GROWTH, DEVELOPMENT, AND VERTEBRATE AND INVERTEBRATE HERBIVORY OF THE FEDERALLY ENDANGERED *SPIRANTHES PARKSII* CORRELL AND SYMPATRIC CONGENER *SPIRANTHES CERNUA*

A Thesis

by

DESERI DAWN NALLY

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

MASTER OF SCIENCE

Chair of Committee,	Fred E. Smeins
Committee Members,	William E. Rogers
	Micky D. Eubanks
Head of Department,	Kathleen Kavanagh

December 2016

Major Subject: Ecosystem Science and Management

ABSTRACT

Spiranthes parksii Correll, a terrestrial orchid protected under the Endangered Species Act, and its congener *Spiranthes cernua* (L.) Rich, were studied in the Post Oak Savanna Ecoregion of Central Texas in 2014 and 2015. The species are sympatric and each produces a single inflorescence in the fall with emergence of a basal rosette during flower senescence or early spring. Objectives of this study were to 1) assess variation in annual and seasonal growth 2) determine the impact of vertebrate and invertebrate herbivores on the rosette and flower phases, and 3) identify invertebrate herbivores that utilize *S. parksii* and *S. cernua*. To assess variation in annual growth patterns between years, an analysis of precipitation, demographic (presence or absence), and growth data (leaf area and inflorescence height) was performed. From 2014 to 2015, there was a reduction in precipitation, plants present, plant height, and the number of flowering plants that survived to seed production. To determine the difference between vertebrate and invertebrate herbivores, a 2 x 3 factorial experiment was conducted. Plants were randomly assigned to one of five treatments: Control (accessible to vertebrates and invertebrates), insecticide with no cage (Vertebrate Only), cage with no insecticide (Invertebrate Only), caged with insecticide (Cage+Insecticide; no vertebrate or invertebrate), and cage with mesh cover and no insecticide (Mesh; access by only small invertebrates). During the flower season, herbivory was visually estimated for plant stalk and inflorescence by 5 percent increments. For rosettes, herbivory was visually estimated for each leaf in 5 percent increments and averaged over the whole rosette. During the first flowering season, vertebrates consumed more reproductive tissue (46%)

than invertebrates (3%), while in the second season, there was no significant difference between the two at 19% and 2%, respectively. There was no significant difference in percent herbivory of rosettes by vertebrates or invertebrates at 9% and 14% in 2014 and 16% and 11% in 2015. Invertebrates that were observed consuming *Spiranthes sp.* inflorescences and rosettes were armyworms (Order Lepidoptera: Family Noctuidae), grasshoppers (Family Acrididae), and an unidentified member of the Actiinae subfamily. This experiment confirms that vertebrates have a direct effect on *Spiranthes sp*. fitness through removal of reproductive tissue and an indirect impact by consuming rosettes. In addition, it documents that invertebrate herbivores can have a similar effect on inflorescence and rosettes. This knowledge can be important in understanding the influence of plant-herbivore interactions on conservation and management plans for *S. parksii*.

ACKNOWLEDGEMENTS

I would like to thank my committee chair, Dr. Fred E. Smeins, and my committee members, Dr. William E. Rogers and Dr. Micky D. Eubanks, for their guidance and patience throughout the course of this research.

Thank you to my friend and mentor Richard Bruton for all his time and words of encouragement, and to Tyler Adams for his friendship.

To my friends Glenda Bone, Brent Harris, and Kathy Sunvision who assisted me in my educational adventures before Texas A&M, and my boyfriend Albert Tijerina for his love and support.

Finally, thank you to my father and mother, Jack and Shelia Nally, for their support, encouragement, and unconditional love. I can never express how fortunate I am to have such wonderful parents.

CONTRIBUTORS AND FUNDING SOURCES

Contributors

This work was supervised by a thesis committee consisting of advisor Professor Fred E. Smeins of the Ecosystem Science and Management Department and Professors William E. Rogers of the Ecosystem Science and Management Department and Micky D. Eubanks of the Entomology Department.

All work for the thesis was completed by the student, under the advisement of Professor Fred E. Smeins of the Ecosystem Science and Management Department and Professors William E. Rogers of the Ecosystem Science and Management Department and Micky D. Eubanks of the Entomology Department.

Funding Sources

Graduate study was supported by a grant from Brazos Valley Solid Waste Management Agency, Inc., Texas Department of Transportation, and the Lady Bird Johnson Wildflower Center Endangered Species Grant Program.

TABLE OF CONTENTS

Page

Page

LIST OF FIGURES

Page

LIST OF TABLES

Page

CHAPTER I

INTRODUCTION AND OBJECTIVE

Spiranthes parksii Correll is a federal and state listed endangered terrestrial orchid endemic to 13 counties in east central Texas. Of these 13 counties, the largest documented colonies are located in Grimes and Brazos counties with the majority of *S. parksii* found within a specific habitat of the Post Oak Savanna ecoregion (Wonkka et al. 2012). In the last century anthropogenic activities such as agriculture, urbanization, timber harvest, crude oil and natural gas extraction, and lignite mining have increased in Grimes and Brazos counties (Jackson 2010) which has caused fragmentation and reduced habitat for *S. parksii* populations (USFWS 2009; Wonkka 2010).

In 2007 the Brazos Valley Solid Waste Management Agency (BVSWMA) began construction of the Twin Oaks waste management facility in Grimes County. Environmental surveys conducted to meet federal regulatory requirements in 2000 and 2001 by HDR, Inc. located 750 *S. parksii* on the Twin Oaks property (Hammons 2008). For mitigation purposes, BVSWMA created 13 deed-restricted areas to protect known populations of *S. parksii* from being damaged during development of the landfill and supplied funds for research on *S. parksii* and its congeners. This research contributed to knowledge of *S. parksii* demographics, life history, mycorrhizal associations, habitat, and associated plant community dynamics (Hammons 2008, Wonkka et al. 2012; Ariza 2013). Additional work focused on the improvement of *S. parksii* survivability through habitat manipulations and transplantation of individuals into protected conservation sites

(Hammons et al. 2010; Bruton 2014). Despite a decade of research at Twin Oaks, knowledge gaps still exist. One is the lack of empirical data to describe the role of insect herbivore on *S. parksii* survivability, fitness, and life history.

During transplanting research conducted by Hammons (2008) and large herbivore experiments by Wonkka (2010) insects were noted utilizing *S. parksii*, but the extent of their impact remained unknown. Insect herbivores can have considerable influence on individual plant fitness and plant community dynamics (Crawley 1998; Huntly1991; Delaney & Macedo 2001; Kessler & Baldwin 2002). Louda (1994) and Kindlmann & Balounová (2001) suggest that relationships between herbivores (vertebrate or invertebrate) and plants are complex with each species responding differently to tissue removal. Thus, the impact of insect herbivores on each species of interest should be investigated to create effective conservation plans.

The objective of this research was to determine if invertebrate herbivores utilize *S. parksii* and differentiate the impacts between vertebrate and invertebrate herbivory on the survivability of the species. Information gained will provide insight into the life history of *S. parksii* and assist in determining if herbivore management should be incorporated into conservation efforts.

CHAPTER II

LITERATURE REVIEW

The influence of herbivores on plants is as complex and diverse as the species involved in the process. Herbivory can have a negative or positive impact on plant survival (Batzli & Pitelka 1970; McNaughton 1984; Prins & Nell 1990; Huntly 1991; Rosenthal & Kotanen 1994). For example, Gomez & Zamora (2000) found that *Lolium multiflorum* seed production is greatly reduced by consumption of vegetative material by herbivores, while Paige & Whitham (1987) discovered that *Ipomopsis aggregata* seed production is increased by herbivory.

Plant-herbivore interaction is further complicated by the type of herbivores. Herbivores range in size from microscopic to megafauna. They can be generalist feeders, consuming any above and below ground plant material, or selective feeders that consume a specific species or plant part (Huntley 1991).

Except in cases of outbreaks, insects have often been considered minor consumers of plant tissue, but studies have suggested the extent of their pressure on plants is underestimated (Crawley 1989). For example, the damage done by insects with chewing mouthparts is easily identified by observation of missing tissue, while injuries from piercing mouthparts are not as obvious. Insects with piercing mouthparts can directly consume xylem and phloem which reduces a plants' ability to access water and nutrients, transmit diseases, and inject growth-altering chemicals (Huntly 1991; Stone & Schönrogge 2003). Crawley (1989) and Huntly (1991) concluded that through their

diversity of utilization, selective feeding, feeding styles, and temporal scale, insect herbivory could significantly impact individual plants and plant community dynamics.

The majority of plant-insect interaction research on members of Orchidaceae has focused on pollinator or pests of cultivated species under controlled conditions (Darwin 1862, Delaney & Macedo 2001). However, orchids experience interactions with insects other than as pollinators and receive greater pressures in natural ecosystems than in greenhouse experiments. These pressures come from a variety of herbivores, mycorrhizal associations, tuber carbon storage, and a number of biotic and abiotic factors (Strauss & Agrawal 1999; Delaney & Macedo 2001). Endangered orchids could be especially vulnerable to these pressures. It is important to study the effects of these variables on each species to facilitate development of the best management practices.

There is no known research that focuses on insect herbivory of *S. parksii*. Research conducted by Wonkka (2010) showed that large herbivores directly affect *S. parksii* by consuming the inflorescence during the flowering season. This reduces seed production and could affect population size. Wonkka also concluded that herbivores could indirectly affect *S. parksii* flower and seed production by reducing rosette leaf surface area and access to photosynthetic energy and nutrients. In field studies, insects have been observed removing photosynthetic and reproductive material of *S. parksii*, but the amount of herbivory has not been documented (M.C. Ariza and D.D. Nally, personal communication and observations; Hammons 2008).

CHAPTER III

STUDY SITE

Research was conducted at the BVSWMA Twin Oaks landfill site (Twin Oaks), a 246 ha parcel of land located in north Grimes County, Texas (96°8'51.66" W 30°35'47.25" N). For conservation of *S. parksii*, 56 ha of land on the Twin Oaks site have been excluded from the landfill footprint and divided into 13 deed-restricted areas (DRAs). In accordance with conservation requirements set by United States Fish and Wildlife Service, yearly surveys are conducted on the DRAs to track population density. Surveys conducted during the 2013 flowering season were evaluated to determine DRAs with large populations of newly discovered *S. parksii* and its congener *S. cernua*. Of the 13 DRA's, DRA 11 (~16 ha), had the largest population of *S. parksii* and *S. cernua* and was selected as the focus for this study (Fig. 1).

The study site is located within the Post Oak Savanna Ecoregion. Climate of the study area is classified as humid subtropical with an average annual precipitation of 88.9 to 114.4 cm from west to east. Precipitation falls in a bimodal pattern with peaks in late May through early June and October. Annual average temperature low and high are 11°C (January) and 35 °C (August) (USDA 1996, Bruton 2014, SRCC 2012).

The regional geology for the site is underlain by the Wellborn formation (USGS 1993). Soils on the site are mapped as the Burlewash soil series intermixed with Boonville, Hatliff, Robco, Tabor and a small patch of Koether-rock (NCSS 2007).

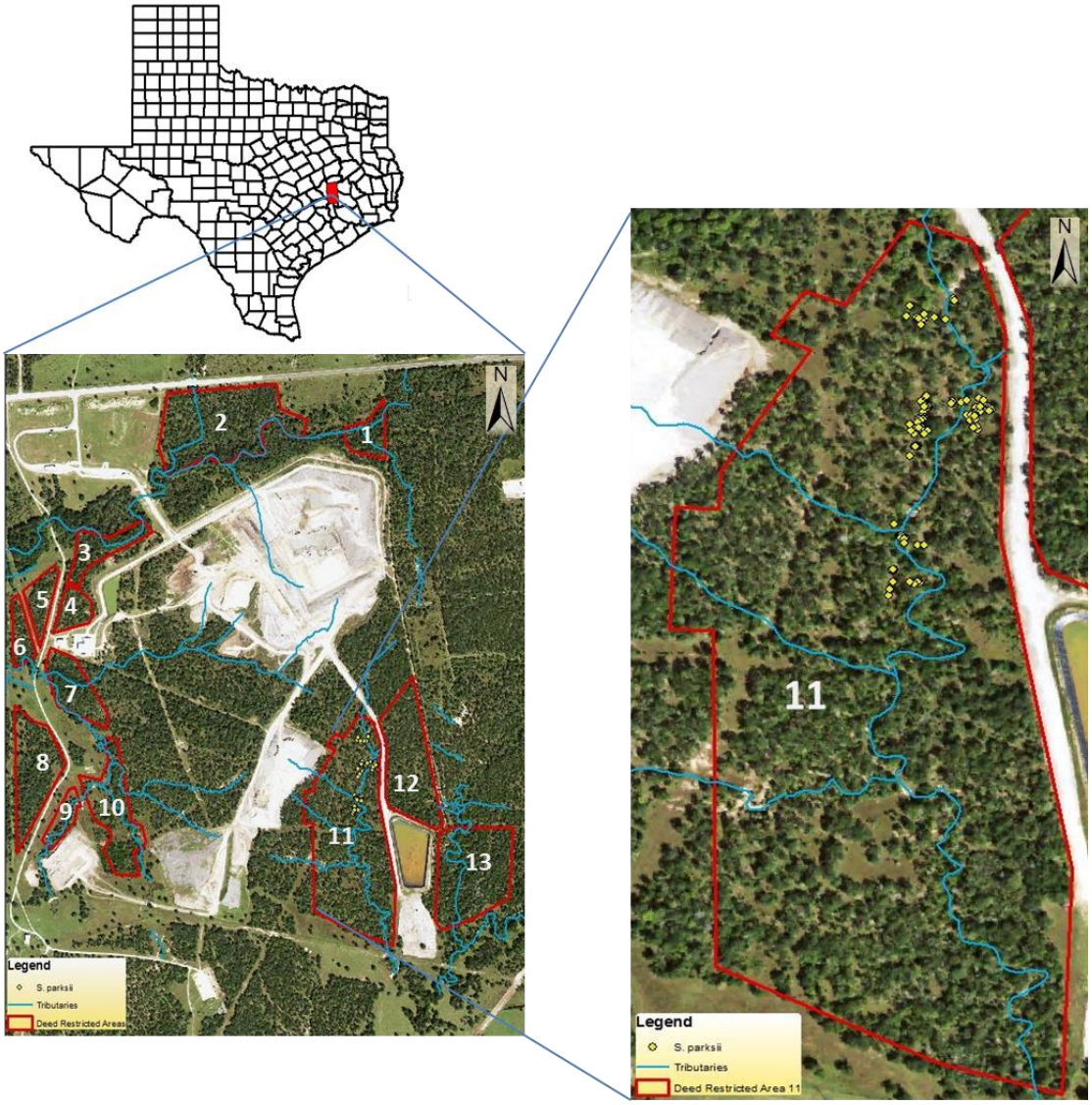

Figure 1. Thirteen deed-restricted areas at BVSWMA's Twin Oaks Location in Grimes County, Texas with DRA 11 enlarged to emphasize plant locations (yellow points).

