters representing significant differences (p < .05)

Table 7. Mean pre- to post-treatment percent woody cover (below 1 m) change by plot treatment 2010-2012.

	Plot Treatment					
	Control (n = 28)	**5 Meter (n = 63)**	**10 Meter (n = 64)**	**15 Meter (n = 60)**	**Chi-square**	**P-value**
Pre-treatment	19.5 ± 17.0	22.9 ± 26.4	27.0 ± 22.7	27.2 ± 24.5	5.96	0.1136
Post-treatment	26.3 ± 24.5	27.5 ± 23.7	26.5 ± 23.5	26.4 ± 24.2	0.23	0.9727
Change	6.8 ± 29.9	4.5 ± 31.7	-0.6 ± 33.1	-0.8 ± 35.1	3.22	0.3589

Across group analysis conducted with a Kruskal-Wallis test (mean ± SD). Differences in the means tested with a Wilcoxon Each Pair test with different uppercase letters representing significant differences ($p < .05$)

Mean herbaceous cover change was assessed by treatment (Table 8). Pre-treatment there was no significant difference in herbaceous cover. Post-treatment, the control and 15 meter treatments had the lowest amount of herbaceous cover (25.5% and 37.5%, respectively) and were not statically different. The 5 meter treatment had higher cover (45.2%) than the 15 meter treatment, though these two treatments were not statistically different. The 10 meter treatment had the highest amount of herbaceous cover (50.2%) post-treatment, but this amount was not significantly different from that found within the 5 meter treatment. The relative change in herbaceous cover was similar between the control and 15 meter treatments (9.3% and 17.9%, respectively) and between the 5 meter and 10 meter treatments (28.0% and 35.6%, respectively). However, these two groups were statistically different; the 5 meter and 10 meter treatments showed significantly more change in herbaceous cover compared to the control and 15 meter treatments.

Table 8. Mean pre- to post-treatment percent herbaceous cover change by plot treatment 2010-2012.

	Plot Treatment					
	Control (n = 28)	**5 Meter (n = 63)**	**10 Meter (n = 64)**	**15 Meter (n = 60)**	**Chi-square**	**P-value**
Pre-treatment	16.3 ± 13.5	17.2 ± 13.3	14.7 ± 13.2	19.6 ± 18.9	2.73	0.4348
Post-treatment	25.5 ± 19.8 C	45.2 ± 24.7 AB	50.2 ± 22.8 A	37.5 ± 27.5 BC	22.09	<.0001
Change	9.3 ± 23.8 B	28.0 ± 28.4 A	35.6 ± 23.0 A	17.9 ± 26.7 B	24.83	<.0001

Across group analysis conducted with a Kruskal-Wallis test (mean ± SD). Differences in the means tested with a Wilcoxon Each Pair test with different uppercase letters representing significant differences (p < .05)

Only little bluestem and narrow-leaf wood-oats had sufficient sampling values to allow for across treatment comparisons. The data showed the two species responded similarly across treatments, decreasing abundance to other species over 65% of the time (Table 9). For example, within the pre-treatment sampling points located in the control treatment, little bluestem was most abundant at 7 of the 28 sampling points, while post-treatment it remained most abundant within only 1 of the 28 sampling points. Many of the sampling points which had either little bluestem or narrow-leaf wood-oats as their most abundant species pre-treatment, shifted towards increased abundance by species within the "other" category. This category is comprised of such species as; beaked panicum (*Panicum anceps*), splitbeard bluestem, bahia grass, sand lovegrass (*Eragrostis trichodes*), and various rosette grasses (*Dichanthelium* spp.) and forbs such as; dog fennel (*Eupatorium capillifolium*) and woolly croton (*Croton capitatus*). Post-treatment the species which played the largest role were; various rosette grasses, sand lovegrass, beaked panicum and woolly croton.

Herbicide influence

Using the definition of resprouting woody vegetation described previously, the only sampling points which contained resprouting woody vegetation were those located within treated circles. The percentage of those treated circles which contained resprouting woody vegetation was determined and the data are used as a proxy for herbicide efficacy. Of the 104 total sampling points located within cleared circles across the study, 49% had resprouting woody vegetation (Table 10). When these sampling points are assessed by treatment, the values ranged from 48% to 51%.

Table 9. Sampling points pre-treatment where *Schizachyrium scoparium* or *Chasmanthium laxum* var. *sessiliflorum* was the most abundant by plot treatment and those sampling points post-treatment variation 2010-2012. (See Table 4 for list of abbreviations)

Plot Treatment		Most Abundant Species Post-treatment						
		LBS	**NWO**	**SDG**	**PTP**	**BSD**	**PNG**	**other**
Control (n = 28)	***Schizachyrium scoparium* (n = 7)**	1*	2	1	2	0	0	1
	***Chasmanthium laxum* var. *sessiliflorum* (n = 14)**	0	4	7	1	0	0	2
5 Meter (n = 63)	***Schizachyrium scoparium* (n = 14)**	0	6	1	0	1	0	6
	***Chasmanthium laxum* var. *sessiliflorum* (n = 32)**	8	11	2	0	0	1	10
10 Meter (n = 64)	***Schizachyrium scoparium* (n = 8)**	1	0	1	1	0	0	5
	***Chasmanthium laxum* var. *sessiliflorum* (n = 36)**	7	6	1	1	2	0	19
15 Meter (n = 60)	***Schizachyrium scoparium* (n = 6)**	1	2	1	0	2	0	0
	***Chasmanthium laxum* var. *sessiliflorum* (N = 25)**	4	2	5	0	2	0	12

*Underlined numbers represent the number of sampling points with no shift in species abundance.

Table 10. Percentage of sampling points located within treated circles which contained resprouting vegetation in May of 2013.

Plot Treatment	Resprouting Woody Vegetation
Control (n = 0)	0%
5 Meter (n = 37)	51%
10 Meter (n = 40)	50%
15 Meter (n = 27)	48%
Total (n = 104)	49%

Spiranthes response

Table 11 is a summary of the counts of flowering individuals found across the experiment as of the 2012 flowering season. The 2001 column is to designate whether the plot location encompassed *S. parksii* historically. The data shows that all *S. parksii* located across the experiment were found in plots associated with the 2001 polygons. Plots located on 2001 polygons contained 38 of the 50 orchids found experiment wide, with nearly half of the orchids found in non-2001 plots being *S. lacera*. Additionally, *S. parksii* were found within treated plots only. The ratio of *S. parksii* to *S. cernua* was roughly 1 to 5.

Table 12 represents the distribution of orchid species across the experiment by habitat. It suggests that *S. parksii* had no affinity to shaded habitat, even when located on a drainage. Otherwise *S. parksii* tended to be evenly distributed across the other habitats. *S. cernua* preferred a dripline or dripline along a drainage, but like *S. parksii* few were found in shaded habitats.

Data were collected on multiple variables, but due to the low number of *Spiranthes* spp. throughout the study, the effect of these variables was not statistically assessed. These variables included; the number of flowers produced per inflorescence, the difference in flower color between the two species, the difference in rosette leaf length and width, the percentage of herbivory sustained, the type of canopy cover, and the depth of litter in which they were found.

Table 11. Distribution of flowering orchid species across treatment plots based on presence or absence of *S. parksii* 2001 polygons in 2012.

2001 Polygon	Plot	Treatment	*S. parksii*	*S. cernua*	*S. lacera*	Total
Absent	**21**	**Control**	-	-	1	1
	22	**5 Meter**	-	-	1	1
	24	**10 Meter**	-	3	-	3
	8	**15 Meter**	-	3	-	3
	20	**15 Meter**	-	-	3	3
	23	**15 Meter**	-	1	-	1
Sub-total			0	7	5	12
Present	**16**	**Control**	-	6	-	6
	3	**5 Meter**	2	-	-	2
	19	**5 Meter**	3	6	-	9
	2	**10 Meter**	-	1	-	1
	17	**10 Meter**	2	13	-	15
	5	**15 Meter**	1	4	-	5
Sub-total			8	30	0	38
Total			8	37	5	50

Table 12. Distribution of flowering orchid species across habitat categories within treatment plots in 2012.

Habitat	*S. parksii*	*S. cernua*	*S. lacera*
Open (n = 11)	2	6	3
Open-drainage (n = 7)	2	5	0
Dripline (n = 20)	3	15	2
Dripline-drainage (n = 8)	1	7	0
Shade (n = 3)	0	3	0
Shade-drainage (n = 1)	0	1	0

Discussion

Vegetation response

Pre-treatment there was no statistical difference in mean woody canopy cover across treatments. Post-treatment there was no difference between the 5 meter, 10, meter and 15 meter treatments, though this group did have significantly less mean woody canopy cover compared to the controls. Despite this, the relative change in woody canopy cover across treatments was not significantly different. While not statistically different, Table 6 shows the relative decrease in mean woody canopy cover was greater within the 5 meter, 10 meter and 15 meter treatments (-15.4% to -17.9%) compared to the controls (2.4%). The high standard deviations reported may help explain the lack of a statistical difference.

The amount of mean woody cover (below 1 m) prior to woody removal was similar across all treatments. Following treatment this trend continued with no statistical differences found between treatments in either mean total cover or relative change in cover. The lack of reduction in woody cover (below 1 m) within the control treatment was expected, but was not expected within the other treatments as all woody vegetation within treated circles received an application of herbicide. Poor herbicide efficacy might be an explanation of this response (Scifres 1982; Stritzke 1991).

Pre-treatment there was no difference in the amount of mean herbaceous cover across treatments. Post-treatment statistically significant differences existed in the total amount of mean herbaceous cover between treatments with all showing increases including the unmanipulated control. These post-treatment increases resulted in values of relative change in cover which divided the treatments into two different groups. Group one consisted of the control and 15 meter treatments which showed less of an increase (9.3% and 17.9%, respectively) then group two which consisted of the 5 meter and 10 meter treatments (28.0% and 35.6%, respectively).

The increased mean herbaceous cover seen within the control treatment was unexpected. This increase might be explained by woody/herbaceous interactions which resulted from the severe 2011 drought (Table 1). It is possible that stress incurred by the woody vegetation created an environment which temporarily favored the herbaceous layer through mechanisms such as; increased light and water availability caused by a reduction in woody leaf cover and/or an increase in nutrient and water availability made available by a reduction in demand from the stressed woody vegetation. Anderson et al. (1969) suggested that even in situations where the forest canopy is dense, light is not often the limiting factor and in situations of canopy opening development, increases in throughfall, not light, are often the determinant factor in the subsequent increased herbaceous response. Regardless, there is generally and often a strong, negative correlation between herbaceous and woody cover (Scholes & Archer 1997), therefore, the increased herbaceous cover found might relate to the drought stress incurred by the woody vegetation.

The lack of statistical difference between the control and 15 meter treatments in both post-treatment total mean herbaceous cover and the relative change in mean herbaceous cover is an unexpected result and does not agree with visual plot assessments which suggested the 15 meter response was on par with those found within the 5 meter and 10 meter treatments. Low sample sizes or the fixed nature of the sampling points within each plot may account for this discrepancy.

Vegetation sampling issues

The high standard deviation values found within the woody canopy cover results and the lack of statistical difference in both the post-treatment total mean herbaceous cover and the relative change in mean herbaceous cover between the control and 15 meter treatments might be explained by issues inherent in the general vegetation sampling methodology.

During post-treatment data analysis it became apparent that the 5 meter, 10 meter and 15 meter plots received two different treatments, with the control plots representing a third treatment. The 5 meter, 10 meter, and 15 meter treated plots received two treatments; random portions (~40%) received woody removal within treated circles while the remaining portions (~60%) received varying amounts of indirect woody removal, mainly through the girdling of large overstory trees within adjacent treated circles of the same plot. This indirect effect was not considered prior to the pre-treatment vegetation sampling and therefore was not designed into the sampling scheme. Adding to the complexity, the location of the 10 sampling points were fixed which resulted in their varied placement relative to the center of the patch circles, with this variation based on the size, location, and treatment applied (Figure 8). Therefore, the sampling point could theoretically fall anywhere within a given circle and represent significantly different treatment effects.

The pre-treatment use of fixed sampling points to characterize the vegetation at the plot level yielded accurate data. However, once the treatments were applied this sampling scheme was no longer appropriate. Given the pre-treatment information is valid, resampling of the study plots using an appropriate sampling methodology would allow an accurate assessment of vegetation response to be conducted. The use of a random sampling design should help to resolve the issues with high variability experienced through the fixed sampling point approach (Heady et al. 1959).

Graminoid diversity

Woody vegetation removal increased graminoid diversity, a response that is well documented within savannas (e.g., Brudvig 2010). This increase came at the expense of narrow-leaf wood-oats and sedge spp. which had reduced abundance within the 5 meter, 10 meter, and 15 meter treatments. This should be expected, as the two genera are generally shade tolerant (Scifres 1982). However, the lack of response of little bluestem especially within the treatments which received woody vegetation removal was not expected and suggests the drought of 2011 may have had a negative impact. Recent site visits (summer 2013) further support this, as little bluestem has substantially increased in abundance since 2012.

Unexpectedly, narrow-leaf wood-oats a shade tolerant and often prolific understory species, showed a large reduction in abundance in the control plots. It was replaced mainly by sedge spp. with which it often coexists, suggesting narrow-leaf-wood-oats may be less drought tolerant. Its' reduced abundance along with the reduction in little bluestem at least within the control plots, may perhaps again be a result of the 2011drought.

Herbicide influence

Though herbicide efficacy was not a focus of this study, crude post-treatment visual estimation suggested it was roughly 50% effective with 48% to 51% of the sampling points located within the treatments' treated circles containing resprouting woody vegetation. This is most likely an underestimation as only vegetation growing directly from a cut stump was counted as resprouting. An occasional poor response of woody vegetation to herbicide treatment is well documented within the literature, with multiple factors presumed to play a role (e.g., Morton 1966; Davis et al. 1968). As alluded to previously, this presumed lack of herbicide efficacy within this study is reiterated by the results of the woody cover (below 1 m) analysis where no significant differences were found pre- to post-treatment. Thus, the herbicide efficacy is most likely the cause of the lack of reduction in woody cover (below 1 m). Regardless, the woody cover (below 1 m) data along with field observations suggests substantial woody regrowth has occurred since treatment.

Spiranthes response

The effect of the various sized patches and their total edge created could not be compared to the orchid response due to the low number of individuals, however, preliminary findings suggest a treatment effect. Plots located on 2001 polygons contained the majority of orchids (76%) post-treatment and were the only to contain *S. parksii*. Furthermore, all *S. parksii* occurred within plots which received woody plant removal, which suggests a positive treatment response. Exactly why *S. parksii* were only found within plots situated on previous 2001 polygons is unknown. While this suggests the potential influence of varying environmental characteristics, it is most likely an artifact of the length of time (yrs.) since the onset of encroachment combined with a seed dispersal effect which promotes establishment within close proximity to the parent plant (Chung et al. 2004). Additionally, data collected on orchid distribution by habitat suggests that not only *S. parksii,* but *S. cernua* and *S. lacera* prefer areas which provide a higher degree of canopy openness, further supporting the potential benefit of woody plant management for *S. parksii*.