Burlewash is the only soil series mapped in DRA 11. Burlewash are well-drained, slightly acidic to acidic fine sandy loam soils over a claypan subsoil with slope ranging from 1to15% (USDA 1996, NCSS2007). The terrain in DRA 11 is gently

sloping at less than 4% with estimated 20 to 40% open woody canopy, and an ephemeral stream that forms gullies from north to south through the middle of the site.

Much of the Post Oak Savanna originally consisted of open grasslands with a variety of grass species such as little bluestem (*Schizachyrium scoparium*), Indiangrass (*Sorghastrum nutans*), and switchgrass (*Panicum virgatum)*[1]. Tree species include post oak (*Quercus stellata*), blackjack oak (*Quercus marilandica*) and winged elm (*Ulmus alata*). With the suppression of the natural fire regime and large grazers that once maintained the open grasslands associated with the Post Oak Savanna, woody encroachment has resulted in an increase in canopy cover. This can affect species that are sensitive to reduced light caused by the increase in woody species canopy cover.

S. parksii habitat on the Twin Oaks site is dominated by little bluestem and post oak, but *Chasmanthium laxum* var. *sessiliflorum, Andropogon ternarius, Drosera annua*, yaupon (*Ilex vomitoria*) and American beautyberry (*Callicarpa americana*) are often associated with the species (Hammons 2008). This matrix of grass, shrub, and tree species forms a slightly open canopy of 20 to 40% with light to medium litter depth, found to be associated with the habitat of *S. parksii* at Twin Oaks. Research conducted on woody encroachment of the Twin Oaks site from 1958 to 2004 noted that yaupon, eastern red cedar (*Juniperus virginiana)*, and other woody species had increased which resulted in less open canopy for *S. parksii* (Hammons 2008).

The Twin Oaks site hosts several small and large mammalian herbivores such as

[1]Taxonomic nomenclature follows Diggs et al. 1999 (Plants)

white-tail deer (*Odocoileus virginianus*), eastern cottontail rabbits (*Sylvilagus floridanus*), field mice (*Peromyscus spp.*), and feral hogs (*Sus Scrofa*)[2]. Insect herbivores such as leafhoppers (Family Cicadellidae), katydids (Family Tettigoniidae), and grasshoppers (Family Acrididae)[3] have been observed at the site. Common within the leaf litter on the Twin Oaks site are mites from the family Tetranychidae and detritivores from the family Armadillidiidae (D.D. Nally, personal observations).

[2]Taxonomic nomenclature follows Schmidly 1994 (Mammals), [3]Triplehorn & Johnson 2005 (Insects)

CHAPTER IV

METHODS

Since 2007, undergraduate and graduate students associated with the Department of Ecosystem Science and Management at Texas A&M University in College Station, Texas have conducted yearly surveys to monitor populations of *S. parksii* and identify *S. cernua* in the 13 DRAs at the Twin Oaks site. These surveys are conducted at the peak of the Spring rosette and Fall flowering season.

To reduce the influence of environmental differences between DRAs, the 2013 Fall flower survey focused on locating a sufficient population of *S. parksii* in one DRA. Once the 2013 flower surveys were concluded, DRA's with large populations of *S. parksii* were evaluated for homogeneity of terrain and clustering of individual plants, but an insufficient population of *S. parksii* was located within any single DRA. To increase sample size, *Spiranthes cernua* was included in the experiment.

In central Texas, *S. parksii* and *S. cernua* often share a similar habitat, but *S. cernua* is not restricted to the Post Oak Savanna and can be found from Canada to Texas. Aside from the difference in distribution and habitat restrictions, both species are genetically alike and research conducted by Ariza (2013) found many similarities in life history and phenology. To determine if the *S. parksii* and *S. cernua* in this experiment shared the phenological similarities in inflorescence height and rosette leaf area found by Ariza, an analysis was conducted at the end of the experiment to compared growth of the two species. Analysis was run using Microsoft Excel's two sample t-test assuming unequal variance. In flowering plant height, there was no significant difference between

S. parksii or *S. cernua* for 2014 (t(57)= -0.3812, p= 0.7045) and 2015 (t(30)= -1.3157, p= 0.1983). There was also no significant difference in total rosette leaf area between the two species in 2015 (t(46)=-0.1167,p=0.9076). Since measurements were not taken on maximum rosette growth in 2014, it was not used for analysis. The growth of the two species and habitat relationships were similar and hence combined for the analysis.

In determination of the best site for the experiment, DRA 11 had the largest number of *S. parksii* and *S. cernua* identified, with the majority of the population clustered within 4 hectares (ha) of the northern section of the plot (Fig. 1). This northern section was evaluated with 1.5 ha along an ephemeral stream determined to be mostly homogenous with a sufficient population of *S. parksii* and *S. cernua* (Fig. 2 (A&B)).

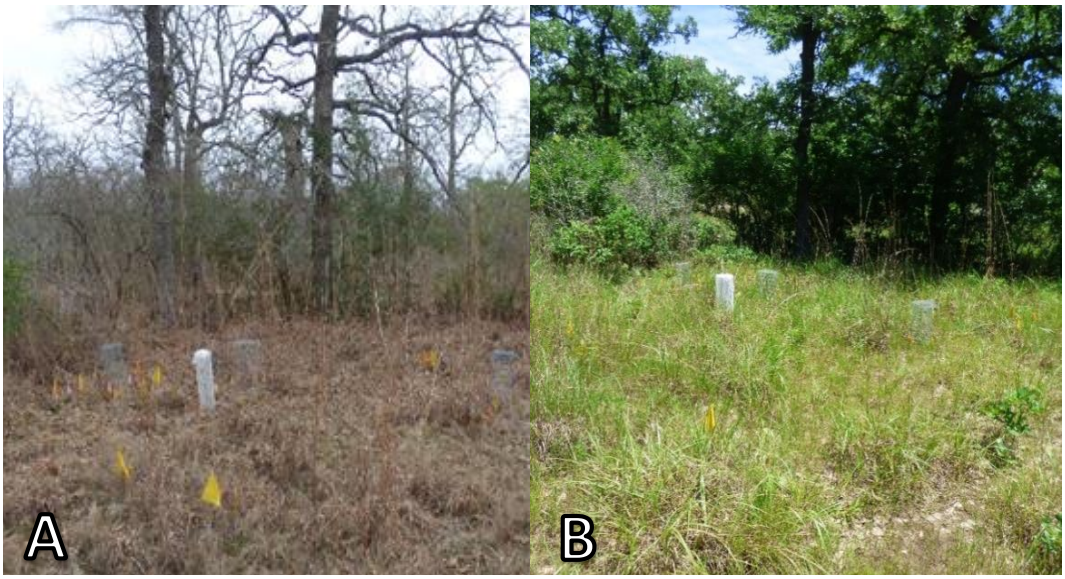

Figure 2. Caged and uncaged (yellow flags) plants near ephemeral stream (not visible) with gentle sloping topography and typical herbaceous vegetation cover for Fall 2014 (A) and Spring 2015 (B).

In the 2014 Spring rosette survey, plants located in the northern section of DRA 11 during the 2013 Fall flower survey were visited with 27 *S. parksii* and 73 *S. cernua* located. The 100 plants were permanently marked and identified by species with two 46 cm plain vinyl steel stake wire flags, each placed 15 cm equidistance to the north and south of the plant. Aluminum 2.54 x 8.89 cm write-on tags were used to label plants with identification numbers and secured to the northern positioned flag.

The 100 plants were randomly assigned to a 2 x 2 factorial treatment of 1) 25 not caged and not treated with insecticide (Control) 2) 25 not caged and treated with insecticide (Vertebrate Only) 3) 25 caged and not treated with insecticide (Invertebrate Only) and 4) 25 caged and treated with insecticide (Cage + Insecticide) using Random.org. The set of caged plants were enclosed in cylindrical 45 cm tall by 15 cm diameter galvanized wire cages with 1 cm^2 openings to exclude vertebrate herbivores such as deer, hogs, rabbits, and mice, while allowing the inclusion of small invertebrates (Hammons 2008) (Fig. 3(A)). For the insecticide treatment, the insecticide was applied to the plant, and a surrounding 15 cm radius, until saturation at 5 to 10 ml of 0.126% Carbaryl (Garden Tech, Sevin ready-to-use spray bottle). For plants not treated with insecticide, the plant and a 15 cm radius around it were saturated with 5 to 10 ml of water. In Fall 2013 and Spring 2014, rosettes were retreated with insecticide every 7 to 10 days until dormancy.

After observations in the field and initial analysis of the 2014 rosette data, it was observed that the size of invertebrates utilizing *S. parksii* and *S. cernua* could influence the type and intensity of herbivory damage. To compare the influence of small and large

invertebrates, a new treatment to exclude large insects was established before the 2014 Fall flower season. This increased the experiment to a 2 x 3 factorial design. Since all identified plants from the 2013 flower surveys were incorporated into the experiment, 25 unidentified plants from the 2014 rosette survey were utilized for the new treatment. Rosettes were enclosed in a cylindrical 45 x 20 cm, 1 cm^2 galvanized wire cage encompassed by off-white 1 mm nylon matte tulle mesh (Fig. 3(B)). Rosettes were marked with flags and aluminum tags similar to plants in the caged and not caged experiment. Plants were later identified as 22 *S. cernua* and 3 *S. parksii*.

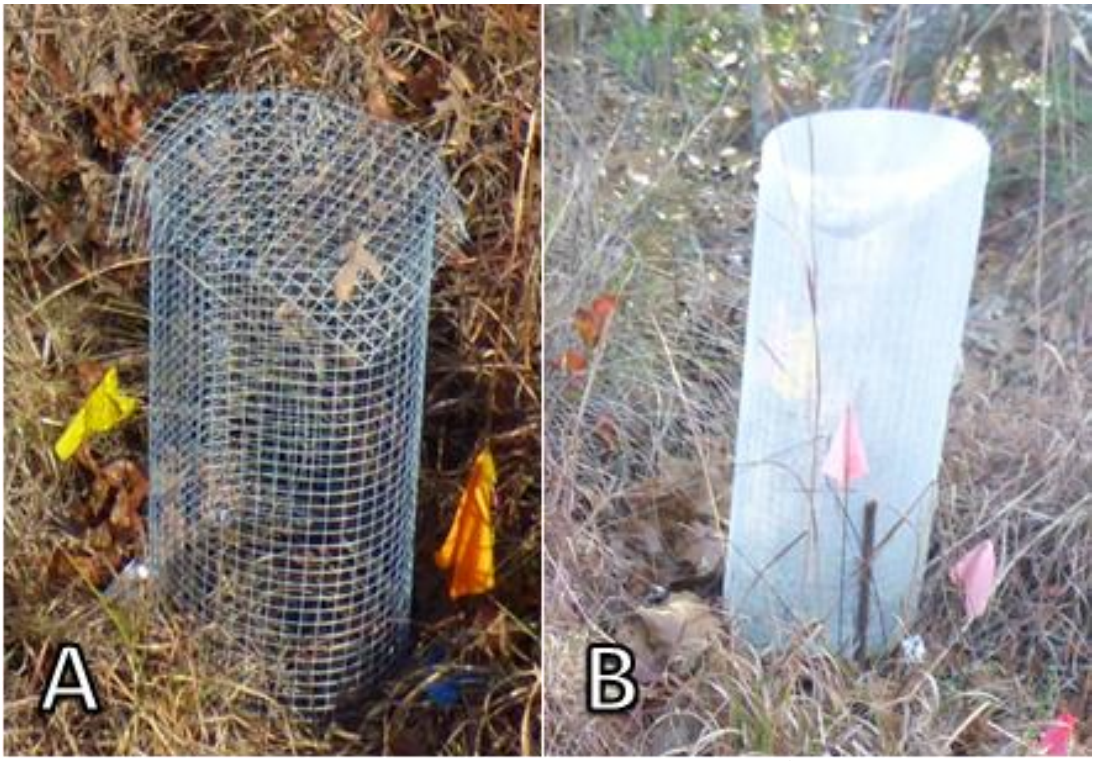

Figure 3. Cylindrical 45 cm tall and 20 cm in diameter, 1 cm^2 galvanized wire cage (A) and the same size cage lined with off white 1 mm nylon matte tulle mesh (B).

Rosette growth was observed weekly in spring 2014 with demographic data and growth measurements taken only once during the estimated maximum growth in May. Measurements included number of leaves produced, leaf length and width at the primary axis of each rosette, and percent herbivory per leaf. For analysis, leaf area of the whole rosette was calculated by the equation 0.7854Ld with (L) the leaf length, (d) the leaf width, and 0.7854 to represent the elliptical area of *S. parksii* and *S. cernua* (Wonkka 2010). Percent herbivory was visually assessed, in situ, of tissue removed from each rosette leaf, based on the prediction of the elliptical area of the leaf. Estimation of material removed for each leaf was in 5 percent increments and averaged over the whole rosette for percent herbivory used in rosette herbivory analysis (Wonkka 2010).

After observed stress of rosettes sprayed with insecticide during the 2014 rosette season, and suggested overtreatment by the insecticide manufacturer, the period between insecticide treatments was increased from 7 to 10 days to every 14 to 17 days for the remainder of the experiment. Demographic data and growth measurements were increased to a 7 to 14 day rotation until dormancy, with a total of 10 sample periods taken, in order to collect details on herbivory and the growth cycle of *S. parksii* and *S. cernua* in the experiment.

Demographic data and growth measurements for the Fall 2014 and Fall 2015 flower season was recorded for plant presence, plant height, inflorescence length, stalk diameter, and number of flowers open and closed. Stalk length was calculated by subtracting plant height and inflorescence length. In 2014, leaf like bracts (stalk leaves) were observed encasing the inflorescence during plant emergence. To determine the

possible contribution of the stalk leaves to inflorescence emergence, number of leaves present and percent herbivory in increments of 5 was recorded during emergence and development of the flowering plant. Herbivory was recorded separately for plant stalk and inflorescence, based on estimated tissue removed from a fully intact plant part by 5 percent increments. Whole plant herbivory was calculated by the average tissue removed from plant stalk and inflorescence.

Spring 2015 rosette demographic data and growth measurements were recorded weekly by procedures established in the spring 2014 rosette season until dormancy, with 36 sample periods recorded. During this time, observation and demographic data was also collected on emerging seedlings from the 2014 flower season. At time of analysis, 2016 rosettes had not reached their maximum leaf area or herbivory and only demographic data is presented to show consistency of rosette presence throughout growth seasons.

At the end of the 2015 rosette season, new growth was observed emerging from rosettes previously identified as dormant. Length and width Measurements and presence/absence were recorded weekly on this new growth.

In conjunction with growth measurements, invertebrate and vertebrate observations and data were collected. Invertebrates found utilizing, or within a 15 cm diameter of, *Spiranthes sp.* were collected for identification. Invertebrates were collected by hand, insect nets, or syphoning tubes and stored in a 20 ml glass vial filled with 70 to 90% Isopropyl until identified. When collection was not successful, observation of known invertebrates was noted and combined with collected specimens to categorize

frequency of families utilizing *Spiranthes sp*. Collected invertebrate were classified as common (>5 observations), uncommon (3-5 observations), or rare (1-2 observations) related to 36 sample periods in the rosette season and 11 in the flower season. Identification of vertebrate utilizing the plant was done through visual identification, tracks, or scat near plants with damaged tissue in the same sample periods.

During the 2015 rosettes season, roly poly (*Armadillidium vulgare*, family Armadillidiidae) activity was observed near several emerging seedlings with herbivory (Fig. 4). Research has indicated that roly polies consume seeds and seedlings of agriculture species in greenhouses. To determine if roly polies consume *Spiranthes sp.* seedlings, an observation only, limited feed choice experiment was implemented (Mariani & Alcoverro 1999; Saska 2008; Sutton 2013). Roly polies were collected in DRA11 and placed into 5 cages similar to the Mesh treatment, with 10 roly polies per cage. The 5 cages were placed over 4, 5, 4, 4, and 5 seedlings, consecutively, that had no prior herbivory with the cages buried 1 cm into the soil to discourage burrowing. Before cages were installed, vegetation was removed from the area with only *Spiranthes* sp. seedling, leaf litter, and less than 5% live vegetation inside. Plants were revisited within 24 hours with seedling herbivory and roly poly presence recorded.

Figure 4. Suspected leaf herbivory of *Spiranthes parksii* seedlings (red arrows) by Armadillidiidae.

Plants require essential elements to grow, but access to some nutrient can be limiting factors to their development and reproduction. Macro and micronutrients can also attract herbivores that require a specific element. There is no information in the literature of the micro and micronutrient makeup of *S. parksii* or *S. cernua*. To gain insight into essential elements that may be limiting factors to *Spiranthes sp*. growth and possibly attract herbivores, a macro and micro nutrients analysis was conducted. In spring 2016, leaves from 10 *S. parksii* and *S. cernua* rosettes located and identified in fall 2015 were collected for analysis. Of the 10 plants, 15 rosette leaves were collected before peak growth with less than 10% herbivory and no more than one third of the

whole plant removed. Leaves were collected and combined into one sample for each species and immediately taken to the Texas A&M Agrilife Extension Service Soil, Water, and Forage testing laboratory for analysis.

To analyze possible influence of weather on *Spiranthes sp.* growth and herbivory, temperature and precipitation data was collected daily during the study period by a Davis Vantage Pro 2, located 800 meters from DRA 11. The weather data was collected and averaged for the week prior to each growth measurement.

Spring 2014 rosette analysis, rosette leaf area (LA) and percent herbivory of *Spiranthes sp.* were recorded for only one sampling period during estimated peak rosette growth (May). Data for growth and herbivory was collected 10 times for the Fall 2014 and 2015 flower season and 36 times for the 2015 rosette season. To compare approximate peak growth and herbivory between 2014 and 2015 rosettes, one sample point in May 2015 that corresponded to the sample date in 2014 was used to test for significant differences between years. Plants absent during data collection were not used for analysis, giving varied sample sizes per treatment.

Data was analyzed using JMP Statistic Software, and data normality evaluated using the distribution platform and Shapiro-Wilks test. Normality assumptions were rejected and data reassessed using transformation methods (Ott & Longnecker 2010). Transformation was unsuccessful for all sample periods, and the nonparametric Wilcoxon Each Pair test was used to determine significant difference between treatments at alpha $p<0.05$ (Schmid & Trede 1996; Ott & Longnecker 2010).

CHAPTER V

RESULTS

Flower Presence, Growth, and Herbivory

The number of flowering plants present and that survived to seed maturity decreased from 2014 to 2015 (Table1). In 2014, of the 125 plants across all treatments, 103 (83%) produced an inflorescence, with an average of 21 blooms, while 100 (80%) produced seeds. During 2015, flowering plants present dropped to 71 (57%) with 13 blooms per inflorescence and only 40 (32%) producing seeds.

Table 1. Original number of flowering plants in experiment by year, subdivided into number per treatment (n=25). The flower column represents plants present at beginning of season used in growth, herbivory and demographic analysis/plants survived to produce seed.

Treatment	Flowers 2014 (n=125)	Flowers 2015 (n=125)
Control (=25)	14/13	12/5
Vertebrate Only (n=25)	17/15	11/4
Invertebrate Only (n=25)	23/23	12/8
Cage+Insecticide (n=25)	24/24	16/10
Mesh (n=25)	25/25	20/13
Total	103/100	71/40

Precipitation in the flower season (August-October) was below the 30-year average for 2014 and 2015. In fall of 2014 the Twin Oaks site received 4.82 cm compared to the long-term average of 8.87 cm, while 2015 received only 1.65 cm (Fig. 5 (A)). A decrease in flowering plants presence and growth was also seen with the reduced precipitation in 2015 (Table 1). All treatments produced similar flowering

growth patterns in 2014 and 2015 with an average height of plants caged and not caged at 9 to 10.4 cm^2 and the latter at 3 to 4.5 cm^2 (Fig. 5 (B)), respectively.

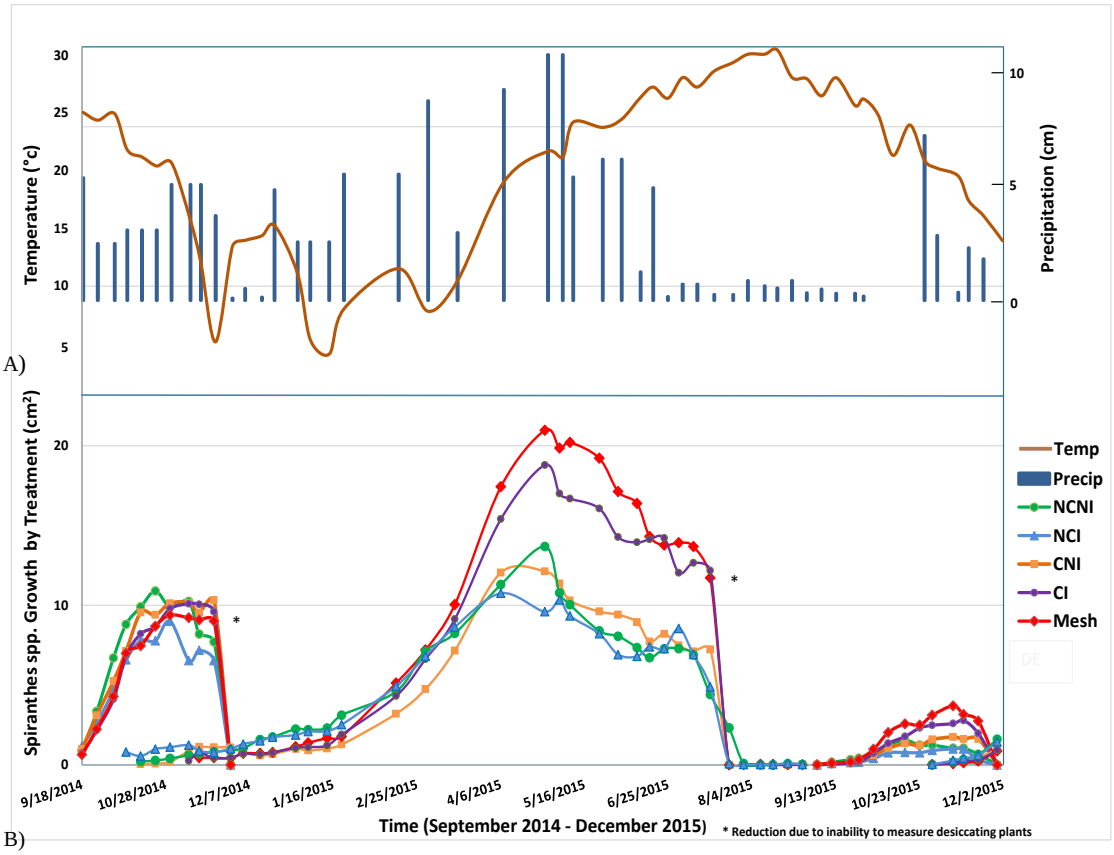

Figure 5. Precipitation (cm) and temperature (°C) for the study period at the Twin Oaks site from September 2014 to December 2015 (A), and Growth cycle of *Spiranthes sp.* from September 2014 to December 2015, by treatment (B). Plant growth expressed as area in centimeters squared.

In 2014 there was no significant difference in maximum plant height, stalk length, or inflorescence length between treatments with mean plant height that ranged from 23 to 26cm (Table 2). Mean flower production ranged from 17 to 19 blooms per

inflorescence with no significant difference in maximum flower production between treatments.

In 2015, flowering plants present above ground decreased by 31% with a 60% decrease in survival to seed production and a significant reduction in mean plant height that varied in growth between caged and not caged plants at 7 to 14cm when compared to 23 to 26cm in 2014 ($p<0.0001$). This could be due to the lack of precipitation just before and at the beginning of inflorescence emergence (Fig. 5 (A)). The Mesh treatment had the most growth with a significant difference in maximum plant height from Vertebrate Only ($p=0.0197$), and between Vertebrate Only and Cage+Insecticide ($p=0.0483$) (Table 2). Stalk length was significantly different in Vertebrate Only and Mesh ($p=0.0111$) and in inflorescence length between Control, Mesh ($p=0.0142$), and Cage+Insecticide ($p=0.0435$).

In flower production there was a significant difference between Control and Mesh ($p=0.0484$) with mean flower production that ranged from 9 to 16 blooms per inflorescence.

Table 2. Maximum plant height, stalk length, and inflorescence length in centimeters (mean ± SE) with significant difference by treatment for 2014 and 2015 flower season. Significant difference calculated by Wilcoxon Each Pair test and indicated in uppercase letters.