Despite these favorable observations which suggest a treatment effect, the results of this study should be considered preliminary; first, due to the inherent seasonal variability seen in many terrestrial orchids (Tamm 1972; Hutchings 1987; Gregg 1991; Light & MacConnaill 1991; Falb & Leopold 1993; Kindlmann & Balounova 1999; Brzosko 2002) and in *S. parksii* (Hammons 2008); second, due to the limited two year duration of this experiment and; third, because a significant portion of the orchids recorded (15/50) including two *S. parksii* occurred in a portion of 1 plot (plot 17) which received negligible amounts of actual woody plant removal and therefore differed little pre- to post-treatment. Additionally, no orchids were recorded during the 2011 flowering or 2012 spring rosette seasons, which paralleled the 2011 drought, a period when orchid populations responded poorly across the entire BVSWMA site.

CHAPTER V

TRANSPLANTATION

Transplantation Analysis

Introduction

The development of a successful transplantation methodology for use in transplanting extant *S. parksii* individuals from threatened into protected environments could prove useful in enhancing the conservation of the species. However, little work has been done to assess the potential feasibility and success of transplanting *S. parksii* (Parker 2001; Hammons 2008, Hammons et al. 2010), *Spiranthes* spp. (Pileri 1998), or terrestrial orchids (Brumback & Fyler 1996; Ferry 2008; Steinauer 2008).

The opportunity to study transplantation of *S. parksii* was created through the mitigation requirements set forth by the United States Fish and Wildlife Service (USFWS) on the Brazos Valley Solid Waste Management Agency (BVSWMA) through their Twin Oaks property (USFWS 2006). This property, which is located on potential habitat of *S. parksii*, was purchased by BVSWMA for the construction of a landfill. The property was subsequently surveyed by HDR Engineering, Inc. (HDR) in 2001 at which time they located several populations of *S. parksii* (HDR 2002; Hammons 2008). As part of the mitigation process, it was deemed necessary to transplant *S. parksii* from threatened areas within the landfill footprint into protected deed-restricted areas (DRA's) (USFWS 2006).

This study was designed to follow previous work in which *S. parksii* were successfully transplanted using a soil intact method which utilizes the excavation of a 4712.4 cm^3 soil core directly centered on an individual plant (Parker, 2001; Hammons 2008, 2010). The transplant methodology was replicated, while an assessment of the effects of transplanting into areas which received prior woody plant removal was added. Transplantation of *S. parksii* into the DRA 2 evergreen shrub and sapling removal plots (Figure 3) was conducted in the spring of 2010 to assess the effects of transplantation and plot treatments on their survivability.

Methods

In the spring of 2010, 83 known *S. parksii,* which were originally located between 2007 and 2009, were relocated from the landfill footprint into the DRA 2 research plots using the soil-intact method. A 20 cm diameter PVC pipe was cut into 15 cm lengths and was used during excavation of individuals to maintain intact soil around root tubers. The PVC pipe was beveled at the bottom so it could be hammered into the soil around a rosette. Once the PVC was driven to a 15 cm depth, a shovel was inserted below the PVC pipe and the core was removed. Less than 2 hours elapsed between excavation and transplantation.

A minimum of 3 excavated plants were transplanted into each of the evergreen shrub and sapling removal plots. They were positioned on a line which bisected the 20 x 20 m plots from north to south; one transplant was placed randomly at one of three (5, 10, or 15 m) locations along line (Figure 10). A hole was carefully dug into the soil to fit the diameter and depth of the transplant inside the PVC pipe. After placing the transplant and PVC pipe in the pre-dug hole, the PVC pipe was removed and soil was fed into the cracks around the transplant to fill any large air spaces. It should be noted that in several occasions more than one *S. parksii* was captured in one PVC sleeve resulting in multiple individuals in some of the transplant locations, in which case the individuals were followed separately. These additionally captured *S. parksii* explain why the total number of transplants was 83 rather than 63 (21 plots by 3 transplants per plot).

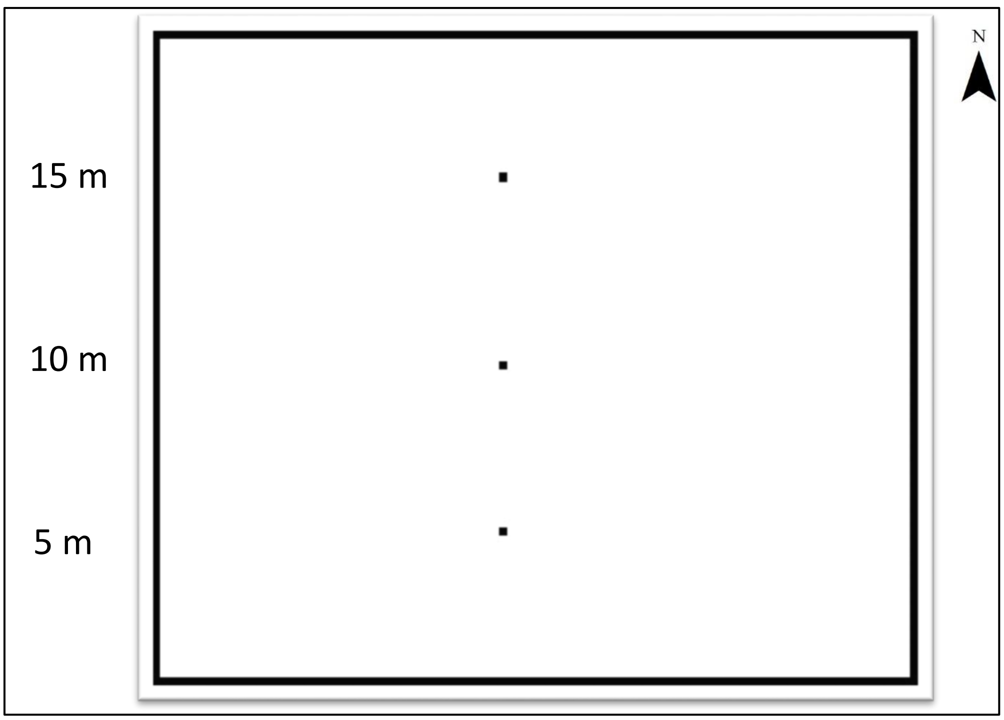


Figure 10. Transplant locations within evergreen shrub and sapling removal plots.

Data collected on transplanted *S. parksii* was the same as that collected in conjunction with other orchids monitored in the evergreen shrub and sapling removal study (see methods page 17). Post-transplant measurements included *S. parksii* rosette and flowering data collected from fall 2010 to the spring of 2013. The number of rosette leaves, leaf length and width, and a visual estimation of the percentage of herbivory damage during periods of peak rosette growth were recorded. Flowering individuals were recorded, stalk and inflorescence lengths were measured, the numbers of flowers per stalk were counted, flower color was noted (white versus cream) and the percentage of herbivory damage was visually assessed. Habitat data were collected including measurements of litter depth, estimates of canopy cover type (shade; under the canopy of the tree/shrub layer, dripline; between the tree/shrub canopy and the open savanna, or open; in the open savanna), and assessments of whether the orchid was situated along a riparian drainage.

Rainfall data was obtained for Easterwood Airport, College Station, TX which is located approximately 13 mi west of the Twin Oaks site.

Statistical analysis

A statistical analysis was conducted to test the effect of the evergreen shrub and sapling removal plot treatments upon the flowering versus rosette producing transplants and to assess differences by year in which the transplant was originally located to the post-transplant production of rosettes, flowers, or the times absent. Test for normality was conducted using a Shapiro-Wilk W test with transformations attempted where data did not meet parametric assumptions. In all instances the data was not normally distributed and in no instances were transformations successful. Test of homogeneity of variances was conducted using four tests (O'Brien, Brown-Forsythe, Levene and

Bartlett). All analyses were conducted using nonparametric methods. Across group differences were analyzed using a Kruskal-Wallis test.