Treatment	Height 2014	Stalk 2014	Inflorescence 2014	Height 2015	Stalk 2015	Inflorescence 2015
Control	25.8±5.8 **A**	20.1±0.9 **A**	6.7±0.6 **A**	10.5±1.8 **AB**	8.5±1.5 **AB**	2.4±0.5 **A**
Vertebrate Only	23.8±5.5 **A**	18.5±10.0 **A**	5.6±0.5 **A**	7.6±1.9 **A**	5.4±1.3 **A**	2.4±0.7 **AB**
Invertebrate Only	26.1±6.3 **A**	20.0±10.0 **A**	7.0±0.4 **A**	12.2±1.9 **AB**	8.4±1.3 **AB**	4.1±0.6 **AB**
Cage + Insecticide	24.4±7.1 **A**	18.7±1.1 **A**	7.4±10.0 **A**	12.6±1.6 **B**	8.6±1.0 **AB**	4.4±0.6 **B**
Mesh	25.3±5.0 **A**	19.2±0.6 **A**	6.8±0.4 **A**	14.5±1.3 **B**	10.1±0.9 **B**	4.7±0.5 **B**

In the 2014 flower season, there was a significant difference in herbivory of plant (stalk + inflorescence), stalk, and inflorescence between caged and not caged treatments. Maximum plant herbivory was significantly different between Control, Invertebrate Only ($p<.0001$), Cage+Insecticide ($p<0.0001$), and Mesh ($p<0.0001$), as well as between Vertebrate Only, Invertebrate Only ($p=0.0026$), Cage+Insecticide ($p=0.0008$) and Mesh ($p=0.0034$) (Table 3). Stalk herbivory was significantly different between Control, Invertebrate Only ($p=0.0060$), Cage+Insecticide ($p=0.0008$), and Mesh ($p=0.0109$), as well as between Vertebrate Only, Invertebrate Only ($p=0.0029$), Cage+Insecticide ($p=0.0003$) and Mesh ($p=0.0052$). The significant difference between treatments in inflorescence herbivory was Control, Invertebrate Only ($p<.0001$), Cage+Insecticide ($p<.0001$), and Mesh ($p<.0001$), as well as between Vertebrate Only, Invertebrate Only ($p=0.0021$), Cage+Insecticide ($p=0.0017$) and Mesh ($p=0.0003$).

Herbivory in the 2015 flowering season showed a significant difference in plant herbivory between Control, Cage+Insecticide (p=0.0352) and Mesh (p=0.0390) (Table 3). Interestingly, though Invertebrate Only received less herbivory than the Mesh treatment, it was not significantly different than Control as indicated for Cage+Insecticide and Mesh. Stalk herbivory had no difference between treatments while there was a significant difference in inflorescence herbivory between Control, Invertebrate Only (p=0.0129), and Cage+Insecticide (p=0.0273). Thought there was a significant difference of herbivory in treatments each year, there was no difference between 2014 and 2015.

Table 3. Maximum percent plant, stalk, and inflorescence herbivory (mean ± SE) with significant difference by treatment for 2014 and 2015 flower season. Significant difference calculated by Wilcoxon Each Pair test and indicated in uppercase letters.

Treatment	Plant 2014	Stalk 2014	Inflorescence 2014	Plant 2015	Stalk 2015	Inflorescence 2015
Control	50.2±12.4 **A**	57.7±13.3 **A**	50.0±48.6 **A**	21.5±8.7 **A**	14.5±7.4 **A**	30.4±12.0 **A**
Vertebrate Only	44.8±11.7 **A**	45.3±11.8 **A**	45.9±48.0 **A**	17.4±8.8 **AB**	15.3±8.6 **A**	19.2±11.1 **AB**
Invertebrate Only	2.9±0.5 **B**	1.5±0.6 **B**	2.0±2.9 **B**	4.6±1.7 **AB**	7.1±3.2 **A**	1.7±1.3 **B**
Cage +Insecticide	2.4±0.7 **B**	1.9±0.9 **B**	1.7±4.3 **B**	2.3±0.8 **B**	1.6±0.8 **A**	2.5±1.1 **B**
Mesh	2.9±0.3 **B**	0.8±0.4 **B**	.8±1.9 **B**	12.0±6.1 **B**	8.0±4.1 **A**	15.8±8.1 **AB**

The *S. parksii* flowering plant is often described as a single leafless flowering stem, but at the beginning of flower emergence the plant may have 3-7 leaves present (Fig. 6 (A)). The leaves act like a sheath that covers the inflorescence during emergence

(Fig. 6 (B)). In 2015 these leaves receive up to 30% herbivory with no damage observed to the inflorescence (Fig. 6 (C)).

Figure 6. *S. parksii* leaf emergence at the beginning of the flower season (A), inflorescence encased by protective leaves (B), and inflorescence emerging from leaves (indicated by red arrow) with herbivory (indicated by black arrows) (C).

Rosette Presence, Growth, and Herbivory

In 2014, the increase in leaf area of rosettes occurred in a short period of time similar to the bolting phase of flowering plants, with 95 of 100 (95%) rosettes present at the beginning of the season (Table 4). In 2015, 121 (97%) rosettes were present and the rosette growth pattern was similar for all treatments with peak growth in early to mid-April. Rosette growth was initiated in mid-October but little growth occurred until temperatures and favorable precipitation occurred in late January and early February. The Mesh and Cage+Insecticide treatment produced the greatest peak growth of 21.7cm^2

and 19.7cm^2. The remaining three treatments peaked at about 12 to 14cm^2. Growth continued in all treatments until late July with some regrowth in August.

Table 4. Original number of rosettes in experiment by year, subdivided into number per treatment (n=25). Rosettes in 2014 include number of plants present in January/plants present for growth, herbivory and demographic analysis at peak growth in May.

Treatment	Rosettes 2014 (n=100)	Rosettes 2015 (n=125)	Rosettes 2016 (n=125)
Control (n=25)	25/18	23	21
Vertebrate Only (n=25)	22/13	23	21
Invertebrate Only (n=25)	23/20	25	22
Cage+Insecticide (n=25)	25/22	25	24
Mesh (n=25)	NA*	25	25
Total * Not added to experiment until 2014 flower season.	95/73	121	116

In the 2014 rosettes growth there was no significant difference in leaf area between treatments (Table 5). Though no significant difference was detected, Invertebrate Only and Cage+Insecticide plants had the largest mean leaf area at 13.8 and 13.6 cm^2, with Control and Vertebrate Only at 10 and 8.7 cm^2 respectively.

In May 2015 there was no significant difference in leaf area between treatments (Table 5). Leaf area increased slightly in 2015 with a range from 9 to 16 cm^2, but was not significantly different than 2014 ($p=0.5831$).

In maximum leaf area for 2015, the numbers of rosettes present was similar throughout treatments with a variation of growth that ranged from an average of 13 to 22 cm^2. Across all treatments, Mesh plants had the largest maximum leaf area with

significantly more growth in Mesh than Control (p=0.0452), Vertebrate Only (p=0.0093) and Invertebrate Only (p=0.0153) (Table 5).

In comparing maximum leaf area of 2015 to the sample period in May 2014, there was no significant difference (p=0.0894). However, there was a significant difference between May 2015 and maximum leaf area in 2015 (p=0.0340).

In the 2015 rosette season, seedling demographic data was collected with significantly more Mesh cages containing seedlings than Control (p=0.0347). Though no significant difference was detected in number of seedlings between treatments, Mesh and Cage+Insecticide had the most seedlings at 67 and 41 compared to Control and Vertebrate Only at 23 and 25, respectively.

Table 5. Leaf area in centimeters squared (mean ± SE) and significant difference of rosettes by treatment for May 2014, May 2015, and maximum growth (MAX) in 2015. Significant difference calculated by Wilcoxon Each Pair test and indicated in uppercase letters.

Treatment	May 2014	May 2015	MAX 2015
Control	10.0±9.6 **A**	10.0±9.8 **A**	14.3±7.4 **A**
Vertebrate Only	8.7±7.7 **A**	9.3±6.6 **A**	12.7±7.0 **A**
Invertebrate Only	13.8±7.9 **A**	10.3±9.7 **A**	14.0±10.3 **A**
Cage+Insecticide	13.6±9.1 **A**	16.7±14.3 **A**	19.7±15.1 **AB**
Mesh ***Not added to experiment until 2014 flower season**	*	*	21.7±12.0 **B**

Herbivory for rosettes in May of 2014 was significantly greater in Control compared to Cage+Insecticide ($p=0.0041$) (Table 6). Plants protected from vertebrates and invertebrates received the least herbivory, while damage received by rosettes from insect or mammals alone was not significantly different. Percent herbivory between Vertebrate Only, Invertebrate Only, and Cage+Insecticide treatments ranged from 9% to 15% compare to Control at 39%.

Similar herbivory was observed in May of 2015 with significantly more herbivory in Control than Cage+Insecticide ($p=0.0237$) (Table 6). There was significantly more herbivory in 2015 than 2014 ($p=0.0225$) with a range from 15% to 36% for Vertebrate Only, Invertebrate Only, and Cage+Insecticide with Control the same as 2014 at 39%.

In the 2015 rosettes season, the dynamics between treatments increased in complexity. Maximum percent herbivory in 2015 was significantly less in Mesh compared to Control ($p=0.0003$) and Vertebrate Only ($p=0.0005$). A similar reduction in herbivory was seen when comparing Cage+Insecticide to Control ($p=0.0161$) and Vertebrate Only ($p=0.0142$). Interestingly, there was also a significant difference found between Mesh and Invertebrate Only ($p=0.0295$) (Table 6).

Table 6. Percent herbivory (mean ± SE) and significant difference of rosettes by treatment for May 2014, May 2015, and maximum (MAX) percent herbivory in 2015. Significant difference calculated by Wilcoxon Each Pair test and indicated in uppercase letters.

Treatment	May 2014	May 2015	MAX 2015
Control	38.8±39.8 **A**	39.0±32.6 **A**	58.5±36.4 **A**
Vertebrate Only	15.8±26.1 **AB**	27.2±29.4 **AB**	56.7±37.7 **A**
Invertebrate Only	11.0±20.3 **AB**	36.0±34.1 **AB**	38.1±37.6 AB
Cage+Insecticide	9.4±14.7 **B**	14.8±29.8 **B**	32.6±37.5 **BC**
Mesh ***Not added to experiment until 2014 flower season**	*	*	22.7±32.7 **C**

In the life history of *S. parksii*, the observed growth cycle has been recorded as bimodal with rosettes in the spring followed by a dormant phase before the flower season in the fall. However, recent observations have recorded aboveground growth from June to August that shows *Spiranthes sp*. can remain active without a dormant period (Fig. 7). This growth may appear shortly after spring rosettes have desiccated in June or July but does not resemble rosette tissue.

Figure 7. Meristematic growth of *Spiranthes parksii* observed on August 20th 2015, (highlighted by red circle) and desiccated rosette leaves from Spring rosette season (indicated by red arrows).

Invertebrate and Vertebrate Observations

Insects and other invertebrate collected and observed throughout the experiment varied in their presence on *Spiranthes sp*. (Table 7). Coleoptera were frequently seen within a 10 cm diameter of *Spiranthes sp*., but rarely observed utilizing the plant. Lepidoptera larvae and Hymenoptera frequented both rosettes and flowers, with Lepidoptera larvae varied between seasons.

Table 7. Identified invertebrate and frequency observed of presence on or within 15 cm of *Spiranthes sp.* Categories of frequency are: common (>5 observations), uncommon (3-5 observations), or rare (1-2 observations) for 36 observations in 2014 rosette and 11 observations in 2015 flower season. ** indicates insects observed consuming plant tissue.

Order	Family	Common Name	Presence (10 cm diameter)	Presence on Rosettes	Presence on Flowers
Coleoptera			Common	Rare	None
	Carabidae	Ground Beetles			
	Cicindelidae	Tiger Beetles			
Diptera			Uncommon	Rare	Rare
Hemiptera			Common	Common	Rare
	Blissidae				
	Cicadellidae	Leafhopper			
	Corimelaenidae	Shield Bugs			
	Membracidae	Treehoppers			
	Pentatomidae	Stink Bugs			
	Rhyparochromidae	Seed Bugs			
Hymenoptera			Common	Common	Common
	Formicidae	Ants			
Isopoda	Armadillidiidae	Roly Poly or Pill bug	Common	Common	None
Lepidoptera			Common	Common	Common
	Arctiinae**	Tiger Moth			
	Geometridae	Inchworms			
	Noctuidae**	Owlet Moths			
Thysanoptera		Thrips	Unknown	None	Uncommon
Trombidiformes	Tetranychoidea	Spider mite	Common	Common	Common
Orthoptera					
	Acrididae**	Short Horned Grasshoppers	Common	Uncommon	Uncommon
	Gryllidae	Crickets	Rare	None	None
	Tettigonidae	Katydid	Common	Rare	None

During the spring, armyworms (Lepidoptera:Noctuidae) were the most common larvae observed consuming rosettes while armyworms and larvae from the Arctiinae

family were observed consuming flower and stalk tissue of infloresence in the fall (Fig. 8). Of lepidoptera larvae capture or observed throughout the experiment, 30% were recorded in October 2015 during the flowering season.

Figure 8. Armyworm larvae burrowing into immature inflorescence (A) and Armyworm consuming xylem and phloem tissue (B) after removing inflorescence (indicated by red arrow).

Vertebrates were difficult to observe actually consuming *Spiranthes sp.* tissue, but several mice, rabbits, and deer were observed near freshly damaged plants. Mice (order: Rodentia family: Muridae) activity was observed near tubers that received herbivory, with 8 plants tubers damaged in total. In addition to tissue removal by herbivores there was several observation of tubers uprooted by feral hogs (Fig. 9 (A)), and consumption of *Spiranthes sp*. roots by mice (Fig. 9 (B)) in the rosettes season.