The difference in the number of times individuals (flower versus rosette only producing) were present was statistically analyzed. Test for normality was conducted using a Shapiro-Wilk W test with transformations attempted where data did not meet parametric assumptions. In all instances the data was not normally distributed and in no instances were transformations successful. Test of homogeneity of variances was conducted using four tests (O'Brien, Brown-Forsythe, Levene and Bartlett). All analyses were conducted using nonparametric methods. Across group differences were analyzed using a Wilcoxon test.

The relationship between several summed rainfall totals and orchid production was assessed using a simple linear regression. Assessments of; total annual rainfall, rainfall during the month of August, and the separate assessments of the combined total rainfall during the months of July and August and January through March were all conducted.

Statistical analyses were conducted using JMP 10 (SAS 2010).

Results

The behavior of 83 transplanted *S. parksii* over five seasons is shown in Figure 11. Seasonally, the number of individuals which did not produce above ground growth ranged from 31 (37%) to 70 (84%) from fall 2010 through spring 2013. Rosette production during the flowering seasons varied from 0 to 46 individuals and during the two spring rosette seasons, production varied from 21 to 29 individuals. Flowers were produced by 6 (7%), 1 (< 1%), and 13 (16%) individuals in 2010, 2011, and 2012, respectively.

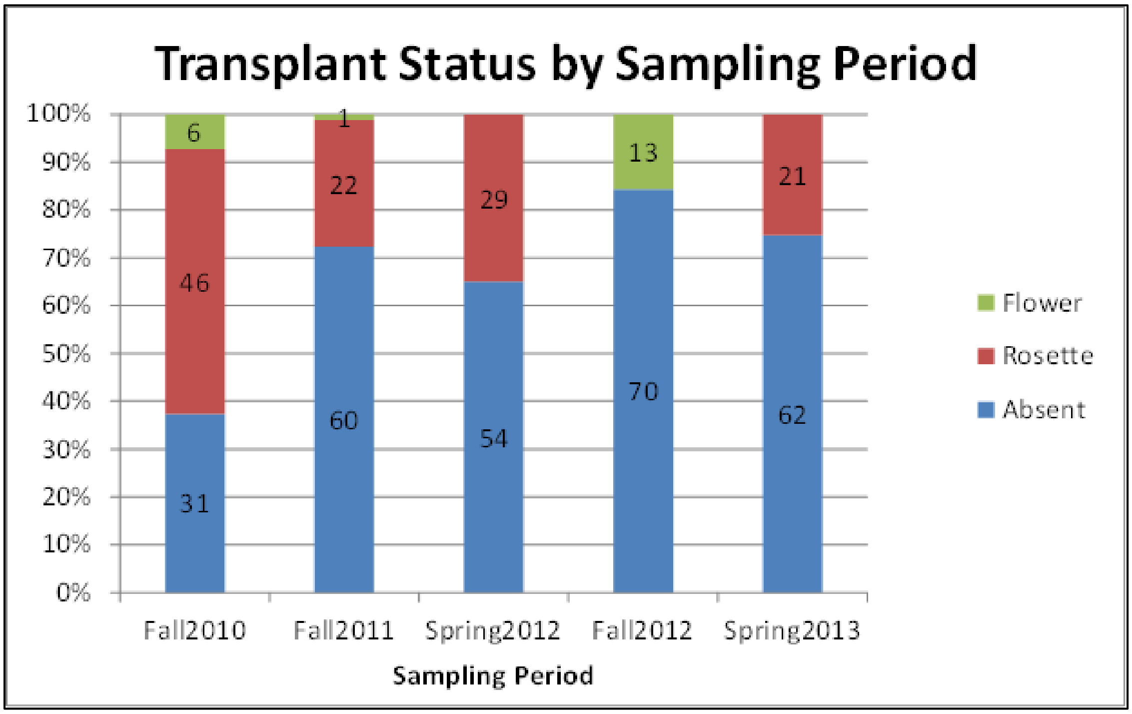


Figure 11. Status of spring 2010 transplants by sampling period as a percentage of total (n=83) for each sampling period.

Of the original 83 *S. parksii* transplanted in the spring of 2010, 27% have not been observed above ground since transplantation and are presumed dead. Of the remaining transplants, 19% have flowered at least one time and the remaining 54% have appeared only as a rosette during the fall 2010 to spring 2013 sampling period (Table 13). The majority of the rosette-only producing individuals (26/24) were above ground only one season with 81% (21/26) of those seen in the fall of 2010. The others produced a rosette during 2, 3, or 4 of the seasons; none of the rosette-only individuals were present aboveground during all 5 seasons. In contrast, 50% of the flowering individuals produced a flower or rosette during all five seasons.

To assess the difference in the number of times individuals (flower versus rosette only producing) were present across the five sampling periods a Wilcoxon test was used. There was a sharp contrast in the seasonal presence of flower producing individuals as compared to rosette-only producing individuals (Table 14). The times observed above ground during the study duration were significantly higher among flower producing individuals as compared with individuals that produced rosettes only with means of 76% and 34%, respectively.

Table 13. Number of times transplants were present or absent as flowers or rosettes from 2010 through spring 2013 with percent of totals in parenthesis.

	Times Observed					
	0	**1**	**2**	**3**	**4**	**5**
Absent* (n = 22)	22	-	-	-	-	-
Flowered (n = 16)	-	2	2	1	3	8
Rosette (n = 45)	-	26	7	11	1	-
Total (n = 83)	22 (27%)	28 (34%)	9 (11%)	12 (14%)	4 (5%)	8 (10%)

*Not present during any sampling visit post-transplant.

Table 14. Number of times individuals (flower vs. rosette producing) were present from 2010 through spring 2013.

	Percent Of Times Present
Flower (n = 16)	76.0 ± 30.0
Rosette (n = 45)	34.0 ± 18.0

Analysis conducted with a Wilcoxon test (mean ± SD).
*Chi-square = 19.95, P-value = < 0.0001

Seasonal fluctuations in plant response were found within the 16 transplants which produced a flower post-transplant (Table 15). Only 3 individuals flowered more than one time with only 1 individual flowering all three flowering periods. Three of the 6 individuals which flowered in 2010, did not produce a flower after that period, and all but one of those were absent thereafter. The 2012 flowering season was more than twice as productive as 2010 or 2011, with 13 flowers produced. Interestingly, all but 2 or 85% of the individuals that produced a flower in 2012 produced a rosette the next spring.

Table 15. Status of the 16 flowering S. parksii after four years (R = rosette, F = flower, A = absent).

Plant	Plot	Treatment	Fall 2010	Fall 2011	Spring 2012	Fall 2012	Spring 2013
1	4	Control	R	A	R	F	R
2	10	Control	R	R	R	F	R
3	15	Control	F	A	A	A	A
4	19	Control	R	R	R	F	R
5	19	Control	F	A	R	A	A
6	19	Control	F	A	A	A	A
7	21	Control	R	A	A	F	A
8	8	Cut	F	R	R	F	R
9	8	Cut	R	A	R	F	R
10	8	Cut	R	R	R	F	R
11	20	Cut	R	R	R	F	R
12	1	Herbicide	R	A	A	F	R
13	2	Herbicide	R	R	R	F	A
14	7	Herbicide	R	R	R	F	R
15	9	Herbicide	F	F	R	F	R
16	18	Herbicide	F	R	R	F	R

The individuals transplanted were originally located between 2007 through fall 2009 (Table 16). The relevance of the year the plant was originally located was compared to the production of rosettes, flowers, or the times absent with no significant differences found, however, low sample sizes may be affecting this outcome.

Table 16. The year the individuals transplanted were originally located 2007 through fall 2009.