Figure 9. Rooting by feral hogs (A) and mouse hole with exposed *S. parksii* roots with herbivory (roots indicated by red arrow) (B).

In the Armadillidiidae seedling experiment conducted during the 2015 rosette season, 2 cages experienced complete consumption of at least one seedling with some of the remaining plants receiving 10 to 45% herbivory (Fig. 10). The remaing three cages had roughly 2-3 plants with 5 to 25% herbivory with the remaining seedlings untouched.

Figure 10. *Spiranthes sp.* seedlings from feed experiment with herbivory indicated by red arrows and Armadillidiidae indicated by black arrows.

Rosette Macro and Micro Nutrient Analysis

In Spring 2016, an analysis was conducted to provide a potential measure of the forage quality of *S. parksii* and *S. cernua*. Plant analysis of *S. parksii* and *S. cernua* indicate high amounts of Nitrogen, Phosphorus, Potassium, Zinc, and Copper (Table 8), with *S. parksii* higher in Potassium. In contrast, both species had low amounts of Magnesium, Iron, and Manganese, with *S. cernua* higher in Manganese. Percent nitrogen was multiplied by 6.25 to calculated crude protein at 16.7% for *S. parksii* and 16.8% in *S. cernua* (Crisan 1978).

Table 8. Micro and macro nutrient analysis based on 100% dry matter of *S. parksii* and *S. cernua* rosette leaves collected at peak growth during spring 2016.

Nutrient	%/ppm	Measurment *S. parksii*	Measurment *S. cernua*
Nitrogen	%	2.67	2.69
Phosphorus	%	.17	.20
Potassium	%	3.81	2.97
Calcium	%	1.52	1.04
Magnesium	%	.38	.40
Sodium	ppm	1213	1612
Zinc	ppm	169	164
Iron	ppm	83	74
Copper	ppm	31	24
Manganese	ppm	79	142
Sulfur	ppm	1578	1577
Boron	ppm	17	16

CHAPTER VI

DISCUSSION AND CONCLUSION

Precipitation Influence on Flower Presence, Growth, and Herbivory

Terrestrial orchids that occur in regions with dry summer conditions rely on wet cool winters and early spring for carbon and nutrient storage (Rasmussen 1995, Ariza 2013). Specifically in *S. parksii* and *S. cernua*, a correlation has been observed between precipitation in the rosettes season from January to March and before flower emergence in August to flower emergence in September at the Twin Oaks site (Hammons 2008, Ariza 2013, Bruton 2014).

Within this study, precipitation recorded in 2014 and 2015 fell in previously observed measurements for January to March, but lower than average precipitation occurred during August and September in 2015. The decrease in plants present and survival in 2015 shows that January through March precipitation may assist with flower emergence, but August and September precipitation is potentially critical for plants to break dormancy or persist through inflorescence initiation to seed production. This study also suggests a critical precipitation and growth threshold for *Spiranthes sp*. In 2013, Ariza recorded average plant height to be 25.1 cm with above average precipitation. In 2014, precipitation was half the 30-year average at 4.82cm, but plant average remained at 25 cm. In 2015, precipitation was reduced to less than a fourth of the 30-year average at 1.62 cm, and plants averaged half the height of 2014 at 12 cm. This indicated that there is a precipitation threshold between 4.82cm and 1.64 cm that influences the height of *Spiranthes sp*. It was also observed in both years that plants below 9 cm did not

flower and that flower production is moderately correlated to plant height (r^2=0.552). Though these observations only cover two years and a small sample size, it could indicate that *Spiranthes* has a height threshold for reproduction that is influenced by precipitation. If this is proven correct, fluctuation in precipitation due to global climate change could influence persistence of the species.

Precipitation can also have an indirect influence on plant-insect interaction. There are many hypotheses that indicate the complexity of plant-insect interaction dealing with plant stress and allocation of nutrient that may influence herbivory. The Plant Stress hypothesis indicates that plants under physiological stress may be vulnerable to herbivory while in contrast the Plant Vigor hypothesis states that herbivores prefer healthy plants (Price 1991). An even more specific theory states that plants able to acquire the optimal balance of nutrients for growth are less susceptible to herbivores (Beanland et al. 2003).

The herbivory observed in this research tends to support the Plant Vigor and Plant Stress hypothesis depending on the herbivore. The majority of herbivory observed in 2014 was by large herbivores when precipitation was closer to the 30-year average, and plants were presumably under little water stress with large visible inflorescences. White tail deer have been specifically indicated as targeting large or flowering plants of some herbaceous species (Knight et al 2009). In the 2015 flower season there was an 80% decrease in precipitation from the 30-year average and large herbivore activity decreased while herbivory increased in the insect inclusion treatment. Inflorescences were visibly weaker and smaller than the previous year and therefore may have not been

as attractive to large herbivore, while nutrients may have been concentrated in the stressed plant thereby more attractive to insects (Mattson & Haack 1987). In both cases, the amount of reproductive tissue removed by vertebrates and invertebrates in 2015 was similar to damage incurred by only vertebrates in 2014. Indicating that invertebrate can cause considerable damage to flowering plants in times of stress from environmental pressures.

Flower Presence, Growth, and Herbivory by Treatment

In Fall 2014, the mean plant height of inflorescence was comparable to those observed by Ariza (2013) with no difference between treatments. In fall 2015, there was a distinct difference between plants present in caged plants compared to plants not in a cage with the most growth and inflorescence emergence seem in the Mesh treatment. The mesh could reduce light intensity and moisture loss while increasing temperature similar to what is seen in other insect exclusion-inclusion experiment (Hand & Keaster 1967), and provided a favorable microclimate for plant emergence.

Although mesh treatment had the greatest growth and flower production, Mesh received a high percentage of herbivory during the 2015 flower seasons compared to 2014. This could be due to the type of insects utilizing *Spiranthes sp*. and their development stages. During the 2015 flowering season more army worms were observed at the study site than in 2014. Armyworms are considered serious pest of agricultural crops but have a wide host range. They consume foliage and burrow into buds, stunting potential growth (Fig. 6). In ideal conditions, larvae will disperse in large numbers consuming all vegetation within their range. In their early stage of

development, the width of armyworm head capsules is roughly 0.35 mm and can easily pass through the holes of mesh fabric and consume plant material while the mesh may protect them from larger predators (Mayer & Babers 1944). As seen in the no significant difference in herbivory when comparing herbivory in 2014 and 2015, there was also no significant difference between Invertebrate Only and Vertebrate Only in 2015. Once again, this indicates that during times of low precipitation or other stressors, vertebrate and invertebrate may damage similar amounts of reproductive material.

Rosette Presence, Growth, and Herbivory by Treatment

Unlike inflorescences, rosettes have a tendency to be present consistently from year to year in the experiment. Research has indicated that *S. parksii* and *S. cernua* have a high cost of reproduction (Antlfinger & Wendel 1997, Ariza 2013), while in *S. cernua* rosettes can contribute 92% of seasonal carbon storage (Antlfinger & Wendel 1997). Rosettes being the main contributor to carbon storage required for reproduction could explain their consistent presence. In the 2014 and 2015 rosette season, the average leaf area of caged plants was the greatest, presumably from lack of damage by large herbivores. Surprisingly of the uncaged plants, Vertebrate Only plants had lower growth that the control plants exposed to tissue removal by all herbivores.

In 2015, the maximum leaf area was greater than recorded in May 2014 and significantly higher than May 2015. This indicated that measurements should be taken throughout the rosette season, instead of only one sample period, to correctly evaluate growth dynamics of the species. Similar to the reduced growth observed in 2014, maximum leaf area was lower in Vertebrate only compared to the other treatments. A

higher percentage of herbivory was observed on the Vertebrate Only rosettes two weeks before noted in the other treatments. This early herbivory could have influenced the plants potential growth. There is also a suspicion that the insecticide treatment may have influenced the reduction of leaf area. Though not definitive, the fact that it had the lowest leaf are but received less herbivory that the Control treatment in both years could confirm the suspicion of an inhibitory effect. There was also a slight difference between the mesh treatment and Invertebrate Only that was unexpected. Observations in the field indicate that the protective cages were too small and may have inhibited growth with leaves desiccating where they touched the cage wire. Leaves in the Mesh treatment were usually observed resting on the mesh fabric without contact with wire.

Overall treatment assessment

Caged plants achieved a greater leaf area than plants not caged, while herbivory decreased in plants caged. Except for the 2014 flower season, there was not a significant difference between plants accessible to vertebrate and invertebrate and plants protected from vertebrates alone. This indicates that insects may consume portions of plant material similar to vertebrates.

There are different intensities and seasonal patterns of herbivory between the rosette and flower stage of *Spiranthes sp*. This could be due to temporal variability with rosettes above ground, and available to herbivores, for a longer period of time (~7 months). They are also exposed during spring when typically high temperature and moisture is conducive to vertebrate and invertebrate activity. For example, during the beginning of the rosettes phase when temperature are cooler (December – January) there

is little herbivory except for occasional low (chronic) herbivory. Only 18% of the insects observed and collected were during this period. In the spring rosette season, herbivores became more active and acute and chronic herbivory was observed. During the flowering season, plants in the experiment where above ground for a much shorter time (~2.5 months) and received more acute herbivory by full removal of inflorescence. The acute herbivory received on the inflorescence could be due to temporal and spatial variability. Temporally, *S. parksii* inflorescence could be visibly targeted during the fall flower season when fewer flowering plants are present. Spatially, herbivores may not choose a plant by random coincidence, but by consuming the nearest neighbors when plants are grouped together (Gross et al. 1995). Since *S. parksii* are often found along game trail in small groups, vertebrate herbivores could target the easily accessible plants and ignore those off the path. Winged insect herbivores may also target specific patches on the landscape for consumption or depositing eggs.

Rooting by feral hogs and consumption of *Spiranthes sp*. tubers by mice occurred during the rosette and dormant phase. It has been documented that herbivory by vertebrates can make a plant produce compounds that attracts and benefit specific insect herbivores (Martinsen 1998). The reverse could be the case for *Spiranthes sp.* rosettes that when stressed by herbivory or resource limited they produce a chemical that attracts vertebrates.

Growth Observations

Understanding the growth cycle of a species can influence how it is managed. The tissue observed above ground during the normal dormant phase of *Spiranthes sp*. could be an important clue to how the species responds to biotic and abiotic stressors before flowering. Though it has not been verified, it is suspected that this new growth may be meristematic tissue. Meristematic tissue is undifferentiated cells that can develop into rosettes or inflorescence depending on environmental variables (Okamuro 1996; Clark 2001). Since this tissue was observed a month before normal inflorescence activity, it could indicate that conditions during this time have the most influence on the plants ques to flower.

The meristematic tissue could also influence the growth and development of the leaves observed encasing the emerging inflorescence. In some epiphytic orchids, sheath like bracts and cataphyll form around developing inflorescence and offer protection from herbivores and abiotic influences. Within a month of recording meristematic tissue above ground, the leaves observed encasing *Spiranthes sp*. inflorescence begin to emerge. If the meristematic tissue is triggered by favorable or unfavorable conditions it could cue the emerging plant to form a more protective barrier around the plant, or trigger it not to reproduce. It is unclear if the meristematic tissue develops into the leaves and inflorescence, but the new growth can come from the same area of the tuber as the rosette and inflorescence. Herbivory observed on the emerging leaves indicated they may offer some protection from consumption.

Understanding the influence of leaf emergence with inflorescence and the role of the unknown summer vegetation could help increase knowledge of *S. parksii's* life cycle to improve conservation efforts.

Growth Variability

Plant growth can vary due to microclimate, developmental stages of plants, and their ability to intake and allocate resources (Jones 1993). In this study, there was wide variability in plant growth and herbivory. Even among the same species there are plants that outperform and underperform for a variety of reasons such as microclimate and plant development stage. Though the study site was chosen for homogeneity, variability in soil properties, shade, moisture and other environmental variables can influence individual plants on a microclimate scale that could affect growth. For *Spiranthes sp.* there was significant variability in rosettes and flower plants size. In 2014 the average growth of flower plants was 25 cm, but some plants achieved height over 35 cm and under 13 cm. One plant observed in a particularly favorable microclimate reached a height of 36.5 cm. This was also noted in rosettes where one rosette reached a maximum leaf area of 58 cm when the average range was from 14 to 22 cm.

This variability was also noted in percent herbivory where a plant may experience only 5 percent herbivory and another 100 percent. This variability could be due to the spatial and temporal selection by herbivores, where a group of plants may be easily accessible or highly visible and consumed, while another group is missed.

This wide range of growth and extreme outliers did not allow for normality even after transformation attempts or equal variance requiring the analysis to be completed by

non-parametric methods. This explains the difference seen between calculated mean growth and the statistical analysis. For example, in Table 5 the mean Leaf Area in May 2014 looks significantly different between Vertebrate Only and Invertebrate Only, but the non-parametric analysis which uses the median growth did not detect a difference with the influence of the outliers. This is also seen in the 2015 plant herbivory analysis where the mean herbivory ranges from 2.3 to 21.5 percent but extremes ranged from 0 and 100 percent herbivory. This caused the median to indicate no significant difference between Invertebrate Only and Control when the mean indicated there should be a difference.

Continuation of this study should include data collection and analysis of potential influential environmental variables to account for variation in future analysis.

Invertebrate Observation

At the Twin Oaks site, insects where often seen on or near plants, but it was uncertain of their utilization. For example, there was an observation of fire ants attacking and killing an armyworm on a *S. parksii*. The carcass was stripped of skin, but half of the flesh was left, leaving the observer to wonder if they simply killed it due to territory or were hunting specific prey on the plant. Arachnids were often seen on or near the plant, but it was undetermined if this was due to predation of insects on *Spiranthes sp.* or simply resting on the plant.

In the roly poly experiment, there was indication that the species could be a major contributor to seed and seedling mortality. Since this consisted of a mostly observation analysis, due to no control cages, this is an area that requires more research.

Some insects, however, were very clear in their purpose on the plant. The majority of other insect herbivores may have injured healthy tissue or reduced fecundity, but only members of the Acrididae and Noctuidae family directly affect reproduction by removing inflorescence before seed production. Though they do not consume as many plants as large herbivores, these species are just as detrimental to individual plants ability to reproduce.

Nutrient Analysis

Micro and macronutrients are compounds required for plant growth and reproduction. Deficiency in nutrients can affect the development, size, and ability of a plant to reproduce. These nuances of nutrients required by a plant are specific to each species and have to be studied independently. To date there is no known research on the nutrient requirements of *S. parksii* or *S. cernua*. Based on general plant biochemistry, *Spiranthes sp*. falls above the norms in macronutrients such as Nitrogen (1.5%) and Potassium (1.0%), but is below average in Phosphorus (0.2%) (Epstein 1965). Wonkka (2012) found that during the flower season, *S. parksii* biomass is increased with phosphorus addition but not Nitrogen. The below average phosphorus in rosette tissue indicates that phosphorus is a limiting nutrient, and may be related to their association with mycorrhizal fungi (Bolan 1991). *Spiranthes sp*. is also above average in the micronutrients Sulfur (1,000 ppm). Sulfur volatiles are known to be a deterrent against herbivores and can be combined with nitrogen to form other volatiles.

The nutrient in a plant can also affect the behavior of herbivores. Plants that are high in nutrients are often targeted by mobile herbivores and parental insect selection of

egg deposit sites. For insect plant nutrients are specifically crucial since some may spend most of their life cycle on a single plant or have to expend immense energy to relocate.

This can be either directly by a plant dying due to lack of nutrients, or indirectly by the plant unable to store enough carbon for reproduction by having to reallocate nutrients for survival during times of stress. With elevated levels, some nutrients can be toxic to plant or reduce the ability to uptake another element.

A negative influence on most endangered plants species survival is anthropogenic activity (Wilcove et al. 1998). The identification and reduction of these natural pressures on endangered species could potentially mitigate some of this influence. In the reproductive stage of *Spiranthes sp*., this experiment showed that vertebrates can consume up to 50% of the inflorescence tissue causing a direct impact on reproduction and fitness of the species. In the rosettes stage, invertebrates and vertebrates were shown to consume 39% to 58% leaf area of rosettes. This reduction in leaf area could reduce *Spiranthes sp*. ability to flower, number of flowers produced, and seed production (Wonkka 2013).

Herbivores can have an even stronger effect on plant fitness in savannas where cycles of normal precipitation are often followed by drought. This was seen in 2015 when drought reduced inflorescence production and the few plants that flowered were eaten by herbivores before they could seed. Although this study did not address how a reduction in flowering plants may influence *Spiranthes sp.* persistence, it did prove that vertebrate and invertebrate herbivory can decrease the seedbank and the ability of individual plants to reproduce. This indicated that *Spiranthes sp.* should be protected

from large herbivores in the flowering phase to improve chance of seed dispersal, and invertebrate impact managed during the rosette season to promote root carbon storage.

The majority of studies on plant-insect herbivory interactions with orchids have been conducted under controlled greenhouse conditions. However, the complex and dynamic interactions observed in this study show the importance of understanding the influence of herbivores, especially invertebrates, on orchids in their native habitat. The knowledge gained in this experiment can not only be used to improve conservation and management plans for *S. parksii*, but may provide clues that lead to the discovery of invertebrate impacts on other species.

REFERENCES

Antlfinger A., Wendel L. 1997. Reproductive effort and floral photosynthesis in *Spiranthes cernua* (Orchidaceae). American Journal of Botany **84**: 769-769.

Ariza MC. 2013. Mycorrhizal associations, life history, and habitat characteristics of the endangered terrestrial orchid *Spiranthes parksii* Correll and sympatric congener *Spiranthes cernua*: implications for conservation. Doctoral dissertation, Department of Ecosystem Science and Management, Texas A&M University, College Station, Texas.

Batzli GO, Pitelka FA. 1970. Influence of meadow mouse populations on California grassland. Ecology **51**:1027-1039.

Beanland L, Phelan PL, Salminen S. 2003. Micronutrient interactions on soybean growth and the developmental performance of three insect herbivores. Environmental Entomology **32**: 641-651.

Bolan NS. 1991. A critical review on the role of mycorrhizal fungi in the uptake of phosphorus by plants. Plant and Soil **134**:189-207.

Bruton RK. 2014. The effects of woody plant management on habitat conditions, plant demography, and transplantation success of the endangered orchid *Spiranthes parksii* Correll. Masters of Science Thesis, Department of Ecosystem Science and Management, Texas A&M University, College Station, Texas.

Clark SE. 2001. Cell signaling at the shoot meristem. Nature Reviews Molecular Cell Biology **2**:276-284.

Crawley MJ. 1989. Insect herbivores and plant population dynamics. Annual Review of Entomology **34**:531-562.

Darwin C. 1862. On the various contrivances by which British and foreign orchids are fertilized by insects, and on the good effects of intercrossing. London, United Kingdom.

Delaney KJ, Macedo TB. 2001. The impact of herbivory on plants: yield, fitness, and population dynamics. Biotic stress and yield loss. CRC Press, Boca Raton, Florida, United States.

Diggs GM, Lipscomb BL, O'Kennon RJ, Mahler WF, Shinners LH. 1999. Shinners' and Mahler's illustrated flora of North Central Texas. Botanical Research Institute of Texas (BRIT). Fort Worth, Texas, United States.

Epstein E. 1972. Mineral nutrition of plants: principles and perspectives. John Wiley and Sons, New York, New York.

Gomez JM, Zamora R. 2000. Differential impact of vertebrate and invertebrate herbivores on the reproductive output of *Hormathophylla spinosa*. EcoScience **7**:299-306.

Gross JE, Zank C, Hobbs NT, Spalinger DE. 1995. Movement rules for herbivores in spatially heterogeneous environments: responses to small scale pattern. Landscape Ecology **10**:209-217.

Hammons JR. 2008. Demographic, life cycle, habitat characterization and transplant methods for the endangered orchid, *Spiranthes parksii* Correll. Masters of Science Thesis, Department of Ecosystem Science and Management, Texas A&M University, College Station, Texas.

Hammons JR, Smeins FE, Rogers WE. 2010. Transplant methods for the endangered orchid, *Spiranthes parksii* Correll. North American Native Orchid Journal **16**:38-46.

Hand LF, Keaster AJ. 1967. The environment of an insect field cage. Journal of Economic Entomology **60**:910-915.

Huntly N. 1991. Herbivores and the dynamics of communities and ecosystems. Annual Review of Ecology and Systematics **22**:477-503.

Jackson CC. 2010. "GRIMES COUNTY," Handbook of Texas Online(**http://www.tshaonline.org/handbook/online/articles/hcg11**), accessed July 27, 2015. Uploaded on June 15, 2010. Published by the Texas State Historical Association.

Jones MB. 1993. Plant microclimate. In Photosynthesis and Production in a Changing Environment. Springer Science, Netherlands.

Kessler A, Baldwin IT. 2002. Plant responses to insect herbivory: the emerging molecular analysis. Annual Review of Plant Biology **53**:299-328.

Kindlmann P, Balounová Z. 2001. Irregular flowering patterns in terrestrial orchids: theories vs. empirical data. Web Ecology **2**:75-82.

Knight TM, Caswell H, Kalisz S. 2009. Population growth rate of a common understory herb decreases non-linearly across a gradient of deer herbivory. Forest Ecology and Management **257**:1095-1103.

Louda SM. 1984. Herbivore effect on stature, fruiting, and leaf dynamics of a native crucifer. Ecology **65**:1379-1386.

Mariani S, Alcoverro T. 1999. A multiple-choice feeding-preference experiment utilizing seagrasses with a natural population of herbivorous fishes. Marine Ecology Progress Series **189**:295-299.

Martinsen GD, Direbe EM, Whitham TG. 1998. Indirect interactions mediated by changing plant chemistry: beaver browsing benefits beetles. Ecology **79**:192-200.

Mattson WJ, Haack RA. 1987. The role of drought in outbreaks of plant-eating insects. BioScience **37**:110-118.

Mayer EL, Babers FH. 1944. Head-capsule measurements of southern armyworm larvae (Prodenia eridania (Cramer)). Annals of the Entomological Society of America, **37**:214-220.

McNaughton SJ. 1984. Grazing lawns: animals in herds, plant form, and coevolution. American Naturalist **124**:863-886.

NCSS (National Cooperative Soil Survey). 2007. Digital soil survey for Grimes County. Available from Texas Natural Resources Information System (TNRIS) at http://www.tnrid.state.tx.us/ accessed February 2016.

Okamuro JK, Den Boer BG, Jofuku KD. 1993. Regulation of *Arabidopsis* flower development. The Plant Cell, **5**:1183.

Ott L, Longnecker M. 2010. An introduction to statistical methods and data analysis. Sixth edition. Duxbury Press, Belmont, California, United States.

Paige KN, Whitham TG. 1987. Overcompensation in response to mammalian herbivory: the advantage of being eaten. American Naturalist **129**:407-416.

Price PW. 1991. The plant vigor hypothesis and herbivore attack. Oikos **62**:244-251.

Prins AH, Nell HW. 1990. Positive and negative effects of herbivory on the population dynamics of *Senecio jacobaea* L. and *Cynoglossum officinale* L. Oecologia **83**:325-332.

Rasmussen HN. 1995. Terrestrial orchids: from seed to mycotrophic plant. Cambridge University Press, Cambridge.

Rosenthal JP, Kotanen PM. 1994. Terrestrial plant tolerance to herbivory. Trends in Ecology & Evolution **9**:145-148.

Saska P. 2008. Granivory in terrestrial isopods. Ecological Entomology **33**:742-747.

Schmid F, Trede M. 1996. Testing for first-order stochastic dominance: a new distribution-free test. The Statistician **45**:371-380.

Schmidly DJ, 1994. The Mammals of Texas: Revised Edition. University of Texas Press, Austin, Texas.

SRCC (Southern Regional Climate Center). 2012. Climotograph for southeastern Texas: Temperature and Precipitation Normals (1971-2000). Available from http://www.srcc.lsu.edu/climate_normals.html (accessedAugust 2015).

Stone GN, Schönrogge K. 2003. The adaptive significance of insect gall morphology. Trends in Ecology & Evolution **18**:512-522.

Strauss SY, Agrawal AA. 1999. The ecology and evolution of plant tolerance to herbivory. Trends in Ecology & Evolution **14**:179-185.

Sutton S. 2013. Woodlice (Invertebrate type). Pergamon Press Ltd., Elmsford, New York.

Triplehorn CA, Johnson NF. 2005. Borror and DeLong's introduction to the study of insects. Seventh edition. Thomson, Belmont, California, United States.

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

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

USGS. 1993. Texas Geology Web Map Viewer. http://txpub.usgs.gov/DSS/texasgeology/, accessed July 20, 2014.

Wilcove DS, Rothstein D, Dubow J, Phillips A, Losos E. 1998. Quantifying threats to imperiled species in the United States. BioScience **48**:607-615.

Wonkka CL. 2010. Large herbivore impacts on demographic characteristics and population dynamics of an endangered orchid (*Spiranthes parksii* Correll). M. S. Thesis, Department of Ecosystem Science and Management, Texas A&M University, College Station, Texas.

Wonkka CL, Rogers WE, Smeins FE, Hammons JR, Ariza MC, Haller SJ. 2012. Biology, ecology, and conservation of Navasota ladies-tresses (*Spiranthes parksii* Correll): an endangered terrestrial orchid of Texas. Native Plants Journal **13**:236–243.