Year Located	Number Transplanted (n = 83)
2007	13
2008	56
2009	14

The number of flowering individuals (16) was not sufficient to allow between year statistical comparisons of mean stalk length, inflorescence length, or number of flowers per inflorescence, therefore, the raw data was assessed. Mean stalk length across the three flowering periods ranged from 10.8 to 15.5 cm and mean inflorescence length ranged from 2.2 to 4.9 cm (Table 17). The mean number of flowers per inflorescence ranged from 10 to 15 between the three flowering periods. On average, the inflorescences in 2012 were shorter in length (2.2 cm), but they produced a larger number of flowers per inflorescence (15).

Table 17. Mean stalk length, inflorescence length, total flower length and number of flowers per inflorescence by year 2010-2012.

Year	Mean Stalk Length (cm)	Mean Inflorescence Length (cm)	Total Flower Length (cm)	Mean Number of Flowers
2010 (n = 6)	15.5	4.9	20.4	14.7
2011 (n = 1)	10.8	2.3	13.1	10.0
2012 (n = 13)	13.1	2.2	15.3	15.0

There was a significant relationship between total yearly rainfall and total flowers produced (Figure 12). When total rainfall summed from January through March was compared to total flower production, the relationship was not significant (p = 0.12).

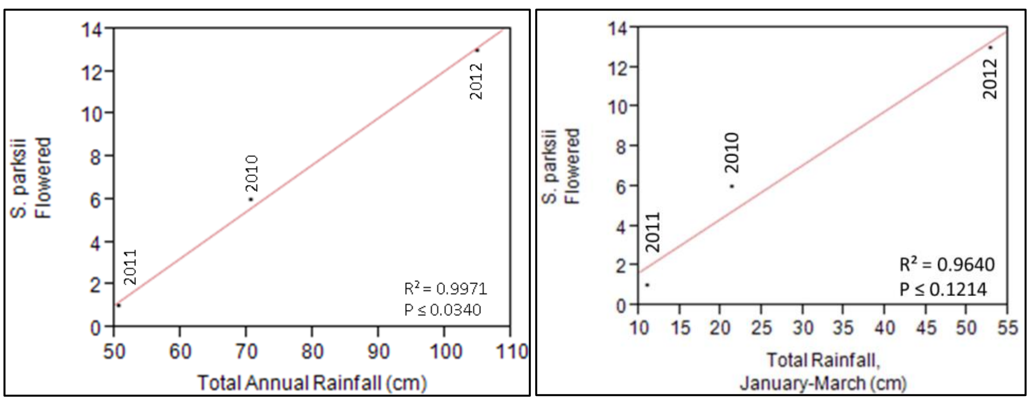


Figure 12. Simple linear regression of number of transplanted S. parksii that flowered each year 2010-2012 with total annual rainfall (cm) and total combined rainfall January-March (cm) each year for Easterwood Airport, College Station, TX.

Additional analyses were conducted which resulted in no significant findings. The evergreen shrub and sapling removal treatments had no statistically significant effect on the number of flowers or rosettes produced by the transplants. The post-transplant habitat (dripline versus shade) under which the transplants were placed was assessed with again no differences found for either flower or rosette production.

Discussion

The data suggests at least 50% of the transplants are still surviving after 3 years. Post-transplant, 27% of the original 83 transplants never appeared aboveground and may be presumed dead for a first year survival rate of 73%. If individuals which produced a rosette during the fall of 2010 (first sampling period) with their subsequent absence are presumed dead, the survival rate would decrease to 48% of the original transplants. This percentage agrees with the findings of Hammons (2008) where roughly 60% of the *Spiranthes* spp. produced a spring rosette (appeared aboveground) one year after transplant. However, orchids are known to remain dormant belowground for multiple seasons (Wells 1967; Tamm 1972) and specifically for periods up to 5 years in *S. parksii* (Parker 2001; Hammons 2008; Ariza 2013). Therefore, the state of the absent individuals can only be speculated without re-excavation or additional monitoring.

Many factors could affect survivability of a transplanted orchid. The age of each transplant was unknown thus many individuals could have potentially been near the end of their life cycle, a period which has been estimated to be approximately 6 years (Ariza 2013). Stress from transplantation combined with environmental factors such as rainfall (2011 drought) and temperature (Wells et al. 1998; Inghe & Tamm 1988; Light & MacConaill 1991) or the suitability of the transplant location (Hammons 2008; Hammons et al. 2010) may have caused their premature demise. Soil loss has been observed in several instances with depressions forming in transplant locations.

Therefore, minimizing the disturbance created at the new location by monitoring soil stability and making proper additions in the weeks following transplantation may help to increase survivability. Additionally, disturbance at the new location produced by transplantation, combined with the use of marking flags and the lingering human scent may have attracted predators, such as feral hogs (Burke 2005). Field observations of rooting nearby and directly at transplant locations has been documented, which further suggests disturbance may have affected survivability.

The higher frequency of appearance of flower producing individuals as compared to those which only produced rosettes suggests they had increased vigor, a trend which has been found in other orchids (Hutchings 1987; Shefferson 2003). If an individual produced a flower at least one time post-transplant, it was recorded above ground on average 76% of the time as compared to 34% for rosette only producers. Though these flower producing individuals may have had an advantage in habitat placement (Hammons 2010) or may simply have been younger. Unfortunately, a detailed pre-transplant history is unknown for the transplanted individuals and therefore could not be used to better understand their post-treatment response.

Measurements of various floral structural characteristics were collected. The 2012 season had on average a smaller flower, but with more flowers per inflorescence than the other seasons. This may be explained by the presence of a few individual flowers in 2012 which had above average inflorescence lengths and number of flowers per inflorescence. An above average production of flowers per inflorescence may reference the presence of favorable environmental conditions, considering this year (2012) produced the majority of the flowers (13/20).

Correlation was found between total annual rainfall and the production of flowering transplants. No correlation was not found for total rainfall January through March ($p = 0.12$) as was found within the evergreen shrub and sapling removal study (Figure 4). However, there were only three flowering periods assessed and sample numbers were low. The specifics driving this relationship are unknown, but rainfall has been determined to contribute to orchid flowering patterns in many (Wells et al. 1998; Inghe & Tamm 1988; Light & MacConaill 1991), but not all studies (Falb & Leopold 1993; Coates et al. 2006).Therefore, further study regarding the effects of seasonal and/or annual rainfall fluctuations on transplanted *S. parksii* should be conducted.

The results of this study further support the use of the soil-intact transplant method, described most recently by Hammons et al. (2010), for conservation of *S. parksii*. The data suggests a survival rate of at least 50% after 3 years, an approximation which needs to be verified through re-excavation of the individuals with long periods of dormancy or continued monitoring of all transplanted individuals. Based on the data, selection of individuals for transplantation should consider their recent flowering history as they may be more vigorous, with recently flowering individuals taking priority. The ability of this transplant method to establish a stable population is unknown, but this study further demonstrates the methods promise.

CHAPTER VI

SUMMARY AND CONCLUSIONS

Seasonal variability in orchid numbers was common across all studies, not only for *S. parksii*, but for all *Spiranthes* spp. encountered. Seasonal variability occurs in many orchids (Tamm 1972; Hutchings 1987; Gregg 1991; Light & MacConaill 1991; Falb & Leopold 1993; Kindlmann & Balounova 1999; Brzosko 2002) which makes it difficult to discern population numbers and temporal variability.

Seasonal rainfall patterns have been correlated to variability in other orchid populations (Wells et al. 1998; Inghe & Tamm 1988; Light & MacConaill 1991) and might explain some of the variability seen across these studies. A graph depicting the relationship between total rainfall from January through March of a given year to the number of flowers subsequently produced across the three studies conducted in this investigation (evergreen shrub and sapling removal, woody patch analysis, and transplantation studies) is insightful (Figure 13). While not a statistical comparison, it does strengthen the argument that orchids across the three studies followed a similar flowering pattern. Furthermore, the graph suggests rainfall as a major driver in this pattern and thus should be studied in more detail.