APPENDIX

2014 Flowering Plant Height, Stalk Length, and Inflorescence Length by Treatment							
Treatment	**-Treatment**	**p-Value**			**Z-score**		
		Plant	Stalk	Inflor.	Plant	Stalk	Inflor.
Control	Vertebrate Only	0.361	0.239	0.279	-0.913	-1.178	-1.083
Control	Cage+Insecticide	0.378	0.522	0.794	-0.378	-0.640	0.261
Control	Invertebrate Only	0.639	0.850	0.607	0.470	0.189	0.514
Control	Mesh	0.803	0.586	0.941	-0.249	-0.545	0.074
Vertebrate Only	Mesh	0.287	0.512	0.145	1.064	0.656	1.456
Vertebrate Only	Invertebrate Only	0.213	0.216	0.133	1.245	1.237	1.504
Vertebrate Only	Cage+Insecticide	0.427	0.550	0.119	0.794	0.598	1.558
Invertebrate Only	Cage+Insecticide	0.530	0.455	0.877	-0.628	-0.747	0.155
Mesh	Cage+Insecticide	0.992	0.928	0.833	0.010	-0.090	-0.315
Mesh	Invertebrate Only	0.710	0.431	0.753	-0.372	-0.787	-0.212

2014 Flowering Plant Height, Stalk Length, and Inflorescence Length by Treatment							
Treatment	**-Treatment**	**Score Mean Difference**			**Standard Error Difference**		
		Plant	Stalk	Inflor.	Plant	Stalk	Inflor.
Mesh	Control	-0.947	-2.061	0.278	3.803	3.783	3.760
Cage+Insecticide	Control	-1.413	-2.375	0.961	3.736	3.713	3.685
Invertebrate Only	Control	1.723	0.689	1.838	3.668	3.651	3.574
Vertebrate Only	Control	-2.995	-3.842	-3.516	3.280	3.263	3.247
Mesh	Vertebrate Only	4.101	2.520	5.534	3.854	3.841	3.800
Invertebrate Only	Vertebrate Only	4.654	4.603	5.473	3.738	3.721	3.638
Cage+Insecticide	Vertebrate Only	3.014	2.261	5.828	3.796	3.781	3.740
Cage+Insecticide	Invertebrate Only	-2.511	-2.980	0.596	4.000	3.987	3.855
Mesh	Cage+Insecticide	0.041	-0.367	-1.265	4.082	4.069	4.018
Mesh	Invertebrate Only	-1.502	-3.172	-0.834	4.043	4.028	3.946

2014 Flowering Plant Height Quantiles by Treatment							
	Quantiles						
Treatment	**Minimum**	**10%**	**25%**	**Median**	**75%**	**90%**	**Maximum**
Control	13.5	16.5	22.8	24.7	30.8	33.9	35.3
Vertebrate Only	16.2	16.6	18	24	27.5	31.3	35.6
Invertebrate Only	7.5	18.6	23.3	26.2	29	35.3	36.5
Cage+Insecticide	5.9	12.2	21.2	25.5	29.6	32.3	33.7
Mesh	17.5	17.9	20	26.4	29.1	31.5	32.8

2014 Stalk Length Quantiles by Treatment							
	Quantiles						
Treatment	**Minimum**	**10%**	**25%**	**Median**	**75%**	**90%**	**Maximum**
Control	15	16	17	19	22.25	26	27
Vertebrate Only	13	13	14.5	18	21	23.2	28
Invertebrate Only	7	13.8	17	20	23	25.6	28
Cage+Insecticide	5	10	16	19.5	22	26	26
Mesh	13	13	16	19	22.5	24.4	25

2014 Inflorescence Length Quantiles by Treatment							
	Quantiles						
Treatment	**Minimum**	**10%**	**25%**	**Median**	**75%**	**90%**	**Maximum**
Control	3	3.5	5	6.5	9	10	10
Vertebrate Only	2	2	4	6	7.5	8	8
Invertebrate Only	1	5	6	7	7	11	11
Cage+Insecticide	1	2.5	6	7	8	9	28
Mesh	3	4.6	5	7	8	9	13

2015 Flowering Plant Height, Stalk Length, and Inflorescence Length by Treatment							
Treatment	**-Treatment**	**p-Value**			**Z-score**		
		Plant	Stalk	Inflor.	Plant	Stalk	Inflor.
Control	Vertebrate Only	0.434	0.224	0.908	-0.782	-1.217	-0.116
Control	Cage+Insecticide	0.254	0.709	0.043*	1.141	0.373	2.019
Control	Invertebrate Only	0.683	1.000	0.064	0.408	0.000	1.852
Control	Mesh	0.065	0.285	0.014*	1.843	1.069	2.452
Vertebrate Only	Mesh	0.020*	0.011*	0.072	2.333	2.540	1.798
Vertebrate Only	Invertebrate Only	0.281	0.372	0.218	1.078	0.892	1.232
Vertebrate Only	Cage+Insecticide	0.048*	0.080	0.068	1.974	1.752	1.827
Invertebrate Only	Cage+Insecticide	0.610	0.693	0.626	0.511	0.395	0.488
Mesh	Cage+Insecticide	0.454	0.316	0.975	0.748	1.003	0.032
Mesh	Invertebrate Only	0.213	0.129	0.413	1.246	1.518	0.818

2015 Flowering Plant Height, Stalk Length, and Inflorescence Length by Treatment							
Treatment	**-Treatment**	**Score Mean Difference**			**Standard Error Difference**		
		Plant	Stalk	Inflor.	Plant	Stalk	Inflor.
Mesh	Vertebrate Only	7.961	8.665	6.129	3.412	3.411	3.409
Mesh	Control	6.346	3.680	8.440	3.442	3.443	3.441
Cage+Insecticide	Vertebrate Only	6.136	5.446	5.676	3.107	3.108	3.106
Mesh	Invertebrate Only	4.266	5.200	2.800	3.424	3.424	3.422
Cage+Insecticide	Control	3.625	1.185	6.413	3.178	3.177	3.176
Invertebrate Only	Vertebrate Only	3.049	2.526	3.484	2.829	2.831	2.829
Mesh	Cage+Insecticide	2.643	3.543	0.112	3.532	3.532	3.530
Cage+Insecticide	Invertebrate Only	1.604	1.239	1.531	3.140	3.139	3.139
Invertebrate Only	Control	1.201	0.000	5.448	2.945	2.945	2.941
Vertebrate Only	Control	-2.265	-3.524	-0.335	2.895	2.896	2.892

2015 Flowering Plant Height Quantiles by Treatment							
	Quantiles						
Treatment	**Minimum**	**10%**	**25%**	**Median**	**75%**	**90%**	**Maximum**
Control	1.6	1.8	3.2	10.6	16.6	18.6	18.9
Vertebrate Only	1.3	1.3	1.5	5.1	13.1	17.7	17.7
Invertebrate Only	0.7	1.2	7.7	12.4	16.0	22.6	25.2
Cage+Insecticide	2.2	2.3	6.6	13.5	17.7	20.7	23.3
Mesh	2.2	4.2	10.5	15.8	17.9	20.7	24

2015 Flower Stalk Length Quantiles by Treatment							
	Quantiles						
Treatment	**Minimum**	**10%**	**25%**	**Median**	**75%**	**90%**	**Maximum**
Control	0.9	1.2	2.3	7.4	11.5	15.7	16.1
Vertebrate Only	0.5	0.54	1.3	4.1	10	10.5	10.6
Invertebrate Only	0.3	0.75	5.5	8.1	10.2	16.0	17.6
Cage+Insecticide	1.6	1.6	4.3	9.3	11.3	13.2	15.6
Mesh	1.7	3.2	7.0	10.1	13.1	15.1	17.4

2015 Inflorescence Length Quantiles by Treatment							
	Quantiles						
Treatment	**Minimum**	**10%**	**25%**	**Median**	**75%**	**90%**	**Maximum**
Control	0.3	0.46	0.8	2	3.2	5.3	6.5
Vertebrate Only	0.2	0.3	0.8	1	4.6	7.1	7.2
Invertebrate Only	0.4	0.49	2.15	3.8	5.5	7.0	7.6
Cage+Insecticide	0.6	0.67	1.7	4.5	6.9	7.8	8.1
Mesh	0.5	0.76	2.2	5.1	6.4	6.6	7.2

2014 Flowering Plant Herbivory by Treatment							
Treatment	**-Treatment**	**p-Value**			**Z-score**		
		Plant	Stalk	Inflor.	Plant	Stalk	Inflor.
Control	Vertebrate Only	0.555	0.862	0.265	-0.5903	0.174	-1.114
Control	Cage+Insecticide	<.0001*	0.0008*	<.0001*	-4.216	-3.359	-4.456
Control	Invertebrate Only	<.0001*	0.0060*	<.0001*	-4.184	-2.750	-4.393
Control	Mesh	<.0001*	0.011*	<.0001*	-4.184	-2.547	-4.886
Vertebrate Only	Mesh	0.0034*	0.0052*	0.0003*	-2.929	-2.796	-3.636
Vertebrate Only	Invertebrate Only	0.0026*	0.0029*	0.0021*	-3.011	-2.974	-3.075
Vertebrate Only	Cage+Insecticide	0.0008*	0.0003*	0.0017*	-3.370	-3.581	-3.141
Invertebrate Only	Cage+Insecticide	0.274	0.155	0.955	-1.095	-1.423	-0.056
Mesh	Cage+Insecticide	0.183	0.052	0.415	1.333	1.947	-0.814
Mesh	Invertebrate Only	0.7183	0.5799	0.372	0.36076	0.554	-0.892

2014 Flowering Plant Herbivory by Treatment							
Treatment	**-Treatment**	**Score Mean Difference**			**Standard Error Difference**		
		Plant	Stalk	Inflor.	Plant	Stalk	Inflor.
Mesh	Cage+Insecticide	5.104	7.145	-2.327	3.670	3.828	2.857
Mesh	Invertebrate Only	1.293	1.920	-2.546	3.468	3.586	2.852
Vertebrate Only	Control	-1.900	0.543	-3.393	3.129	3.219	3.045
Cage+Insecticide	Invertebrate Only	-4.087	-5.108	-0.170	3.590	3.734	3.040
Invertebrate Only	Vertebrate Only	-10.690	-10.332	-10.281	3.474	3.550	3.343
Mesh	Vertebrate Only	-10.771	-9.931	-11.957	3.552	3.678	3.288
Cage+Insecticide	Vertebrate Only	-12.410	-12.913	-10.601	3.606	3.683	3.374
Invertebrate Only	Control	-14.568	-9.090	-14.869	3.305	3.481	3.384
Mesh	Control	-15.200	-8.652	-16.544	3.397	3.632	3.385
Cage+Insecticide	Control	-15.298	-11.740	-15.298	3.495	3.631	3.433

2014 Flowering Plant Herbivory Quantiles by Treatment							
	Quantiles						
Treatment	**Minimum**	**10%**	**25%**	**Median**	**75%**	**90%**	**Maximum**
Control	3	3	5	53	98	99	99
Vertebrate Only	0	0	3	10	98	99.2	100
Invertebrate Only	0	0	3	3	3	5	10
Cage+Insecticide	0	0	0	1.5	4.5	5	15
Mesh	0	0	3	3	4	5	5

2014 Stalk Herbivory Quantiles by Treatment							
	Quantiles						
Treatment	**Minimum**	**10%**	**25%**	**Median**	**75%**	**90%**	**Maximum**
Control	0	2	5	5	95	98	98
Vertebrate Only	0	0	5	10	95	98.2	99
Invertebrate Only	0	0	0	5	5	8	10
Cage+Insecticide	0	0	0	0	5	7.5	10
Mesh	0	0	2.5	5	5	10	10

2014 Inflorescence Herbivory Quantiles by Treatment							
	Quantiles						
Treatment	**Minimum**	**10%**	**25%**	**Median**	**75%**	**90%**	**Maximum**
Control	0	2	5	100	100	100	100
Vertebrate Only	0	0	0	10	100	100	100
Invertebrate Only	0	0	0	0	5	5	10
Cage+Insecticide	0	0	0	0	3.75	5	20
Mesh	0	0	0	0	0	5	5

2014 and 2015 Flowers Produced by Treatment					
Treatment	**-Treatment**	**p-Value**		**Z-score**	
		2014	2015	2014	2015
Control	Vertebrate Only	0.871	0.739	0.161	0.333
Control	Cage+Insecticide	0.533	0.073	-0.622	1.787
Control	Invertebrate Only	0.552	0.106	-0.594	1.613
Control	Mesh	0.621	0.0484*	-0.493	1.973
Vertebrate Only	Mesh	0.285	0.270	-1.067	1.102
Vertebrate Only	Invertebrate Only	0.203	0.445	-1.272	0.762
Vertebrate Only	Cage+Insecticide	0.229	0.331	-1.201	0.971
Invertebrate Only	Cage+Insecticide	0.948	0.651	0.064	0.451
Mesh	Cage+Insecticide	0.710	1.000	0.371	0.000
Mesh	Invertebrate Only	0.634	0.498	0.476	0.676

2014 and 2015 Flowers Produced by Treatment					
Treatment	**-Treatment**	**Score Mean Difference**		**Standard Error Difference**	
		2014	2015	2014	2015
Control	Vertebrate Only	0.502	0.825	3.106	2.476
Control	Cage+Insecticide	-2.312	5.322	3.716	2.978
Control	Invertebrate Only	-2.167	4.416	3.645	2.737
Control	Mesh	-1.870	6.466	3.788	3.276
Vertebrate Only	Mesh	-4.053	3.600	3.797	3.264
Vertebrate Only	Invertebrate Only	-4.681	2.016	3.678	2.643
Vertebrate Only	Cage+Insecticide	-4.495	2.843	3.740	2.925
Invertebrate Only	Cage+Insecticide	0.255	1.385	3.987	3.066
Mesh	Cage+Insecticide	1.511	0.000	4.033	3.445
Mesh	Invertebrate Only	1.920	2.266	4.033	3.349

2014 Flowers Produced Quantiles by Treatment							
	Quantiles						
Treatment	**Minimum**	**10%**	**25%**	**Median**	**75%**	**90%**	**Maximum**
Control	11	11.4	13.5	17	22	25.8	27
Vertebrate Only	11	11.6	16	19	21	27.8	35
Invertebrate Only	0	10.4	14	16	20	29	37
Cage+Insecticide	0	4	14	16	20	25.5	30
Mesh	8	11	14	17	19	24	25