Another interesting relationship suggested in the graph (Figure 13) is the response of the woody patch analysis plots compared to the two other studies. In fall 2013, orchid flowering numbers increased from the previous year within the woody patch analysis study, while the orchid populations within the other studies followed the rainfall totals January through March and decreased. The results of the woody patch analysis study suggested that clearing of woody vegetation and altering of patch size, pattern and/or edge effects may have increased orchid production. Therefore, the varying response of *Spiranthes* spp. to the rainfall patterns further suggests a treatment effect existed.

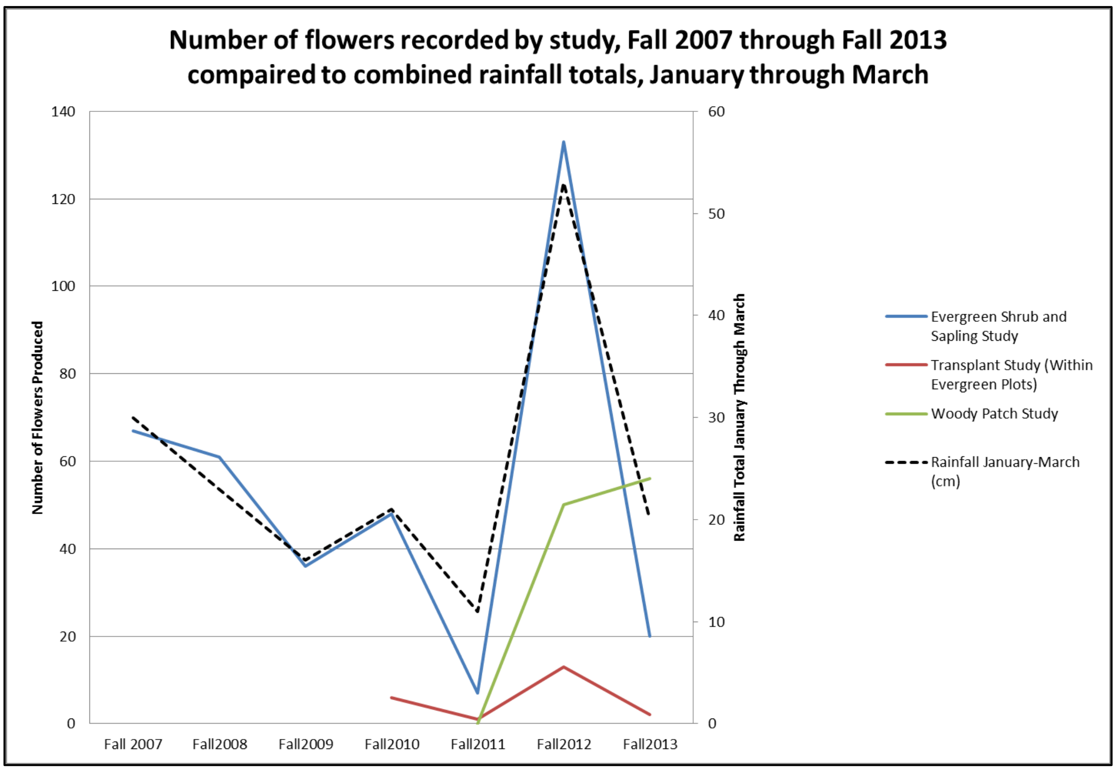


Figure 13. Number of flowers produced per year by study related to total rainfall (January-March), fall 2007 through fall 2013.

The woody patch analysis study makes an argument for the need to manage woody vegetation for *S. parksii* preservation. However, it raises the question of habitat suitability versus seed dispersal limitations. Plot surveys determined the majority of all orchids (76%) and all *S. parksii* within the study were located within plots with previously recorded orchids (2001 polygons). The need to determine if any major environmental differences exist between the two plot types (polygons/no polygons) is significant as this might further define the suitable habitat for *S. parksii* as well as describe differences between *S. parksii* and *S. cernua*.

The woody patch analysis study utilized herbicide as much of the woody vegetation is capable of resprouting and therefore top removal alone would be ineffective. The data suggests poor herbicide efficacy which demonstrates the need to retreat the resprouting vegetation. If left unmanaged, the woody vegetation will dominate within a few seasons which neutralizes treatment objectives.

Drought conditions in 2011 may have positively influenced the orchid population within the three studies. Orchid flower production was substantially higher in 2012 across all studies which also paralleled the herbaceous cover increases found within the woody patch analysis study. Whether the same mechanisms should be credited is unknown, but it could be hypothesized both benefited through increased light and or rain throughfall or increased nutrient and soil moisture availability induced through stress incurred by the woody vegetation.

Similar to the woody patch study, the evergreen shrub and sapling study suggested the removal of woody vegetation may have positively influenced orchid production. However, due to the substantial increases in orchid numbers seen across treatments, combined with the uneven distribution of those orchids across plots, this result is less conclusive.

Regardless of the effectiveness of management inputs such as woody plant control, urban expansion and subsequent *S. parksii* habitat loss is inevitable and therefore transplantation methods should be perfected. Similar to the findings of Hammons et al. (2010), transplantation of *S. parksii* via the soil-intact method was relatively successful and resulted in at least 50% survival after 3 years. Survival rates potentially would improve by selecting individuals for transplant that have produced a flower, as these individuals seem to have increased vigor. Additionally, minimizing the disturbance created at the new location by monitoring soil stability and making proper soil additions in the weeks following transplantation may help to increase survivability. The ability of transplanted individuals to establish a stable population is unknown and was not the focus of the study; however this should be the long term goal.

Due to the seasonal and annual variability inherent in *S. parksii* numbers there is a strong need to maintain these studies if long-term population stability is to be understood. Additionally, to determine if rainfall or other cyclical climatic patterns are driving *S. parksii* population dynamics, studies will be required which span multiple weather oscillations.

REFERENCES

Abrams, M. D. 1986. Historical development of gallery forests in northeast Kansas. Vegetation **65**:29-37.

Agee, J. K. 1996. Fire ecology of Pacific Northwest forests. Island Press, Washington, D.C.

Anderson, L. J., M. S. Brumbaugh, and R. B. Jackson. 2001. Water and tree-understory interactions: a natural experiment in a savanna with oak wilt. Ecology **82**:33-49.

Anderson, R. C., O. L. Loucks, and A. M. Swain. 1969. Herbaceous response to canopy cover, light intensity, and throughfall precipitation in coniferous forests. Ecology **50**:255-263.

Anderson, R. C., J. E. Schwegman, and M. R. Anderson. 2000. Micro-scale restoration: a 25-year history of a southern Illinois barrens. Restoration Ecology **8**:296-306.

Archer, S. 1995. Tree-grass dynamics in a Prosopis-thornscrub savanna parkland: reconstructing the past and predicting the future. Ecoscience **2**:83-99.

Arditti, J., and A. K. A. Ghani. 2000. Numerical and physical properties of orchid seeds and their biological implications. New Phytologist **145**:367-421.

Ariza, M. C. 2013. Mycorrhizal associations of the endangered terrestrial orchid *Spiranthes parksii* Correll and sympatric congener *Spiranthes cernua*: implications for conservation. Ph.D. Dissertation. Texas A&M University, College Station, TX.

BEG (Bureau of Economic Geology). 2007. 15-Minute Digital GAT QUAD-SE. Jackson School of Geosciences, University of Texas at Austin, Austin, TX.

Belsky, A. J., R. G. Amundson, J. M. Duxbury, S. J. Riha, A. R. Ali, and S. M. Mwonga. 1989. The effects of trees on their physical, chemical and biological environments in a semi-arid savanna in Kenya. Journal of Applied Ecology **26**:1005-1024.

Belsky, A. J. 1990. Tree/grass ratios in East African savannas: a comparison of existing models. Journal of Biogeography **17**:483-489.

Belsky, A. J., S. M. Mwonga, R. G. Amundson, J. M. Duxbury, and A. R. Ali. 1993. Comparative effects of isolated trees on their undercanopy environments in high-and low-rainfall savannas. Journal of Applied Ecology **30**:143-155.

Bridges, E. L., and S. Orzell. 1989. Additions and noteworthy vascular plant collections from Texas and Louisiana, with historical, ecological and geographical notes. Phytologia **66**:12-69.

Brudvig, L. A. 2010. Woody encroachment removal from Midwestern oak savannas alters understory diversity across space and time. Restoration Ecology **18**:17-84.

Brumback, W. E., and C. W. Fyler. 1996. Small Whorled Pogonia (*Isotria medeoloides*) transplant project. Case Study 7. Pages 445-451 in Falk, D. A., C. I. Millar, and M. Olwell, Editors. Restoring diversity: strategies for reintroduction of endangered plants. Island Press, Washington, D.C.

Brumback, W. E., S. Cairns, M. B. Sperduto, and C. W. Fyler. 2011. Response of an *Isotria medeoloides* population to canopy thinning. Northeastern Naturalist **18**:185-196.

Brzosko, E. 2002. Dynamics of island populations of *Cypripedium calceolus* in the Biebrza river valley (north-east Poland). Botanical Journal of the Linnean Society **139**:67-77.

Burke, R. L., C. M. Schneider, and M. T. Dolinger. 2005. Cues used by raccoons to find turtle nests: effects of flags, human scent, and diamond-backed terrapin sign. Journal of Herpetology **39**:312-315.

Catling, P. M., and K. L. McIntosh. 1979. Rediscovery of *Spiranthes parksii* Correll. Swedish International Development Cooperation **8**:188-193.

Chung, M. Y., J. D. Nason, and M. G. Chung. 2004. Spatial genetic structure in populations of the terrestrial orchid *Cephalanthera longibracteata* (Orchidaceae). American Journal of Botany **91**:52-57.

Coates, F., I. D. Lunt, and R. L. Tremblay. 2006. Effects of disturbance on population dynamics of the threatened orchid *Prasophyllum correctum* D. L. Jones and implications for grassland management in south-eastern Australia. Biological Conservation **129**:59-69.

Cooper, C. F. 1960. Changes in vegetation, structure, and growth of southwestern pine forests since white settlement. Ecological Monographs **30**:129-164.

Correll, D. S. 1947. A new *Spiranthes* from Texas. American Orchid Society, Bull. **16**:400.

Correll, D. S. 1978. Native orchids of North America north of Mexico. Stanford University Press. Stanford, CA.

Correll, D. S., and M. C. Johnston. 1970. Manual of the vascular plants of Texas. University of Texas at Dallas, Richardson, TX.

Curtis, J. T. 1959. The vegetation of Wisconsin: an ordination of plant communities. University of Wisconsin Press, Madison, WI.

Davis F.S., M. G. Merkle, and R. W. Bovey. 1968. Effect of moisture stress on the absorption and transport of herbicides in woody plants. Botanical Gazette **129**:183-189.

Davis, M., and D. Faber-Langendoen. 1995. Fire frequency and its effect on woody plant cover at Allison Savanna, East-Central Minnesota. Natural Areas Journal **15**:319-328.

Dickman, A. 1978. Reduced fire frequency changes species composition of a ponderosa pine stand. Journal of Forestry **76**:24-25.

Diggs, G. M. Jr., B. L. Lipscomb, and R. J. O'Kennon. 1999. Shinners' & Mahler's illustrated flora of North Central Texas. Botanical Research Institute of Texas and Austin College, Fort Worth, TX.

Dueck, L. A., and K. M. Cameron. 2008. Molecular evidence on the species status and phylogenetic relationships of *Spiranthes parksii*, an endangered orchid from Texas. Conservation Genetics **9**:1617-1631.

Dyksterhuis, E. J. 1957. The savannah concept and its use. Ecology **38**:435-442.

Falb, D. L., and D. J. Leopold. 1993. Population dynamics of *Cypripedium candidum Muhl*. ex Willd., small white ladyslipper, in a western New York fen. Natural Areas Journal **13**:76-86.

Farrell, L. 1991. Population changes and management of *Orchis militaris* at two sites in England. Pages 63-68 in Wells, T. C. E., and J. H. Willems, editors. Population ecology of terrestrial orchids. SPB Academic Publishing. The Hague, NL.

Ferry, R. L. 2008. Relocating terrestrial orchid plants. North American Native Orchid Journal **14**:179-82.

Garren, K. H. 1943. Effects of fire on vegetation of the southeastern United States. The Botanical Review **9**:617-654.

Gould, F. W. 1969. Texas plants: a check list and ecological summary. Texas Agriculture Experiment Station, College Station, TX.

Gregg, K. B. 1991. Reproductive strategy of *Cleistes divaricata* (Orchidaceae). American Journal of Botany **78**:350-360.

Grundel, R., N. B. Pavlovic, and C. L. Sulzman. 1998. Habitat use by the endangered Karner blue butterfly in oak woodlands: the influence of canopy cover. Biological Conservation **85**:47-53.

Hammons, J. R. 2008. Demographics, life cycle, habitat characterization and transplant methods for the endangered orchid, *Spiranthes parksii* Correll. MSc Thesis. Texas A&M University, College Station, TX.

Hammons J. R., F. E. Smeins, and W. E. Rogers. 2010. Transplant methods for the endangered orchid *Spiranthes parksii* Correll. North American Native Orchid Journal **16**:38-46.

HDR Inc. 2002. Section 401/404 joint application for proposed solid waste landfill, SH 30 site, Grimes County, TX. HDR. Dallas, TX.

Heady, H. F., R. P. Gibbens, and R. W. Powell. 1959. A comparison of the charting, line intercept, and line point methods of sampling shrub types of vegetation. Journal of Range Management **12**:180-188.

Hennessy, J. T., R. P. Gibbens, J. M. Tromble, and M. Cardenas. 1983. Vegetation changes from 1935 to 1980 in mesquite dunelands and former grasslands of southern New Mexico. Journal of Range Management **36**:370-374.

Heyward, F. 1939. The relation of fire to stand composition of longleaf pine forests. Ecology **20**:287-304.

Hutchings, M. J. 1987. The population biology of the early spider orchid, *Ophrys sphegodes.* Mill II. temporal patterns in behavior. Journal of Ecology **75**:729-742.

Inghe, O., and C. O. Tamm. 1988. Survival and flowering of perennial herbs. V Patterns of flowering. Oikos **51**:203-219.

Jacquemyn, H., R. Brys, O. Honnay, and M. Hermy. 2008. Effects of coppicing on demographic structure, fruit and seed set in *Orchis mascula*. Basic Applied Ecology **9**:392-400.

Kindlmann, P., and Z. Balounová. 1999. Flowering regimes of terrestrial orchids: unpredictability or regularity? Journal of Vegetation Science **10**:269-273.

Khavhagali, V. P., and W. J. Bond. 2008. Increase of woody plants in savannah ecosystems. Grassroots: newsletter of the Grassland Society of Southern Africa **8**:21-24.

Ko, L. J., and P. B. Reich. 1993. Oak tree effects on soil and herbaceous vegetation in savannas and pastures in Wisconsin. American Midland Naturalist **130**:31-42.

Light, M. H., and M. MacConaill. 2006. Appearance and disappearance of a weedy orchid, *Epipactis helleborine*. Folia Geobotanica **41**:77-93.

Ludwig, F., H. de Kroon, F. Berendse, and H. H. Prins. 2004. The influence of savanna trees on nutrient, water and light availability and the understory vegetation. Plant Ecology **170**:93-105.