2015 Flowers Produced Quantiles by Treatment							
	Quantiles						
Treatment	**Minimum**	**10%**	**25%**	**Median**	**75%**	**90%**	**Maximum**
Control	0	0	0	0	3.5	16.4	20
Vertebrate Only	0	0	0	0	14.75	20.9	21
Invertebrate Only	0	0	0	8	12.25	21.6	24
Cage+Insecticide	0	0	0	10	18.75	22.3	23
Mesh	0	0	0	10	14.75	21.8	22

2015 Flowering Plant Herbivory by Treatment							
Treatment	**-Treatment**	**p-Value**			**Z-score**		
		Plant	Stalk	Inflor.	Plant	Stalk	Inflor.
Control	Vertebrate Only	0.803	0.974	0.345	-0.249	0.033	-0.945
Control	Cage+Insecticide	0.035*	0.162	0.021*	-2.109	-1.400	-2.309
Control	Invertebrate Only	0.238	1.000	0.013*	-1.179	0.000	-2.486
Control	Mesh	0.039*	0.190	0.061	-2.065	-1.311	-1.876
Vertebrate Only	Mesh	0.06	0.116	0.490	-1.873	-1.572	-0.692
Vertebrate Only	Invertebrate Only	0.394	0.898	0.150	-0.853	-0.129	-1.439
Vertebrate Only	Cage+Insecticide	0.058	0.133	0.328	-1.893	-1.502	-0.979
Invertebrate Only	Cage+Insecticide	0.144	0.074	0.462	-1.461	-1.785	0.736
Mesh	Cage+Insecticide	0.654	0.872	0.891	-0.450	-0.162	0.137
Mesh	Invertebrate Only	0.106	0.068	0.383	-1.617	-1.825	0.872

2015 Flowering Plant Herbivory by Treatment							
Treatment	**-Treatment**	**Score Mean Difference**			**Standard Error Difference**		
		Plant	Stalk	Inflor.	Plant	Stalk	Inflor.
Vertebrate Only	Control	-0.696	0.087	-2.583	2.796	2.663	2.733
Mesh	Cage+Insecticide	-1.406	-0.450	0.393	3.132	2.783	2.865
Invertebrate Only	Vertebrate Only	-2.352	-0.348	-3.333	2.758	2.705	2.317
Invertebrate Only	Control	-3.333	0.000	-6.416	2.826	2.761	2.581
Cage+Insecticide	Invertebrate Only	-4.302	-5.104	1.750	2.943	2.859	2.377
Mesh	Invertebrate Only	-5.133	-5.533	2.266	3.173	3.031	2.599
Cage+Insecticide	Vertebrate Only	-5.599	-4.142	-2.625	2.958	2.756	2.682
Mesh	Vertebrate Only	-5.918	-4.579	-2.000	3.160	2.912	2.890
Cage+Insecticide	Control	-6.343	-3.864	-6.635	3.007	2.761	2.873
Mesh	Control	-6.600	-3.800	-5.800	3.196	2.898	3.092

2015 Flowering Plant Herbivory Quantiles by Treatment							
	Quantiles						
Treatment	**Minimum**	**10%**	**25%**	**Median**	**75%**	**90%**	**Maximum**
Control	0	0	0.75	6.5	44.7	88.2	99
Vertebrate Only	0	0	0	5	25	91	100
Invertebrate Only	0	0	0.75	3	5	17.9	20
Cage+Insecticide	0	0	0	1.5	3	13	13
Mesh	0	0	0	0	3	75	75

2015 Stalk Herbivory Quantiles by Treatment							
	Quantiles						
Treatment	**Minimum**	**10%**	**25%**	**Median**	**75%**	**90%**	**Maximum**
Control	0	0	0	2.5	20	76.1	98
Vertebrate Only	0	0	0	5	10	89.2	99
Invertebrate Only	0	0	0	5	10	31	40
Cage+Insecticide	0	0	0	0	5	13	20
Mesh	0	0	0	0	3.75	50	50

2015 Inflorescence Herbivory Quantiles by Treatment							
	Quantiles						
Treatment	**Minimum**	**10%**	**25%**	**Median**	**75%**	**90%**	**Maximum**
Control	0	0	0	7.5	76.2	100	100
Vertebrate Only	0	0	0	0	21.2	100	100
Invertebrate Only	0	0	0	0	0	12	15
Cage+Insecticide	0	0	0	0	5	8	15
Mesh	0	0	0	0	5	100	100

2014 and 2015 Rosette Growth by Treatment							
Treatment	**-Treatment**	**p-Value**			**Z-score**		
		May '14	May '15	Max '15	May '14	May '15	Max '15
Control	Vertebrate Only	0.410	0.694	0.404	-0.823	0.394	-0.835
Control	Cage+Insecticide	0.783	0.107	0.398	0.276	1.611	0.846
Control	Invertebrate Only	0.675	0.981	0.380	0.420	0.024	-0.877
Vertebrate Only	Invertebrate Only	0.102	0.627	0.934	1.635	-0.486	-0.083
Vertebrate Only	Cage+Insecticide	0.084	0.136	0.173	1.729	1.493	1.362
Invertebrate Only	Cage+Insecticide	0.562	0.121	0.233	-0.579	1.552	1.193
Mesh	Cage+Insecticide	------	------	0.415	------	------	0.815
Mesh	Invertebrate Only	------	------	0.015*	------	------	2.425
Control	Mesh	------	------	0.045*	------	------	2.002
Vertebrate Only	Mesh	------	------	0.009*	------	------	2.600

2014 and 2015 Rosette Growth by Treatment							
Treatment	**-Treatment**	**Score Mean Difference**			**Standard Error Difference**		
		May '14	May '15	Max '15	May '14	May '15	Max '15
Cage+Insecticide	Vertebrate Only	6.181	5.835	5.509	3.573	3.909	4.044
Invertebrate Only	Vertebrate Only	5.600	-1.898	-0.333	3.424	3.909	4.044
Invertebrate Only	Control	1.457	0.092	-3.547	3.469	3.909	4.044
Cage+Insecticide	Control	0.993	6.298	3.422	3.601	3.909	4.044
Cage+Insecticide	Invertebrate Only	-2.195	6.400	4.920	3.789	4.123	4.123
Vertebrate Only	Control	-2.476	1.421	-3.304	3.007	3.605	3.957
Mesh	Vertebrate Only	------	------	10.518	------	------	4.044
Mesh	Invertebrate Only	------	------	10.000	------	------	4.123
Mesh	Control	------	------	8.097	------	------	4.044
Mesh	Cage+Insecticide	------	------	3.360	------	------	4.123

2014 Rosette Growth (cm^2) Quantiles by Treatment							
	Quantiles						
Treatment	**Minimum**	**10%**	**25%**	**Median**	**75%**	**90%**	**Maximum**
Control	2.3	2.3	4.5	12.1	18.7	28.8	30.2
Vertebrate Only	2.4	2.5	3.8	6.6	12.6	24.8	26.3
Invertebrate Only	0.3	2.5	8.7	12.2	19.4	24.6	31.3
Cage+Insecticide	4.7	5.1	6.5	10.8	17.6	30.8	37.4
Mesh	------	------	------	------	------	------	------

2015 May Rosette Growth (cm^2) Quantiles by Treatment							
	Quantiles						
Treatment	**Minimum**	**10%**	**25%**	**Median**	**75%**	**90%**	**Maximum**
Control	0	0.182	4.6	12.7	19.1	27.4	39.2
Vertebrate Only	0.22	0.322	5.0	7.6	15.0	20.0	27.4
Invertebrate Only	0.09	2.162	4.0	8.4	16.5	33.0	38.2
Cage+Insecticide	0.86	1.984	8.3	14.7	30.1	38.4	58.4
Mesh	------	------	------	------	------	------	------

2015 Max Rosette Growth (cm^2) Quantiles by Treatment							
	Quantiles						
Treatment	**Minimum**	**10%**	**25%**	**Median**	**75%**	**90%**	**Maximum**
Control	0.24	4.6	9.2	15.0	19.3	26.1	29.4
Vertebrate Only	3.8	4.3	6.5	11.6	17.	22.6	28.8
Invertebrate Only	3.6	3.6	6.6	10.5	18.0	33.0	38.1
Cage+Insecticide	1.4	2.4	8.3	15.7	30.2	43.4	58.4
Mesh	6.2	8.1	10.2	18.4	32.9	41.1	44.0

2014 and 2015 Rosette Herbivory by Treatment							
Treatment	**-Treatment**	**p-Value**			**Z-score**		
		May '14	May '15	Max '15	May '14	May '15	Max '15
Control	Vertebrate Only	0.056	0.143	0.974	-1.914	-1.466	-0.032
Control	Cage+Insecticide	0.004*	0.024*	0.016*	-2.870	-2.262	-2.407
Control	Invertebrate Only	0.075	0.767	0.060	-1.779	-0.297	-1.883
Vertebrate Only	Invertebrate Only	0.662	0.528	0.061	0.437	0.632	-1.873
Vertebrate Only	Cage+Insecticide	0.402	0.243	0.014*	-0.839	-1.168	-2.453
Invertebrate Only	Cage+Insecticide	0.165	0.086	0.533	-1.390	-1.719	-0.623
Mesh	Cage+Insecticide			0.091			-1.692
Mesh	Invertebrate Only			0.030*			-2.177
Control	Mesh			0.0003*			-3.609
Vertebrate Only	Mesh			0.0005*			-3.457

2014 and 2015 Rosette Herbivory by Treatment							
Treatment	**-Treatment**	**Score Mean Difference**			**Standard Error Difference**		
		May '14	May '15	Max '15	May '14	May '15	Max '15
Invertebrate Only	Vertebrate Only	1.396	2.107	-7.554	3.198	3.335	4.033
Cage+Insecticide	Vertebrate Only	-2.814	-3.815	-9.850	3.355	3.267	4.015
Cage+Insecticide	Invertebrate Only	-4.963	-4.766	-2.560	3.570	2.773	4.106
Vertebrate Only	Control	-5.961	-5.192	-0.127	3.115	3.542	3.987
Invertebrate Only	Control	-6.069	-0.972	-7.676	3.411	3.276	4.076
Cage+Insecticide	Control	-10.151	-7.311	-9.800	3.536	3.231	4.071
Mesh	Cage+Insecticide			-6.920			4.089
Mesh	Invertebrate Only			-8.920			4.096
Mesh	Vertebrate Only			-13.899			4.020
Mesh	Control			-14.659			4.061

2014 Rosette Herbivory Quantiles by Treatment							
	Quantiles						
Treatment	**Minimum**	**10%**	**25%**	**Median**	**75%**	**90%**	**Maximum**
Control	0	4.5	5	15	90	95.3	98
Vertebrate Only	0	1	3.7	5	12.5	75	85
Invertebrate Only	0	0.25	5	5	10	19.2	95
Cage+Insecticide	0	0	2.5	5	5	43.5	50
Mesh	------	------	------	------	------	------	------

2015 May Rosette Herbivory Quantiles by Treatment							
	Quantiles						
Treatment	**Minimum**	**10%**	**25%**	**Median**	**75%**	**90%**	**Maximum**
Control	0	0	10	30.8	65.6	98	98
Vertebrate Only	0	0	5	5	50	64	98
Invertebrate Only	0	0.50	3.7	28.7	56.1	96.8	98
Cage+Insecticide	0	0	0.93	5	13.7	89.9	98
Mesh	------	------	------	------	------	------	------

2015 Max Rosette Herbivory Quantiles by Treatment							
	Quantiles						
Treatment	**Minimum**	**10%**	**25%**	**Median**	**75%**	**90%**	**Maximum**
Control	0	9	23.5	59	95	99	99
Vertebrate Only	0	6.2	20	60	98	98.6	99
Invertebrate Only	0	0.6	6.5	23	80	98	99
Cage+Insecticide	0	0.6	4	15	59	98	98
Mesh	0	0	0	5	35.5	92	95

t-Test for Maximum plant Height between 2014 and 2015		
	Variable 1	**Variable 2**
Mean	25.0	11.9
df	141	
t Stat	13.5	
P(T<=t) one-tail	1.55201E-27	
t Critical one-tail	1.6557	
P(T<=t) two-tail	3.10403E-27	
t Critical two-tail	1.9769	

t-Test for Maximum Plant Herbivory between 2014 and 2015		
	Variable 1	**Variable 2**
Mean	15.8	13.5
df	162	
t Stat	0.4695	
P(T<=t) one-tail	0.3196	
t Critical one-tail	1.6543	
P(T<=t) two-tail	0.6392	
t Critical two-tail	1.9747	

t-Test for May Rosette Growth between 2014 and 2015		
	Variable 1	**Variable 2**
Mean	12.7	11.8
df	154	
t Stat	0.5499	
P(T<=t) one-tail	0.2915	
t Critical one-tail	1.6548	
P(T<=t) two-tail	0.5831	
t Critical two-tail	1.9754	

t-Test for May Rosette Herbivory between 2014 and 2015		
	Variable 1	**Variable 2**
Mean	18.2	30.5
df	117	
t Stat	-2.3123	
P(T<=t) one-tail	0.0112	
t Critical one-tail	1.6579	
P(T<=t) two-tail	0.0225	
t Critical two-tail	1.9804	