Luer, C. A. 1975. The native orchids of the United States and Canada excluding Florida. W.S. Cowell Ltd Butter Market. Ipswich, UK.

MacRoberts, M. H., and B. R. MacRoberts. 1997. Notes on *Spiranthes parksii* Correll (Orchidaceae) in deep east Texas. Phytologia **83**:133-137.

McPherson, G. R. 1997. Ecology and management of North American savannas. University of Arizona Press. Tucson, AZ.

Mordelet, P., and J. C. Menaut. 1995. Influence of trees on above-ground production dynamics of grasses in a humid savanna. Journal of Vegetation Science **6**:223-228.

Morton, H. L. 1966. Influence of temperature and humidity on foliar absorption, translocation, and metabolism of 2,4 5-T by mesquite seedlings. Weeds **14**:136-141.

NCSS (National Cooperative Soil Survey). Digital Soil Survey for Grimes County. Available from Texas Natural Resources Information System (TNRIS) at http://www.tnrid.state.tx.us/ (accessed February 2013).

Nielsen, S., C. Kirschbaum, and A. Haney. 2003. Restoration of midwest oak barrens: structural manipulation or process-only. Conservation Ecology **7**:10.

Parker, K. M. 2001. A compendium on *Spiranthes parksii*, Correll (Navasota Ladies'-Tresses). Tejas Ecological Services, College Station. Unpublished report prepared for Texas Municipal Power Agency, Bryan, TX.

Pileri, V. S. 1998. Root morphology, distribution of mycorrhizae, and nutrient status of the terrestrial orchid *Spiranthes cernua*. MSc Thesis. Department of Biology. University of Nebraska at Omaha, Omaha, NE.

Rasmussen, H. N. 1995. Terrestrial orchids: from seed to mycotrophic plant. Cambridge University Press. Cambridge, UK.

SAS Institute Inc. 2010. Base SAS® 9.3 Procedures Guide. SAS Institute Inc. Cary, NC.

Scholes, R., and S. Archer. 1997. Tree-grass interactions in savannas. Annual Reviews in Ecology and Systematics **28**:517-544.

Scifres, C. J. 1982. Woody plant control in the Post Oak Savannah of Texas with hexazinone. Journal of Range Management **35**:401-404.

Shefferson, R. P., J. Proper, S. R. Beissinger, and E. L. Simms. 2003. Life history trade-offs in a rare orchid: the costs of flowering, dormancy, and sprouting. Ecology **84**:1199-1206.

Smeins, F. E., and D. D. Diamond. 1986. Grasslands and savannahs of East Central Texas: ecology, preservation status and management problems. Pages 381-394 in Kulhavy, D. L., and R. N. Conner, editors. Wilderness and natural areas of the eastern United States: a management challenge. School of Forestry, Stephen F. Austin State University, Nacogdoches, TX.

Steinauer, G. 2008. Transplanting a rare orchid. Nebraska Game and Parks Commission Annual Report of the Wildlife Conservation Fund. Lincoln, NE.

Stout, A. B. 1944. The bur oak openings in southern Wisconsin. Transactions of the Wisconsin Academy of Sciences Arts and Letters **36**:141-161.

Stritzke, J. F., D. M. Engle, and F. T. McCollum. 1991. Vegetation management in the Cross Timbers: response of woody species to herbicides and burning. Weed Technology **5**:400-405.

Taft, J. B. 1997. Savanna and open-woodland communities. Pages 24-54 in Schwartz, M., editor. Conservation in highly fragmented landscapes. Chapman and Hall. New York, NY.

Tamm, C. O. 1972. Survival and flowering of some perennial herbs. II. The behavior of some orchids on permanent plots. Oikos **23**:23-28.

Tamm, C. O. 1991. Behavior of some orchid populations in a changing environment. Observations on permanent plots, 1943–1990. Pages 1-13 in Wells, T. C. E., and J. H. Willems, editors. Population ecology of terrestrial orchids. SPB Academic Publishing. The Hague, NL.

USDA (United States Department of Agriculture). 1996. Soil Survey of Grimes County, Texas. Natural Resources Conservation Services. Texas Agriculture Experiment Station, College Station, TX.

USFWS (United States Fish and Wildlife Department). 1980. Endangered and threatened wildlife and plants; proposal to determine *Spiranthes parksii* (Navasota Ladies'-Tresses) to be an endangered species. Federal Register. 41326-41328. Dep. Interior.

USFWS (United States Fish and Wildlife Department) 1982. Endangered and threatened wildlife and plants; determination of *Spiranthes parksii* (Navasota Ladies'-Tresses) to be an endangered species. Federal Register. 19539-19542. Dep. Interior.

USFWS (United States Fish and Wildlife Department). 1984. Navasota Ladies'-Tresses recovery plan. United States Fish and Wildlife Service, Albuquerque, NM.

USFWS (United States Fish and Wildlife Department). 1993. Navasota Ladies'-Tresses (*Spiranthes parksii*) recovery plan. United States Fish and Wildlife Service, Albuquerque, NM.

USFWS (United States Fish and Wildlife Department). 2006. Final biological opinion for the proposed first phase of construction (Cells 1, 2A, 2B, and 3A) of the Brazos Valley Solid Waste Management Agency (BVSWMA) landfill in Grimes County. United States Fish and Wildlife Service, Austin, TX.

USFWS (United States Fish and Wildlife Department). 2009. Navasota Ladies'-Tresses (*Spiranthes parksii*) 5-year review: summary and evaluation. Unpublished report. United States Fish and Wildlife Service Southwest Region, Albuquerque, NM.

Wells, T. C. E. 1967. Changes in a population of *Spiranthes spiralis* (L.) Chevall. at Knocking Hoe National Nature Reserve, Bedfordshire, 1962-65. Journal of Ecology **55**:83-99.

Wells, T. C. E. 1981. Population ecology of terrestrial orchids. Pages 281-295 in Synge, H., editor. The biological aspects of rare plant conservation. John Wiley and Sons Ltd. New York, NY.

Wells, T. C. E., P. Rothery, R. Cox, and S. Bamford.1998. Flowering dynamics of *Orchis morio* L. and *Herminium monorchis* (L.) R. Br. at two sites in eastern England. Botanical Journal of the Linnean Society **126**:39-48.

Walters, C. 2007. Genetic relationships among *Spiranthes parksii* and congeneric species. Ph.D. Dissertation. Texas A&M University, College Station, TX.

Weltzin, J. F., and M. B. Coughenour. 1990. Savanna tree influence on understory vegetation and soil nutrients in northwestern Kenya. Journal of Vegetation Science **1**:325-334.

Wilson, H. 2006. *Spiranthes parksii* - endangered orchid of the Texas Post Oak Savannah. Available from http://www.csdl.tamu.edu/FLORA/hdwsp/sp_pro.htm (accessed February 2013).

Wonkka, C. L., W. E. Rogers, and D. Twidwell. 2009. The effects of fire and herbivory on an endangered endemic orchid, Navasota Ladies'-Tresses (*Spiranthes parksii*). The 94th ESA Annual Meeting 26-44.

Wonkka, C. L. 2010. Large herbivore impacts on demographic characteristics and population dynamics of an endangered orchid (*Spiranthes parksii* Correll). MSc Thesis. Texas A&M University, College Station, TX.

Wonkka, C. L., W. E. Rogers, F. E. Smeins, J. R. Hammons, M. C. Ariza, and S. J. Haller. 2012. Biology, ecology, and conservation of Navasota Ladies'-Tresses (*Spiranthes parksii* Correll) an endangered terrestrial orchid of Texas. Native Plants Journal **13**:236-243.

Wright, H. A., and A. W. Bailey. 1982. Fire ecology: United States and southern Canada. John Wiley and Sons Ltd. New York, NY.